AN INVESTIGATION INTO AORTIC CALCIFICATION AND ITS RELEVANCE TO SHORT- AND LONG-TERM OUTCOME FOLLOWING COLORECTAL CANCER RESECTION

BY

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Abstract

Cardiovascular disease and colorectal cancer are major causes of death worldwide. The interaction between host and tumour in patients with operable colorectal cancer may be influenced by the presence of cardiovascular disease. Recently, the burden of abdominal aortic calcification has been identified as a potential driver of inferior outcome following abdominal surgery. The present thesis systematically examines the relationship between the degree of aortic calcification and outcome following elective colorectal cancer resection with particular focus on:

- Derivation and validation of an objective score reflecting the burden of calcification
- The relationship between the degree of aortic calcification, postoperative complications and survival
- Response to radiotherapy and tolerance and completion of adjuvant chemotherapy
- The degree of tumour hypoxia evident on immunohistochemical staining
- Potential mediating factors including the presence of systemic inflammation and comorbidity
- The correlation between aortic calcification and dynamic measures of cardiorespiratory fitness
- The use of a novel imaging technique for preoperative assessment of mesenteric flow

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Declaration

I declare that the work presented in this thesis was undertaken by myself. In addition, the following individuals contributed:

Mrs Roseanne Gorman assisted with the illustrations in Table 1.4 and Figure 1.1.

Miss Kate Boland, Mr Daniel Dolan and Mr Allan Golder undertook co-scoring in Chapter 3.

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Dedication

This thesis is dedicated to the memory of Jim, Stuart and Andrew Knight, and to my sister Roseanne, for helping me to see it through.

1 Introduction

1.1 Epidemiology

Colorectal cancer is the third most common cancer and the second most common cause of cancer death worldwide [1]. In 2012, over 1.4 million new cases of colorectal cancer and in excess of 690,000 deaths due to colorectal cancer were recorded globally [1]. The majority of cases occur in developed countries with the highest incidence currently in Australia and New Zealand, followed closely by Europe and North America [2]. The incidence of colorectal cancer in low and middle-income countries is projected to rise significantly by 2030 as Western dietary habits and lifestyle patterns become prevalent in these regions [3].

In the United Kingdom, colorectal cancer is the fourth most common cancer and accounted for 12% of all new cases of cancer in 2016 [4]. In total, 42,555 new cases were recorded in the UK in 2016; of these, 23,529 developed in males and 18,626 in females. There is a degree of variation in incidence across the devolved nations, with the highest crude rate per 100,000 persons in 2016 reported at 63.6 in Scotland, compared with 55.0 in England, 61.7 in Wales and 54.5 in Northern Ireland [4]. A deprivation gradient in the incidence of and mortality from colorectal cancer in Scotland has also been reported. The age-standardised incidence rate was 88.4% in the most deprived regions compared with 77.2% in the least deprived between 2010 and 2014, while age-standardised mortality rates of 39.1% and 31% respectively were reported [5].

Incidence rates have increased by approximately 4% since the 1990s, reflecting stable rates in females and increased rates in males [4]. This is however a smaller increase than that seen in the preceding decade, where rates were around 8%. Survival has gradually improved since the 1970s. Five-year age-standardised survival in men has risen from 25% during 1971-72 to 59% in 2010-11 with a similar trend evident for females, increasing from 24 to 58%. Survival for those diagnosed with colorectal cancer in the UK is currently around 54%, lower than the European average of 56% [4].

The largest proportion of cases affect the rectum, followed by sigmoid colon and caecum. Rectal cancer is more common in males and caecal cancer more

common in females. UK statistics for incidence by site between 2010 and 2012 reported 7,327 new cases of rectal cancer in males and 4,240 new cases in females. By contrast, 3,145 cases of caecal cancer in females and 2,829 cases in males were recorded in the same time period.

In non-hereditary cases, incidence increases with age, with a sharp increase from age 50 onwards. Incidence by age varies according to gender, with the most notable difference occurring between 60 to 65 years old, where the incidence rate in men is 1.7 times that of females[4]. The highest incidence rates are currently in those aged over 85. However, in the last 25 years, an increasing proportion of patients are being diagnosed with colorectal cancer under the age of 50, leading to proposals to lower the age at which screening is offered in certain high-risk groups, such as those with a family history of colorectal cancer.

1.2 Aetiology

1.2.1 Genetic factors

Colorectal cancer (CRC) most commonly arises sporadically but can evolve on a background of inherited genetic mutations which predispose to its development. Germline mutations in certain key genes inherited in an autosomal dominant or recessive pattern are pathognomonic of hereditary CRC. Somatic mutations in non-germ cells acquired throughout a person's lifetime can lead to the development of sporadic colorectal cancer.

In addition to being categorised as sporadic or hereditary, CRC can be broadly regarded as hypermutated or non-hypermutated [6]. Regardless of the overall burden, a minimum number of mutations is required to induce invasive cancer. Such mutations are classified as driver mutations [7] which confer a selective growth advantage to a tumour, increasing the net replication rate of cells. Passenger mutations are found in every cell affected by a driver mutation but do not confer any active advantage to the tumour and occur by chance during the process of cell division and clonal expansion [8,9].

Mutations in tumour suppressor genes and oncogenes are central to the development of cancer. Under normal conditions, control of cell proliferation is achieved by the regulatory function of tumour suppressor genes. Most mutations in tumour suppressor genes are initially recessive, requiring a further mutation affecting the remaining non-mutated allele before progression to CRC may occur. Consequent loss of heterozygosity results in the tumour suppressor gene becoming homozygous for the mutated gene. Oncogenes differ in this regard: a single copy of a mutation can lead to activation and loss of control of cell proliferation. In health, oncogenes provide stimuli which promote cellular replication and often encode cell cycle regulators, growth factors and receptors and signalling molecules. During carcinogenesis, overstimulation can result from changes in oncogenes arising from translocations, point mutations, amplifications or epigenetic alterations. Over-expression or mutations of oncogenes including RAS, MYC, BRAF, EGFR, AKT1 and PIK3CA are key players in colorectal carcinogenesis.

1.2.1.1 Oncogenes – KRAS and BRAF

The oncogenes BRAF and KRAS are key elements of colorectal carcinogenesis. The mitogen-associated protein kinase (MAPK) pathway involves a signalling cascade that influences cell proliferation, differentiation, migration and angiogenesis. The RAS oncogene encodes a small GTP-binding protein which activates several signalling pathways. Of the four known RAS proteins, KRAS is the most potent stimulator of this pathway and the most commonly mutated RAS isoform in cancer.

Following binding of an inducer (cytokine, growth factor or mitogen), RAS transitions to its active form and stimulates RAF proteins, among them BRAF, to move to the cell membrane. In colorectal cancer, KRAS mutations occur with a frequency of approximately 33%, compared with HRAS at 0% and NRAS at 2% [10]. Constitutional activation results from KRAS mutations, leading to promotion of proliferation via BRAF signalling, as well as inhibition of apoptosis mediated by phosphoinositol kinases such as PI3K. The resulting dysregulated growth and survival results in adenoma formation and eventually, progression to invasive cancer [11].

KRAS mutation status is important in determining appropriate chemotherapy in the setting of metastatic colorectal cancer [12]. Epidermal growth factor receptor (EGFR) blockade using monoclonal antibodies prevents ligand-induced activation, blocking signalling via MAPK and PI3K pathways [13]. However, mutant KRAS constitutionally activates these pathways. EGFR blockade therefore is ineffective, as it acts upstream of KRAS and cannot deactivate mutant KRAS-driven cellular proliferation.

BRAF mutations are found in approximately 8 to 12% of patients with metastatic disease [14-18]. The majority result in a valine amino acid substitution in exon 15 (V600E), enhancing BRAF activity in the region of 10 to 100-fold compared to its non-mutated state. BRAF-mutated colorectal cancer is associated with older age of onset [19], female gender [19], proximal colonic location [19-21] and large tumour size [21]. Pathological correlates of BRAF-mutated cancer include poor differentiation [19-21], mucinous histology [22] and a tendency for peritoneal recurrence [23-25].

In sporadic colorectal cancer, BRAF mutations are not regarded as key driver mutations. By contrast, colorectal cancer arising via the serrated pathway is often characterised by BRAF driver mutations, particularly in right-sided serrated sessile cancers and their precursors (right-sided hyperplastic polyps and serrated adenomas) [26]. Similarly, sporadic colorectal cancer with microsatellite instability commonly feature aberrations in BRAF as part of the genomic background [19,20,27]. This does not routinely feature in MSI tumours arising in patients with Lynch syndrome, previously known as hereditary non-polyposis colorectal cancer. Indeed, BRAF status forms a component of the criteria used to distinguish MSI-H tumours arising in Lynch syndrome from those arising sporadically [28].

1.2.1.2 Colorectal stem cells

The precursor lesion to colorectal cancer is the adenoma, a dysplastic lesion which over time accumulates repeated mutations and has the potential to result in the development of colorectal cancer. Adenomas arise from aberrant crypt foci found within the millions of crypts which form the epithelial lining of the colon and rectum.

Stem cells and proliferating progenitor cells are found at the crypt base. At the tip of the crypt, a proportion of differentiated cells (enterocytes, goblet cells and enteroendocrine cells) proceed to apoptosis. In cells with mutated genes, apoptosis does not occur and such cells continue to proliferate, forming aberrant crypt foci.

Cancer stem cells are defined by capacity to undergo extensive self-renewal. Cancer stem cells can also produce differing progeny and can be reprogrammed from one cell type to another through the process of de-differentiation [29,30].

1.2.1.3 Role of clonal expansion

Colorectal cancers are not composed purely of cancer cells each possessing equal proliferative and tumorigenic properties. Instead, sub-populations of

cancer stem cells exist with the ability to initiate and maintain a tumour as well as the capacity to metastasise [31].

Clonal expansion is the process by which cancers progress by selection of more fit tumour populations [32]. Mutations in the initiating cancer stem cell are present in all tumour cells through the process of clonal expansion. Subsequent replication errors generate new mutations that are limited to sub-populations of tumour cells (subclones). The result is a tumour populated by multiple sub-clones. It is this feature that renders each cancer unique and also determines treatment response: selection pressure resulting from genotoxic therapies enables more virulent sub-clones to survive and ultimately develop resistance.

1.2.1.4 The adenoma to carcinoma sequence

Almost 30 years ago, Fearon and Vogelstein described a model of colorectal carcinogenesis consisting of multiple sequential steps characterised by activation of oncogenes and inactivation of tumour suppressor genes on chromosomes 5, 12, 17 and 18 [33]. While several major advances in the intervening years have provided refinement, the adenoma to carcinoma model remains a defining paradigm of colorectal carcinogenesis.

The initiating event involves loss of heterozygosity of the adenomatous polyposis coli (APC) gene located on chromosome 5q21. Inactivation of APC results in activation of the Wnt pathway and has multiple effects on the normal crypt cell function, influencing mitosis, adhesion, migration and apoptosis [34,35]. APC regulates intracellular β-catenin levels, which accumulate in the presence of APC mutations, leading to nuclear translocation and stimulation of transcription of multiple genes involved in tumour growth and formation [36,37]. APC mutations are regarded as driver-gene mutations enabling hyperproliferation and early adenoma formation.

Following the initiation phase, an expansion phase characterised by allelic loss of chromosome 12p, the locus of the ras oncogene, occurs. The subsequent oncogenic activation of KRAS predisposes to the development of an intermediate adenoma. Originally, a further driver-gene mutation occurring through the loss of chromosome 18q was thought to inactivate the tumour suppressor gene

Deleted in Colorectal Cancer (DCC). However, subsequent studies have highlighted inactivation of other tumour suppressor genes located on chromosome 18q such as SMAD4 and SMAD2 [38,39] which exert regulatory functions in cell growth, differentiation and apoptosis. The resulting late adenoma remains a benign tumour. In the final invasive phase, a driver mutation inactivates tumour suppressor genes including TP53, enabling transition from adenoma to high-grade dysplasia and later invasion and metastasis [40].

The temporal order of the events described in the adenoma to carcinoma sequence is important. For example, a KRAS mutation may result in formation of an adenomatous polyp but alone does not induce progression to cancer. In combination with a preceding APC mutation, tumour progression can occur. However, mutations affecting the APC gene are not the sole route of initiation of colorectal carcinogenesis. Mutations in other driver genes in the APC pathway including catenin beta-1 (CTNNB1) [41] and SRY-box transcription factor 9 (SOX9) [42] are recognised as initiating events in the adenoma to carcinoma sequence. Moreover, in a subset of colorectal cancer, the adenoma to carcinoma sequence does not apply, reinforcing that this model likely over-simplifies some of the genetic events leading to tumour development.

1.2.2 Colorectal cancer pathways

Loss of stability enables development of colorectal cancer by facilitating mutations. While colorectal cancer is a genetically heterogenous tumour type, certain pathways of genomic instability commonly characterise its development. These include the chromosomal instability, microsatellite instability and serrated pathways. Overlap between pathways has been documented and new pathways continue to emerge.

1.2.2.1 Chromosomal instability

Chromosomal instability (CIN) is the most common form of genomic instability in colorectal cancer and is responsible for approximately 65-70% of cases of

sporadic cases [43]. Point mutations and loss of heterozygosity are hallmarks of CIN and give rise to aneuploidy, where whole chromosome numbers within a cell differ from the usual euploid state of 46. Copy number alterations are changes to chromosome structure that lead to gain or loss of copies of DNA segments including loss of wild type copy of APC, p53 or SMAD4 which under normal circumstances perform tumour suppressor functions. Changes in chromosome structure, for example by translocation, can lead to rearrangement of genes and activation of oncogenes.

It is not yet clear whether CIN arises as a consequence of the associated mutational changes or provides the landscape that enables initiating events such as APC activation to take place. CIN is involved in the transformation of normal stem cells to cancer stem cells both in vitro and in vivo [44,4546,47] in other tumour types, suggesting it is an early event.

1.2.2.2 Microsatellite instability

DNA replications errors occurring in regions with frequent short tandem base repeats known as microsatellites, of which there are over 100,000 throughout the genome, give rise to microsatellite instability (MSI). Mutations within these regions often do not affect protein-coding domains, but more commonly result in activation or inactivation of proteins essential to the normal control of cell cycle processes. Mismatch repair (MMR) proteins exist to recognise and excise such errors. Approximately 15% of sporadic colorectal cancers and almost all cases of Lynch syndrome, a disorder characterised by germline mutations in MMR genes, are characterised by MSI.

MSI is observed more commonly in young patients, females and the proximal colon and the associated cancer is often less invasive and less likely to be characterised by KRAS mutations [46,47]. In hereditary colorectal cancer, the identification of widespread deletion mutations in microsatellite sequences in the tumours of patients with Lynch syndrome led to the identification of MMR genes [48–52]. Under normal circumstances, DNA mismatches occurring during replication are recognised and bound by MMR proteins. In patients with defective MMR (dMMR) genes, recognition of replication errors and subsequent repair of these mismatches does not occur. A high rate of mutations affecting MMR genes

across the genome is referred to as MSI-high (MSI-H). MSI-low tumours are characterised by methylation-associated abnormalities and are associated with poorer prognosis in stage III colorectal cancer [53]. Microsatellite-stable cancers do not display the characteristics of MSI-H or MSI-L and are often marked by other forms of genetic instability e.g. CIN.

Germline mutations in the MMR pathway are found in Lynch syndrome which is characterised by multiple tumours, most commonly by endometrial, colon and gastric cancers and accounts for approximately 3% of all cases of colorectal cancer [54,55]. Most colorectal cancers with MSI are sporadic and occur in older patients than those affected by Lynch syndrome. Acquired MMR deficiency can arise from epigenetic changes such as methylation of the MLH1 promoter region which results in transcriptional silencing of MMR genes [56,57]. The latter is common in tumours with the CG island methylator phenotype (CIMP).

1.2.2.3 The serrated pathway

Historically, serrated polyps were regarded as benign hyperplastic lesions lacking the potential to progress to CRC. Named due to the histological saw-toothed pattern of their crypt epithelium, serrated polyps were later recognised as a separate entity with significant malignant potential [58]. Serrated colorectal polyps include hyperplastic polyps, traditional serrated adenomas, mixed serrated polyps and sessile serrated adenomas. The latter are associated with abnormal proliferation and the development of serrated adenocarcinoma [59].

Clinically, serrated adenocarcinoma (SAC) is associated with cancers which develop in the interval between colonoscopic examinations (interval cancers). SAC is also associated with synchronous advanced neoplasia [60]. The molecular characteristics of the serrated pathway differ in several ways from the traditional adenoma-carcinoma sequence. BRAF mutations are found in most serrated polyps and are thought to be an early event in serrated aberrant crypt foci [61]. KRAS is less commonly mutated than in conventional adenoma-carcinoma pathway [61]. Aberrant methylation is common and colorectal cancer arising in the region of serrated polyps is often associated with MSI and CIMP status [62].

1.2.3 The phenomenon of epigenetics

Epigenetic instability represents a further pathway which plays a significant role in colorectal carcinogenesis. Epigenetics encompasses heritable changes occurring in the absence of direct alterations to DNA sequences that lead to alterations in gene expression by modifying interactions between the regulatory portions of DNA or messenger RNAs (mRNAs). DNA methylation is an example of an epigenetic alteration in which a methyl group is added to a cytosine base.

CpG islands are clusters of dinucleotides formed by a cytosine nucleotide preceding a guanine nucleotide. They are widespread in gene promoter regions and hypermethylation of these islands can lead to silencing of genes encoding tumour suppressor genes [8,63]. This is recognised as the CpG island methylator phenotype (CIMP) [64]. It is commonly associated with sporadic MSI tumours but can be found in MSS stable tumours [65-67] and is often found in serrated adenomas. In the latter, aberrant methylation mediates transcriptional silencing of several genes within the WNT/β-catenin pathway [68,69].

Interaction between the genetic and epigenetic pathways occurs, for example, when epigenetic alterations result from a mutation or sequence variation in a gene or regulatory element distant from the gene being regulated. These pathways should therefore not be regarded as independent but interconnected in the molecular biology of colorectal cancer [70]. The role of nutrient-derived dietary factors in modulating and preventing epigenetic events such as DNA methylation exemplifies the interaction between environment, genetic and epigenetic pathways [71]. Such interactions may be manipulated through dietary supplementation and modification to potentially reduce the incidence of colorectal cancer.

1.2.4 Inherited syndromes

Early descriptions of colorectal carcinogenesis were largely contingent on observations drawn from forms of hereditary colorectal cancer, most notably Familial Adenomatous Polyposis (FAP) and Lynch syndrome (formerly known as

hereditary non-polyposis colorectal cancer). Germline mutations in APC characterise FAP and are considered the hereditary correlate of sporadic colorectal cancers. Multiple adenomas distributed throughout the colorectum occur in FAP, but only a proportion progress to invasive cancer. The requirement for an enabling characteristic in the form of genetic instability is thus highlighted. However, as demonstrated by the diploid state of MSI cancers compared with the widespread aneuploidy evident in tumours displaying CIN, genetic instability can take differing forms.

The difference between genotype and phenotype is demonstrated in the differing manifestations of FAP in patients with the same germline mutation deletion of chromosome 5q [72]. FAP can be associated with extra-colonic lesions including brain tumours, desmoid lesions and osteomas. However, a relatively small number of patients with FAP develop such manifestations despite having identical germline mutations [73,74]. Attenuated FAP results from truncating mutations of APC and patients affected develop a lower burden of polyps compared with other forms of APC mutations [75]. Pathogenic variants of FAP include MUTYH-associated polyposis which is characterised by defects in base excision repair [76]. Cowden disease, an autosomal dominant disorder characterised by variants in the PTEN tumour suppressor gene, gives rise to hamartomatous polyposis. Among thyroid, breast and renal cell cancer, patients with Cowden disease are also at higher risk of colon cancer [77,78].

Lynch syndrome arises due to germline mutations in MMR genes coupled with somatic inactivation of the wild-type allele [79]. Familial clustering of colorectal and endometrial cancers was initially documented as early as 1913. However, it was not until its genetic aetiology was established in the 1990s that the genotype associated with Lynch syndrome was uncovered. Prior to this, Lynch syndrome was referred to as hereditary non-polyposis colorectal cancer (HNPCC) to differentiate it from FAP. Clinical features associated with Lynch syndrome colonic tumours include proximal tumour location, young age of onset and increased rates of synchronous and metachronous lesions.

Identification of individuals at risk of Lynch syndrome is critical as prevention of colorectal cancer can be achieved through screening colonoscopy which begins

at the age of 18. Järvinen et al reported a 62% reduced risk of colorectal cancer in at-risk individuals who underwent surveillance colonoscopy every 3 years when compared with an at-risk group who declined surveillance [80]. Several other heritable syndromes exist, including Peutz-Jeghers syndrome, juvenile polyposis syndrome and oligopolyposis. Between 2 to 5% of all colorectal cancer cases arise on a background of inherited syndromes [81].

1.3 Risk factors for colorectal cancer

1.3.1 Risk factors

Genetic mutations and epigenetic modifications may form the foundation for development of colorectal cancer but do not act alone it its pathogenesis. Risk of colorectal cancer is increased by non-modifiable factors including advancing age, male gender and a family history of first-degree relatives with colorectal cancer. Modifiable risk factors include smoking, alcohol consumption, obesity, physical inactivity and dietary factors such as high intake of red and processed meat.

1.3.1.1 Gender and colorectal cancer

Trends in colorectal cancer incidence differ by gender. Male gender is consistently associated with higher incidence, with some studies suggesting an association with poorer outcome when compared with female gender [82,83]. Multiple interacting factors are likely at play: lower rates of screening uptake in males may predispose to cancers which are more advanced by the time of diagnosis; higher rates of right-sided colon cancers are seen in women with often more favourable pathological characteristics; oestrogenic hormones are associated with a reduced risk of colorectal cancer [84]. Both overall and cancer-specific survival following colorectal cancer are significantly better in females than males [85]. Female gender has also been associated with positive response to adjuvant chemotherapy [86].

1.3.1.2 Age and colorectal cancer

Age is a further important non-modifiable risk factor for colorectal cancer development and disease outcome. The effect of accumulated somatic mutations, epigenetic silencing and environmental risk factors over time likely contributes to higher rates of colorectal cancer with increasing age [87,88]. Between 2012 and 2014, over 40% of colorectal cancer deaths occurred in

patients aged 80 or older [4]. Advancing age is associated with higher rates of morbidity and mortality following surgical resection, even when adjusted for other patient factors and comorbidities [89]. The incidence of cardiorespiratory complications and prolonged length of stay is higher among patients aged 80 and over undergoing colorectal surgery [90]. In patients over 70 years, the survival benefit from adjuvant chemotherapy is modest when compared with patients aged under 70. Analysis of six trials of contemporary adjuvant regimens suggested no benefit in terms of time to recurrence, disease-free survival or overall survival [91]. Defining the fit older patient from those with comorbidity, functional dependency and/or disability is required to improve patient selection for surgical and cytotoxic therapy.

1.3.1.3 Demographics

The demographics, both geographic and socio-economic, of colorectal cancer are of interest due to their influence on outcome. Swift increases in the incidence of colorectal cancer have been observed in regions including east Asia and eastern Europe, as well as countries such as Spain which were previously regarded as low-risk [92,93]. This is largely attributed to the adoption of a Westernised lifestyle, with its attendant association with physical inactivity and dietary patterns. The counter argument for the influence of Western lifestyle on colorectal cancer incidence arises when considering the stable or in some cases declining rates seen in North America and UK, among other developed countries[92]. This is likely to be influenced by national screening programmes and the increased use of colonoscopy with polypectomy in these regions.

The socioeconomic circumstances of patients who develop colorectal cancer offer an insight into the complex interaction between genetics and the environment. In a prospective cohort of over 500,000 Americans, those with lower levels of educational attainment experienced a disproportionately high risk of developing colorectal cancer [94]. In Scotland, similar health inequalities are manifest: deprivation is independently associated with higher rates of postoperative mortality and poorer 5 year survival [95,96]. Again, environmental factors including diet, physical inactivity and harmful behaviours

such as smoking are likely to be responsible in part for the increased risk and lower survival rates, but host factors such as systemic inflammation are of particular importance. Systemic inflammation is more frequent in those from deprived backgrounds [97], while both smoking and deprivation are related to comorbidity, which in turn is an independent predictor of colorectal cancer survival [98].

It is clear that interventions both to reduce incidence rates of colorectal cancer and optimise outcomes are required. Their design requires comprehensive acknowledgment of both environmental and socioeconomic influences.

1.3.1.4 Smoking

Current smokers experience a higher risk of developing colorectal cancer but are also at risk of poorer outcome should they develop it. Those who smoke 40 cigarettes per day have a 40% increased risk of colorectal cancer compared with non-smokers [99]. In the Cancer Prevention Study II, patients with colorectal cancer who smoked prior to diagnosis were at higher risk of all-cause and cancer-specific mortality [100]. The impact of smoking is also manifest in short-term outcomes: 30 day mortality was significantly higher among current smokers in Walter and colleagues' meta-analysis of 16 studies addressing survival and smoking in colorectal cancer patients [101]. Smoking cessation therefore remains a priority in the public health campaign to reduce colorectal cancer incidence rates.

1.3.1.5 Alcohol

In 2007, the International Agency for Research on Cancer confirmed alcohol as a carcinogenic substance which increased the risk of developing colorectal cancer [102]. Regular daily consumption of approximately 50 grams of alcohol increased the relative risk to 1.4 for colorectal cancer when compared with those who did not consume alcohol [103,104]. While consumption is highest in

Europe, it is rising in other parts of the world including Asia. This may be a contributing factor to the changing patterns of disease incidence which are emerging. It may also be implicated in the higher rates of colorectal cancer seen in men, who are more likely to consume excessive amounts of alcohol than women, although this pattern is also changing with increasing rates in women [105]. Subsequent meta-analyses have confirmed a dose-response relationship, with even light drinkers (<10 grams ethanol/day) having a 7% higher risk of colorectal cancer than non-drinkers, and subsequent higher mortality [106].

1.3.1.6 Diet

Cancers of the gastrointestinal tract are influenced by dietary intake. This reflects the direct contact which ingested agents have with the GI tract, where carcinogens that are not metabolised in the small intestine can interact directly with the colorectal mucosa. According to the World Cancer Research Fund (WCRF), strong evidence supports the link between red and processed meat and increased colorectal cancer risk, although other dietary components may act to reduce the risk[107].

The role of red and processed meats in colorectal carcinogenesis has been investigated extensively. Both types of meat produce carcinogens such as polycyclic aromatic hydrocarbons (PAHs) [108109], heterocyclic amines (HCAs) [110] and N-nitroso compounds (NOCs) [111] when cooked for prolonged durations at high temperatures. The PAH benzo-a-pyrene is generated when grilling meat over an open flame, or during processing that uses smoking methods. Reactive metabolites form during PAH metabolism that can damage DNA. HCAs are mutagenic compounds generated during high-temperature cooking that, when metabolised by the liver, become genotoxic. NOCs are alkylating agents that can institute DNA base changes in target tissues, initiating carcinogenesis [112]. Smoking of meats at high temperatures oxidises nitrogen to nitrogen oxide that then alkylates amines present in the meat resulting in NOC generation. Limiting intake of red meat to 500 grams per week and avoiding processed meat is advised by the WCRF [107].

1.3.1.7 Obesity

Obesity, defined as an excess of body fat, has consistently been associated with higher risk of colorectal cancer. In a recent UK Biobank study of 472,526 subjects, higher body mass index (BMI), waist circumference, waist-to-hip ratio and total body fat percentage were associated with an increased risk of colorectal cancer in men [113]. In women, only waist-to-hip ratio was associated with higher risk suggesting that males are at a greater risk due to overall adiposity, while in females, abdominal adiposity appears to carry significant risk.

Anthropometric measures are readily available but cannot distinguish between lean and fat mass. Bioelectric impedance and dual-energy x-ray absorptiometry are capable of assessing both lean and fat mass, but the most accurate measures are made on cross-sectional imaging such as computed tomography (CT) and magnetic resonance imaging (MRI).

Obesity results in higher circulating insulin levels, which are implicated at cellular level in promotion of cell growth and inhibition of apoptotic processes. When coupled with the pro-inflammatory state induced by excess adiposity, these factors can promote colorectal carcinogenesis. A dose-response relationship is evident, with an increased relative risk per 5kg/m^2 increase in BMI. The relative risk of colorectal cancer increases from 0.98 in those with a BMI of 18, to 1.34 in those with a BMI of over 30 [114].

The effect of weight loss on modulation of colorectal cancer risk is unclear. Reduced risk with intentional weight loss has been reported[115,116] but other large studies do not support this association [117,118]. Data from small observational studies of obese patients following diet-induced intentional weight loss reported reductions in circulating glucose, cholesterol and triglycerides and changes in the inflammatory profile of the colorectal mucosa. Lower levels of TNF-α, IL-1ß and IL-8 were found in rectosigmoid biopsies, combined with down-regulation of proinflammatory signalling pathways and transcription factors [119]. While these results are preliminary, the potential for weight loss obtained through dietary and physical activity interventions to alter the risk of colorectal carcinogenesis is nonetheless promising.

1.3.1.8 Physical inactivity

Physical inactivity is endemic, with 3.2 million attributable deaths each year worldwide [120]. In Scotland, only 65% of adults attain the recommended amount of physical activity each week [121]. It is estimated that 10% of cases of colon cancer are related to physical inactivity [122]. Multiple intrinsic factors such as age, gender and weight interact with extrinsic factors including the built environment and public transport availability to promote inactivity. A meta-analysis of 126 studies reported a potential 7% reduction in cancer risk should the current WHO weekly physical activity guidelines be followed (Table 1.1). The majority of this risk reduction was accounted for by the association between higher leisure-time physical activity and a reduced risk of breast and colon cancer [123].

Patterns of physical activity differ across the spectrum of economic development. In developed countries, approximately 40% of 12.8 million deaths due to cardiovascular disease, diabetes and selected cancers are related to physical inactivity [124]. Of significant concern is the uniformly low activity levels in school-going adolescents, with 80% insufficiently active [125]. Rates of colorectal cancer may rise as this population carry forward their inactive lifestyles into adulthood.

Table 1-1 - WHO physical activity guidelines for adults adapted from Global Recommendations on Physical Activity for Health

<table>
<tr><th>Exercise type</th><th>Duration</th><th>Intensity</th><th>Frequency</th></tr>
<tr><td rowspan="3">Aerobic physical activity</td><td>At least 150 minutes</td><td>Moderate</td><td rowspan="3">Weekly at intervals of at least 10 minutes</td></tr>
<tr><td colspan="2">OR</td></tr>
<tr><td>At least 75 minutes</td><td>Vigorous</td></tr>
</table>

Muscle-strengthening	Not specified	Not specified	2 or more days weekly

1.3.1.9 Inflammatory bowel disease

Severe inflammation, for example in the context of Crohn's disease or ulcerative colitis, disrupts mucosal homeostasis and enables mutations that can lead to increasing degrees of dysplasia and eventually, invasive cancer. Sporadic colorectal cancer develops in otherwise macroscopically normal mucosa. While both are characterised by similar molecular features, differing sequences of molecular events occur: early inactivation of the tumour suppressor TP53 is seen in colitis-associated cancer, an event that occurs late in the development of sporadic colorectal cancer [126]. Conversely, APC mutations are generally initiating events in sporadic colorectal cancer but occur later in colitis-associated cancer.

The incidence of colorectal cancer in patients with inflammatory bowel disease (IBD) is influenced by the time elapsed since diagnosis, the use of maintenance anti-inflammatory therapy such as aminosalicylates and colectomy rate. Emerging evidence points towards a potential reduction in IBD-related cancer risk in patients who received and respond to modern immunologic therapy [127]. IBD-related cancer is thought to account for 2% of colorectal cancer annually [128]. For ulcerative colitis, the probability of developing colorectal cancer is estimated to be 2%, 8% and 18% at 10, 20 and 30 years post-diagnosis respectively [129]. For Crohn's disease affecting the colon, the relative risk of developing colorectal cancer is estimated to be 2.59 [130].

1.3.1.10 Cardiovascular disease and colorectal cancer

As the most common cause of death globally, cardiovascular disease (CVD) is frequently encountered in patients with colorectal cancer. Several studies have demonstrated an association between the presence of coronary arterial disease and colorectal cancer or its precursor lesion, the adenoma. This has been drawn from study populations undergoing colonoscopy as part of screening

programmes. Questionnaires or medical history are most often used to determine the presence of CVD as invasive diagnostic measures are not justified in asymptomatic populations [131]. The true incidence of colorectal neoplasms (advanced adenomas and colorectal cancer) in patients with angiogram-confirmed CVD is unclear. In one cross-sectional study, advanced colonic lesions were associated with angiographic evidence of CVD (OR 2.51, 95% CI 1.43 – 4.35, p<0.001); the presence of metabolic syndrome and smoking were independently related to the co-existence of CVD and colorectal neoplasms [132]. Metabolic syndrome represents a cluster of traditional risk factors implicated in cardiovascular disease and type 2 diabetes mellitus (DM). Its association with obesity, physical inactivity and western dietary patterns mean it is also an important risk factor for colorectal cancer.

1.3.1.11 Metabolic syndrome

Metabolic syndrome is underpinned by insulin resistance and is defined by abnormally high waist circumference, elevated triglycerides, blood pressure and fasting glucose and reduced HDL cholesterol [133]. The hyperinsulinaemia, hyperglycaemia and gluconeogenesis that characterise metabolic syndrome generate excess reactive oxygen species which are genotoxic, driving mutations and altering the cell cycle through prevention of apoptosis and promotion of proliferation [134].

Metabolic syndrome is associated with increased colorectal cancer incidence and poorer prognosis. In an international study of over 500,000 people, the incidence of colorectal cancer incrementally increased with the number of components of the metabolic syndrome present [135]. Mortality from colorectal cancer follows a similar trend: an increased risk of colorectal cancer-related death (HR 2.15, 1.27 – 3.62) was reported in a cohort of 9268 patients with metabolic syndrome followed for 14 years [136]. Taken together, it is plausible that the metabolic derangement acts to promote carcinogenesis [137] and may act as a nexus for the interaction between CVD and colorectal cancer. The potential to modify lifestyle risk factors and to reverse components of the metabolic syndrome is therefore a

key aspect of strategies to reduce the incidence of cardiovascular disease and colorectal cancer.

1.3.1.12 Systemic inflammation

Systemic inflammation is thought to occur when active termination of an acute inflammatory response fails, resulting in a state of sustained chronic inflammation. It is of a lower order of magnitude than an acute inflammatory response [138] and involves stimulation of both the haematopoietic and hepatic systems, resulting in detectable alterations in circulating levels of white cells including neutrophils, lymphocytes, monocytes, platelets and acute-phase proteins, such as C-reactive protein (CRP) and albumin. While many association studies have demonstrated higher circulating levels of inflammatory markers such as CRP and increased risk of incident cancer, it remains unclear whether causative or a result of early neoplastic change. It is also possible that raised CRP levels in study participants reflect inflammation as a result of factors with a pro-inflammatory aetiology such as cardiovascular disease or obesity.

In a longitudinal population study of 10,408 Danish individuals without a history of cancer at inclusion, 1,624 patients developed cancer during the 16 year follow up period. Following adjustment for age, sex, smoking, alcohol intake and BMI, participants with a CRP greater than 3 mg/L had a hazard ratios of 1.3 (95% CI, 1.0 to 1.6) for any cancer type compared with those with a CRP less than 1mg/L [139]. This was not significant when assessed in patients who developed colorectal cancer (HR 1.9 (95% CI 0.8 to 4.6)). Similar findings have been reported in other studies; however, meta-analysis of ten studies suggested a minor increase in risk of developing colorectal cancer in patients with an elevated CRP [140]. Pre-existing systemic inflammation represented by a raised CRP has been strongly linked with later development of colon cancer but not rectal cancer [141]. The latter findings fit with the established association between inflammatory bowel disease and subsequent development of colorectal cancer. It is unclear whether elevated CRP is a cause or effect of increasing dysplastic change in the colonic mucosa. However, higher circulating levels of inflammatory markers have been associated with all-cause, colorectal cancer and cardiovascular mortality [142],

suggesting that regardless of mechanism, systemic inflammation is a risk factor with prognostic utility.

1.3.1.13 Summary

It is clear that there is a multitude of risk factors for colorectal cancer potentially amenable to modification or optimisation. Striking overlap is evident between several of these risk factors, with lifestyle factors such as smoking and repeated exposure to dietary toxins interacting to potentially influence the development of systemic and mucosal inflammation. Cardiometabolic dysregulation is a common feature of the phenotype associated with physical inactivity, obesity and impaired glucose tolerance, features that are shared with colorectal carcinogenesis. The related increase in proliferation and loss of regulatory control over cell cycle processes induced by many of these risk factors likely contributes to higher incidence of colorectal cancer in such individuals. With over 50% of these risk factors related to lifestyle, concentrated efforts in these areas to reduce risk, coupled with progressive translational research, could translate to improvements in the prevention and management of colorectal cancer.

1.4 Investigation and management of colorectal cancer

In asymptomatic patients, colorectal cancer can be detected via screening. Symptomatic presentation most commonly occurs by presentation to primary care but also includes emergency presentation to secondary care. There are several symptoms and signs associated with colorectal cancer, some of which vary with the anatomical location of the tumour. Right-sided tumours often manifest clinically with iron-deficiency anaemia, while left-sided and rectal cancers may present with passage of blood per rectum. Left-sided tumours are more likely to present with obstructive features due to the smaller calibre of the left colon compared with the right colon. Altered bowel habit (diarrhoea or constipation) and abdominal pain can occur regardless of anatomical location. Signs include weight loss and the presence of a palpable abdominal or rectal mass.

Clinical features are of limited predictive value in identifying patients who have colorectal cancer. In a meta-analysis of the diagnostic accuracy of colorectal cancer symptoms which included 19,443 patients, both the positive and negative likelihood ratios of symptoms centred around 1, suggesting symptom presence or absence does not hold reliable value in detecting colorectal cancer [143]. This was echoed by Jellema et al, who reported wide variability in the sensitivity and specificity in risk of detecting a colorectal cancer based on common symptoms alone but found combinations of symptoms, most notably rectal bleeding and altered bowel habit, improved sensitivity and specificity significantly [144].

1.4.1 Screening

The aim of bowel screening is to detect colorectal cancer at an early stage and thereby increase the chance of achieving cure. Polypectomy during colonoscopy can also reduce the risk of future colorectal cancer.

The first phase of the Scottish Bowel Screening Programme began in 2007 and was rolled out to all Scottish NHS health boards in 2010. Faecal occult blood

testing was originally used as the primary mode of screening but was replaced in 2017 with faecal immunochemical testing (FIT). This is offered to individuals aged between 50 and 74. FIT uses an immunoassay of antibodies to the globin moiety of haemoglobin to detect microscopic traces of blood in a faecal sample. A threshold of 80 μgHb/g is used to determine an abnormal result. Individuals with an abnormal result are invited for colonoscopy following an assessment of their suitability for invasive investigation. Individuals with a normal result are screened every 2 years.

Uptake of bowel screening since its inception has typically been in the region of 50%, with lower rates in males and areas of socioeconomic deprivation. Between 2007 and 2014, over 5 million individuals were invited. Of these, 56% completed the initial screening test and 2% returned a positive result. In those undergoing complete colonoscopy, detection rates are 7% for cancer and 37% for adenomas [145].

1.4.2 Investigation for suspected colorectal cancer

Investigation consists of initial investigation to reach a diagnosis followed by staging investigations. The latter are used to determine treatment.

In the UK, patients referred urgently for investigation of symptoms suggestive of colorectal cancer are governed by the two-week wait policy (2WW) [146]. In Scotland, the policy differs: all patients referred urgently on suspicion of cancer should be diagnosed and proceed to treatment within 62 days. Patients deemed fit for colonoscopy without prior clinic review proceed directly to test. The pathway for investigation of suspected colorectal cancer is outlined in Table 1.2, adapted from the Association of Coloproctology of Great Britain & Ireland (ACPGBI) guidelines [147]. A positive result generates a referral to the colorectal MDT.

Table 1-2 - ACPGBI pathway for investigation of suspected colorectal cancer.

Symptom	Age category	2-week wait indication	Recommended investigation
Change in bowel habit	Over 55 years	Loose and/or more frequent stools for more than 6 weeks without rectal bleeding	Colonoscopy
Rectal bleeding	Over 40 years	Rectal bleeding with change in bowel habit as above	Colonoscopy
	Over 55 years	Rectal bleeding without change in bowel habit	Flexible sigmoidoscopy
Mass	All ages	Rectal mass	Flexible sigmoidoscopy
	All ages	Abdominal mass	Colonoscopy or CT colonography
Anaemia	All ages	Males – unexplained Hb less than 110 g/l	OGD + colonoscopy or CT colonography
		Non-menstruating Females - unexplained Hb less than 100 g/l	OGD + colonoscopy or CT colonography
Any of the above	Over 75 years	Change in bowel habit, rectal bleeding, mass or anaemia	2WW outpatient clinic then proceed to test if fit

Abbreviations: OGD oesophagogastroduodenoscopy, 2WW 2 week wait, Hb haemoglobin.

1.4.2.1 Flexible sigmoidoscopy and colonoscopy

Colonoscopy involves examination of the rectum and colon in its entirety and remains the reference standard investigation for suspected colorectal cancer [148]. It enables biopsy and thereby histological confirmation of colorectal cancer. Other relevant pathology including pre-cancerous polyps and inflammatory bowel disease may be visualised and therapeutic procedures including polypectomy carried out. Associated risks include perforation and bleeding from biopsy or polypectomy; a population-based study of over 97,000 patients undergoing outpatient colonoscopy reported these as 0.85 and 1.64 per 1,000 colonoscopies respectively [149]. The procedure requires bowel cleansing with oral osmotic laxatives to enable adequate visualisation.

Flexible sigmoidoscopy aims to assess the lower 65cm of the colon and rectum [150]. It is recommended by both NICE and ACPGBI for the investigation of suspected colorectal cancer presenting with rectal bleeding or a palpable rectal mass. Advantages include the use of a self-administered enema in place of oral bowel preparation and reduced procedure length. However, two large series reported rates of synchronous colorectal cancer in 3.8% and 3.9% respectively [151,152]. Both found an increased risk of synchronous tumours in men, with a third occurring in distinct anatomical locations [152]. Flexible sigmoidoscopy alone, particularly in males, may be associated with failure to identify synchronous cancers.

1.4.2.2 CT colonography

This radiological investigation uses 2D and 3D reconstruction of cross-sectional images of the abdomen and pelvis acquired following bowel preparation and faecal tagging. Visualisation is achieved by insufflation of carbon dioxide or air via a rectal catheter. CT colonography is advantageous in patients with distal stenosing or obstructing lesions which prevent complete colonoscopic examination. Pooled analyses have suggested the sensitivity of CT colonography to be comparable to that of colonoscopy for the detection of cancer and advanced neoplasia (polyps >10mm) [153]. The main disadvantage is the inability to obtain histology for tissue diagnosis.

1.4.2.3 Clinical staging of colorectal cancer

The purpose of staging is to enable risk stratification and define appropriate treatment. Clinical staging commonly involves imaging investigations which characterise the primary tumour and assess the presence and extent of metastatic disease. All patients with colorectal cancer should undergo CT of thorax, abdomen and pelvis to assess for distant metastatic disease (most commonly lung, liver and peritoneum) and provide local staging information. The degree of spread beyond the bowel wall and involvement of adjacent organs and structures can be determined by CT but accuracy is limited by restricted definition of bowel wall morphology [154]. It is therefore recommended that in patients with rectal cancer, locoregional staging by MRI is undertaken in addition to CT. The enhanced visualisation of the mesorectum and circumferential resection margin enables discrimination of patients with resectable disease from those with margin-threatening disease who may benefit from neoadjuvant therapy.

Endorectal ultrasound is an adjunct to MRI in patients with early stage rectal cancer that may be amenable to organ-preserving surgical approaches. Staging accuracy is reported to be at least equivalent to MRI [155] but inter-observer variability and incomplete visualisation of pelvic nodes have led to its use being restricted to early rectal cancers with prior assessment by MRI or in those with contraindications to MRI [147,148].

Additional imaging modalities may be used to further characterise metastatic lesions. MRI of the liver can be useful in defining hepatic lesions which are equivocal on CT. In recent years, use of positron emission tomography (PET) CT has increased as a higher number of patients undergo non-anatomical resection of locally advanced colorectal cancer. Occult metastases may be identified preoperatively using PET CT, facilitating appropriate patient selection [156].

1.4.3 Management

The goal of treatment for cancer is to achieve cure. This is most often attained through surgical resection. In cases of locally advanced rectal tumours, neoadjuvant therapies such as chemo- and/or radiotherapy are administered prior to surgery to increase the likelihood of achieving complete resection.

1.4.3.1 Multidisciplinary team

Accurate identification of patients with primary operable disease and those who may benefit from neoadjuvant therapy are central tenets of the colorectal cancer multidisciplinary team. Teams consist of colorectal surgeons, GI radiologists, pathologists and oncologists as well as palliative care physicians, specialist nurses and administrators. This facilitates integrated review of each patient's clinical, radiological and pathological data and enables a comprehensive discussion of the treatment plan. Multidisciplinary management is governed within the framework of a managed clinical network responsible for auditing performance and ensuring quality.

1.4.3.2 Neoadjuvant therapy

Until recently, neoadjuvant chemotherapy for locally-advanced colon cancer outside the trial setting was not standard practice in the UK. Initial results from the Fluoropyrimidine, Oxaliplatin and Targeted-Receptor preOperative Therapy (FOxTROT) trial point towards a significantly reduced rate of incomplete tumour (R1 or R2) resection and increased rates of pathological down-staging [157]. Full trial results have yet to be published but use of neoadjuvant chemotherapy for high risk colon cancer is gradually increasing.

In rectal cancer with involvement of the circumferential resection margin by tumour, lymph nodes, lymphovascular or perineural spread, neoadjuvant radiotherapy is recommended. High resolution MRI is used to identify such features. Reduced rates of local recurrence have been consistently reported in

randomised trials comparing preoperative radiotherapy to surgical resection by total mesorectal excision (TME) alone in patients with locally advanced rectal cancer [158-161]. However, no benefit in overall survival has been demonstrated.

Radiotherapy is often combined with a radio-sensitising chemotherapeutic agent, most commonly a fluoropyrimidine. This pushes cells into the S-phase, making them more vulnerable to DNA double-stranded breaks induced by radiation. A Cochrane review in 2012 reported a reduction in local recurrence rates without an impact on overall survival in patients who received neoadjuvant chemoradiotherapy prior to TME compared with neoadjuvant radiotherapy [162]. However, one of the trials of 3,480 patients included in the pooled analysis of overall survival was performed before the era of TME. The analysis of local recurrence rates is also subject to the bias introduced by the differing radiotherapy schedules and chemotherapy regimens as well as length of time between completion of neoadjuvant treatment and surgery.

1.4.3.3 Short versus long course radiotherapy

The optimal format of neoadjuvant radiotherapy for rectal cancer is an ongoing source of debate. Short course radiotherapy (SCRT) is delivered in five fractions of 5 Gray while long-course radiotherapy is given in 25 fractions totalling 45 to 50 Gray. Proponents suggest that this is convenient and less expensive while opponents point to the lack of time for tumour response to develop as the major deficiency of short course when compared to long course radiotherapy. It has been suggested that short course radiotherapy may be appropriate for patients with cancer of the mid to upper rectum without circumferential resection margin involvement while long course chemoradiation is preferable in patients with mid to low rectal cancer where the circumferential resection margin is involved by tumour and/or nodal disease [163].

1.4.3.4 Ongoing evolution of neoadjuvant therapy

Neoadjuvant chemoradiation in the UK consists of radiation in combination with a chemotherapy drug that primarily acts to enhance radiosensitivity. With strategies to reduce local recurrence rates established, ways to optimise neoadjuvant therapy to target a reduction in distant recurrence are now being considered. The introduction of systemic chemotherapy to the neoadjuvant treatment schedule has been proposed. By delivering systemic chemotherapy upfront, micro-metastatic disease present preoperatively may be treated. Perceived benefits include increased completion rates of systemic chemotherapy and the eradication of delays to treatment caused by postoperative complications. The recently reported Rectal cancer and preoperative induction therapy followed by dedicated operation (RAPIDO) trial assessed the role of neoadjuvant systemic chemotherapy in combination with SCRT [164]. Lower rates of disease-related treatment failure, defined as the first occurrence of locoregional failure, distant metastasis, new primary colorectal tumour, or treatment-related death, were observed in the experimental arm compared with standard of care (24% vs 30%, p=0.0019), supporting the transition of chemotherapy from the postoperative to preoperative setting as the new standard of care in high risk rectal cancer. The Chemotherapy Alone or Chemotherapy Plus Radiation Therapy in Treating Patients With Locally Advanced Rectal Cancer Undergoing Surgery (PROSPECT) trial examines the use of selective radiotherapy following neoadjuvant systemic chemotherapy in patients with locally advanced rectal cancers (T2N1 or T3N0-1) suitable for low anterior resection[165]. This is due to complete in 2023.

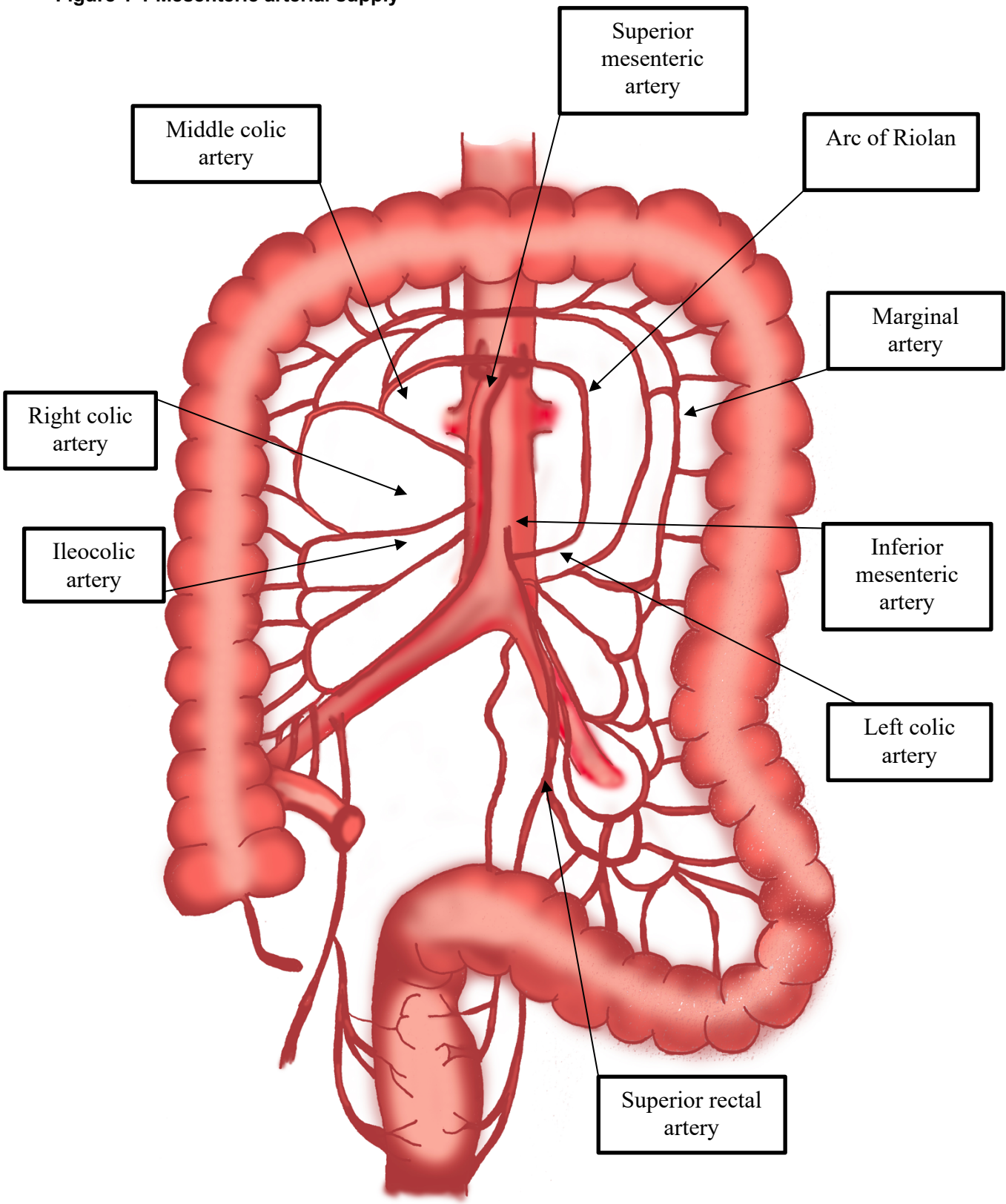
1.4.3.5 Surgical resection

Curative treatment of colorectal cancer centres on surgical resection of the tumour en bloc with the surrounding segment of bowel and its associated lymphovascular pedicle. This may be performed using an open or minimally invasive approach. Right colonic tumours are excised by right hemicolectomy, transverse colonic and splenic flexure tumours by extended right hemicolectomy and left colonic tumours by left hemicolectomy. Subtotal colectomy may be performed for synchronous tumours arising in distinct colonic segments, or for splenic flexure tumours.

The arterial supply of the colorectum is outlined in Figure 1.1. The superior mesenteric artery (SMA) arises from the abdominal aorta at the level of the first lumbar vertebra and supplies the second part of the duodenum to mid-transverse colon. From there, the inferior mesenteric artery (IMA), a more distal aortic branch, forms the arterial supply of the remaining colon down to the superior rectal artery. The arc of Riolan extends from the middle colic vessels arising from the SMA to the left colic branches of the IMA and is present in 7 to 10% of individuals [166]. The marginal artery refers to a series of arterial arcades along the border of the colon formed by terminal branches of the SMA and IMA. The lymphatic drainage follows the arterial supply. Together with the venous drainage, the arteries and lymphatics form lymphovascular pedicles supplying discrete segments of the colon, the borders of which are used in standard colectomy to define the limits of resection.

Avascular planes delineate the retroperitoneum from the colon laterally and posteriorly. Dissection along these planes can be approached from the lateral aspect along the line of Toldt or from a medial perspective. This achieves mobilisation of the colon and is completed by ligating and dividing the lymphovascular pedicle. The colon is then divided at its proximal and distal margins.

Figure 1-1 Mesenteric arterial supply



The concept of complete mesocolic excision (CME) was described by Hohenberger in 2009 as dissection in the embryological plane between the visceral and parietal peritoneum, resulting in an intact mesocolic envelope which is ligated at the root of the arteriovenous supply and incorporates extended lymphadenectomy [167]. The potentially higher morbidity rates associated with CME and the impact on oncologic outcome are ongoing subjects of debates [168,169], with trials designed to address these concerns in progress.

The principles guiding resection of rectal tumours are similar and also influenced by tumour site. The superior rectal artery arises from the IMA, the middle and inferior rectal arteries from the internal iliac artery. Upper rectal cancers may be resected with a mesorectal transection point 5cm from the distal tumour margin [170] while mid and low rectal tumours require TME. The concept of TME was introduced in 1982 [171]. Long-term follow up demonstrated significantly lower rates of local recurrence in patients who had TME compared with patients who had traditional rectal resection in which much of the mesorectum was left in situ [172]. Anterior resection is performed for mid and low rectal cancers. Transanal approaches to TME have been described [173] and are the subject of ongoing randomised trials [174]. Tumours involving or close to the sphincter or levator ani muscles require abdominoperineal resection which may be performed in the intersphincteric or extralevator planes. Locally advanced rectal cancers with involvement of adjacent organs or structures may be amenable to en bloc resection involving multivisceral surgery and reconstruction. Such procedures extend beyond standard anatomical planes and involve multidisciplinary teams including plastic, orthopaedic, urological and other surgical specialities.

Anastomotic healing following colorectal resection requires adequate arterial perfusion and absence of tension among other factors. If perfusion is compromised or a tension-free join is not feasible, end stoma formation may be chosen to avoid the morbidity of anastomotic leak, particularly in patients with significant comorbidity. The lack of non-invasive, readily available preoperative imaging techniques that reliably identify suboptimal arterial perfusion to the colon and rectum means that decisions regarding end stoma formation are often made either intraoperatively or based on subjective assessment in the

preoperative period. The development of dynamic imaging to assess mesenteric flow is an area of unmet clinical need.

1.4.3.6 Arterial supply

Following colonic or rectal resection, the arterial supply is altered. In the right colon, the ileocolic branch of the SMA is ligated and divided. The middle colic branches perfuse the remaining transverse colon. In left-sided colectomy or rectal resection, the IMA is divided and the remaining colon is perfused by branches of the SMA. It can be seen that the health of the arterial supply is integral to sufficient perfusion of the anastomosed region. Moreover, in the setting of rectal resection, a significant distance between the anastomosis and main arterial supply results.

The arc of Riolan and marginal artery branches provide collateral mesenteric circulation in so-called watershed areas such as the splenic flexure (Griffith's point) and the rectosigmoid junction (Sudeck's point) where supply from the branches of the SMA and IMA is tenuous. High ligation of the vascular pedicle is recommended to ensure adequate lymph node yield [167]. In left and rectal resections, this potentially risks the collateral circulation to the proximal anastomotic segment. Failure of anastomotic healing due to ischaemia may be increased in patients with poor collateral circulation. However, the literature on mesenteric arterial disease and outcome following colorectal resection is limited. One single-centre study assessing asymptomatic mesenteric vascular disease in patients undergoing colectomy reported no significant difference in anastomotic leak rate among those with <50% occlusion of the main mesenteric arteries compared with >50% or total occlusion [175].

While arterial perfusion is relevant to the surgical management of colorectal cancer, it is unclear whether arterial supply plays a role in colorectal carcinogenesis. There is no description in the literature of incidence of colon or rectal cancer in relation to well perfused versus watershed areas. However, differences in the outcomes of patients with right versus left colon cancer have been reported and may relate to differing vascular supply. Wang and colleagues

reported higher prevalence of cardiovascular disease, both in terms of risk factors and established disease, in patients with right colon cancer when compared with left [176]. In patients with atherosclerosis of mesenteric arteries, tumours may develop against a backdrop of relative hypoxia compared with those in patients without evidence of mesenteric vascular disease. Similarly, in watershed areas such as the splenic flexure and the rectosigmoid junction where arterial supply may be less consistent, the characteristics of the tumour may differ from those in areas supplied directly by mesenteric arteries. It is unknown whether differences in perfusion characteristics influence clinical outcomes including complications and survival.

1.4.3.7 Surgical approach

The first laparoscopic colectomy was reported in 1991 [177] but translation to oncologic surgery was hampered by an initial report demonstrating a 20% port site recurrence rate [178]. This was subsequently countered by other series [23,179] and by randomised controlled trials showing oncologic safety and equivalence to open surgery [180–183]. The benefit to short-term outcomes including enhanced postoperative recovery and reduced analgesic requirement was also highlighted by these trials.

The uptake of a laparoscopic approach for rectal cancer resection was not straight-forward. The Medical Research Council CLASICC trial reported higher rates of circumferential margin (CRM) positivity in patients with rectal cancer who underwent laparoscopic anterior resection [183]. This was not significant when compared with patients undergoing open anterior resection but prompted further trials. The Colorectal Cancer Laparoscopic or Open Resection (COLOR) II found no difference in CRM positivity rates by either laparoscopic or open approach [184]. Long-term follow up confirmed no significant differences in locoregional recurrence rates, disease-free and overall survival between the two approaches [185].

To provide data on short and long-term outcome within an acceptable time frame, a series of subsequent randomised trials used composite oncological

endpoints and a noninferiority design. Long term follow up data from the American College of Surgeons Oncology Group (ACOSOG) Z6051 trial [186], the COREAN trial [187] and Australasian Laparoscopic Cancer of the Rectum Trial (ALaCaRT) [188] have demonstrated equivocal outcomes between open and laparoscopic rectal cancer resection. The importance of optimal pre-treatment imaging to guide appropriate selection of patients for a minimally invasive approach is a key finding in these trials.

1.4.3.8 Robotic Surgery

Technology continued to evolve in response to the uptake of minimally invasive approaches. Robotic surgery overcomes some of the limitations of laparoscopic surgery including the 2-dimensional view and the restricted dexterity of non-articulating instruments while capitalising on the reduced surgical trauma associated with minimally invasive surgery. Advantages of robotic surgery stem from the three-dimensional view offered by the console headset, a fixed platform for the camera and the increased dexterity of the wristed instruments [189]. Such benefits are most notable in procedures involving restricted areas such as the pelvis and therefore rectal cancer surgery is particularly enhanced by the use of a robotic approach [190]. Disadvantages include longer operating times, the 2-dimensional view available to the assistants and the cost of the system and its maintenance.

Several small randomised controlled trials comparing robotic and laparoscopic rectal cancer resection have been reported from centres in the USA, Europe and Asia. Comparable pathological outcomes including lymph node harvest [191,192] and completeness of TME [191] were noted in the laparoscopic and robotic arms in these studies. Clinical benefits including shorter length of stay [191], reduced conversion rate [193] and better preservation of urinary and sexual function in males [194] offset the longer set up and operative time [192]. The Robotic vs. Laparoscopic Resection for Rectal Cancer (ROLARR) study [195] is the largest randomised controlled trial comparing minimally invasive approaches to date. No significant differences in pathological outcomes including CRM positivity, complications or quality of life at 6 months were noted. To date, only one study

has reported survival outcomes. In 76 patients, Patriti and co-workers reported similar overall and disease-free survival between the laparoscopic and robotic arms [193]. However, follow up was limited, extending to a mean of only 18 months in the laparoscopic group and 29 months in the robotic arm.

1.4.3.9 Enhanced recovery after surgery (ERAS)

Protocol-driven perioperative management has become standard of care following elective colorectal surgery. Key elements include preoperative patient education and optimisation (smoking and alcohol cessation), limited fasting prior to and early mobilisation and diet following surgery [196,197]. Additional components include avoidance of routine bowel preparation, use of carbohydrate-loading drinks preoperatively, tailored anaesthesia and analgesia, restricted use of drains and NG tubes and early removal of catheter and cessation of intravenous fluid supplementation (Table 1.3).

The use of minimally invasive surgery where possible is a guiding principle in minimising surgical trauma and supports the main aim of ERAS in limiting surgical stress and shortening time to functional recovery. The superiority of laparoscopic colectomy combined with ERAS to open colectomy and ERAS was demonstrated in the LAFA trial [198]. Similar findings were reported by the UK-based EnROL trial that included participants undergoing colon and rectal cancer resection [199]. Laparoscopic resection shortened length of stay when combined with ERAS programme when compared with open surgery and ERAS (Median 5, IQR 4-9 vs 7, IQR 5-11, p=0.033).

Randomised controlled trials of enhanced recovery protocols have been challenging to run due to difficulties with blinding and comparison of multimodal pathways consisting of multiple components with traditional care. The latter has evolved in parallel, often encompassing ERAS elements. Reductions in length of stay and postoperative complications have been reported following implementation of ERAS protocols [200,201]. It is unclear which elements contribute directly to improved outcomes. Avoiding unnecessary mechanical bowel preparation [202] and nasogastric decompression [203] are supported by high quality

evidence while preoperative carbohydrate loading and early re-introduction of diet following surgery have been independently associated with reduced morbidity and length of stay [204-206].

Table 1-3 - Enhanced recovery after surgery components

Preoperative	Intraoperative	Postoperative
Patient education	Antibiotic prophylaxis	Early mobilisation
Alcohol and smoking cessation	Goal directed fluid therapy	Early feeding
Carbohydrate loading	Minimally invasive surgery	Discontinuation of IV fluids
Limited fasting	Avoidance of abdominal & NG drainage	Early removal of catheter
	Avoidance of hypothermia	Thromboprophylaxis

1.4.4 Pathological staging

Principles similar to those that guide curative resection of colorectal cancer apply to the pathological staging process. The presence or absence of high-risk tumour features is central to the provision of adjuvant chemotherapy with the intention of minimising the risk of recurrence [207-209]. The third edition of the standard dataset for histopathological reporting of colorectal cancer by the Royal College of Pathologists is in use at the time of writing and incorporates the International Union in Cancer/American Joint Committee on Cancer Tumour, Nodes, Metastases staging system version 5.

The degree of infiltration of the bowel wall by tumour and the presence or absence of locoregional lymph node involvement form the basic components of pathological staging. Cuthbert Dukes of St Marks hospital proposed Dukes' classification of rectal cancer in 1932 [210]. Dukes A described tumours limited to the rectal wall, Dukes B applied to tumours extending beyond the bowel wall into the adjacent tissue but not regional lymph nodes and Dukes C described tumour involving adjacent tissues and regional nodes (Table 1.4). This system and all subsequent staging systems reflect the increasingly poor prognosis as disease stage increases. Dukes stage was refined in 1949 by Kirklin, Docherty and Waugh [211] to acknowledge depth of invasion in relation the layers of the bowel wall (mucosa and muscularis propria) with subdivision of Dukes B to B1 (lesions extending into but not infiltrating muscularis propria) and B2 (lesions penetrating the muscularis propria) (Table 1.4). Astler and Coller of the University of Michigan further subdivided Dukes C into C1 where tumours were confined to the bowel wall with involved nodes and C2 in which tumours penetrated through all layers and had involved nodes (Table 1.4) [212]. In 1957, the American Joint Committee on Cancer (AJCC) derived the tumour, node, metastasis (TNM) cancer staging system to standardise the language used to describe tumour burden (Table 1.5) [213]. Continuous expert review of existing and new data relating to prognosis has led to the release of updated versions reflecting modifications. TNM staging can be applied to clinical data derived from imaging and therefore clinical TNM staging in the preoperative period is possible, in contrast to previous staging which relied on examination of the pathological specimen.

Table 1-4 - Early pathological staging of colorectal cancer.

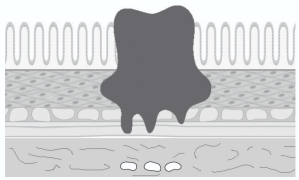
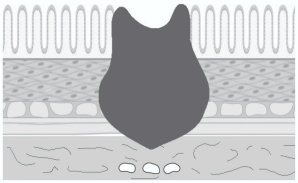
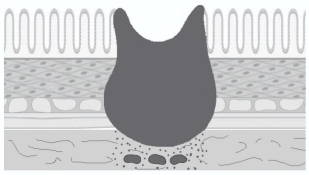
	Dukes' stage [210]	Modified Dukes' stage [211]	Astler and Coller [212]
	A Tumour limited to rectal wall	Tumour limited to mucosa	As Modified Dukes' classification
	B Tumour extending beyond bowel wall into adjacent tissues but not regional lymph nodes	B1 Tumour extending into but not penetrating muscularis propria with negative nodes	As Modified Dukes' classification
		B2 Tumour penetrating muscularis propria with negative nodes	
	C Tumour involving adjacent tissues and regional nodes	B1 or B2 with involved nodes	C1 Limited to bowel wall with positive nodes
			C2 Through all layers of bowel wall with positive nodes

Table 1-5 - AJCC TNM staging of cancers of colon and rectum, 5th edition.

Tumour (T) stage	Tis	Carcinoma in situ: intraepithelial or invasion of lamina propria
	T1	Tumour invades submucosa
	T2	Tumour invades muscularis propria
	T3	Tumour invades through muscularis propria and invades subserosa
	T4a	Tumour invades or perforates the visceral peritoneum
	T4b	Tumour directly invades other organs or structures
Nodal (N) stage	N0	No regional lymph node metastasis
	N1	Metastasis in 1 – 3 regional lymph nodes
	N2	Metastasis in 4 or more regional lymph nodes
Metastasis (M) stage	M0	No distant metastasis
	M1	Distant metastasis detected

1.4.4.1 Prognosis by stage

Currently, data on survival by TNM stage is not published for patients with colorectal cancer in Scotland. Data on survival by stage in England is based on years 2002 to 2006 and is derived from one region in England [214]. As such, applicability is limited as changes in investigation and management as well as the introduction of the Scottish National Bowel Screening programme are likely to have impacted on survival over the last 10 to 15 years. Indicative figures for 5-year overall survival by stage are displayed in Table 1.6 [170, 171].

Table 1-6 - Combined TNM stage and survival for cancers of the colon and rectum.

TNM stage	T	N	M	5-year overall survival	
				Females	Males
I	T1	N0	M0	100%	95%
	T2	N0	M0		
IIA	T3	N0	M0	90%	80%
IIB	T4	N0	M0		
IIIA	T1-T2	N1	M0	65%	65%
IIIB	T3-T4	N1	M0		
IIIC	T (any)	N2	M0		
IV	T (any)	N (any)	M1	10%	5%

Over time, the components of the early staging systems which stratified prognosis according to anatomical features have expanded to include inherent tumour characteristics recognised to confer additional prognostic information.

1.4.4.2 Tumour type

Tumours are typed according to the WHO classification based on histologic appearance rather than molecular characteristics [215]. Most colorectal cancers are adenocarcinomas. The main morphological subtypes of relevance are mucinous adenocarcinomas, containing over 50% extracellular mucin, and signet ring cell adenocarcinomas which consist of more than 50% intracytoplasmic mucin. The latter confers an adverse prognosis that is independent of stage [216] and is associated with advanced disease. Mucinous adenocarcinoma is estimated to account for between 10 and 15% of all colorectal cancers while signet ring cell adenocarcinoma constitutes approximately 1% of all colorectal cancers. Mucinous differentiation has an impact on outcome that depends on the context. MSI-H tumours often show mucinous differentiation [217]. In this setting, survival is improved compared with patients with mucinous microsatellite stable and non-mucinous colorectal cancer [218].

1.4.4.3 Differentiation

In adenocarcinoma, the degree of differentiation has been shown to be a stage-independent prognostic factor [219-221]. It is most commonly determined by assessment of tumour architecture focusing on the degree of gland or tubule formation. Morphological heterogeneity within colorectal tumours is a well-recognised characteristic. To take account of this, low-grade tumours (well or moderately differentiated) are identified by the presence of greater than 50% gland formation while high grade (poorly differentiated) tumours are classified as those exhibiting less than 50% gland formation.

1.4.4.4 Venous invasion

This adverse feature is considered extramural or intramural with the latter consisting of submucosal and intramuscular venous invasion. Histological evidence of venous invasion in over 50% of rectal cancers was reported in 1938 and correlated with the development of distant metastases [222]. Venous invasion can be identified by the presence of tumour within the endothelium-lined compartment surrounded by a rim of muscle or red cells. It can also manifest as rounded satellite regions of tumour next to arteries. Extramural venous invasion is well-established as an independent predictor of haematogenous spread and mortality in colon [223-227] and rectal cancer [226-229]. Intramural venous invasion [225,227,230] has also been reported to be prognostic of inferior survival. Node-negative disease in the presence of venous invasion is associated with inferior survival and can be used to risk stratify such patients and allocate adjuvant therapy. It is therefore critical that venous invasion is accurately recorded. Assessment using elastica staining has been shown to be superior to standard haematoxylin and eosin staining in identification of venous invasion, particularly among non-GI pathologists [226].

1.4.4.5 Lymphatic invasion

The presence of tumour within endothelium-lined spaces of the submucosal layer denotes lymphovascular invasion. Accurately distinguishing lymphatic from venous invasion can be challenging, the main difference being that lymphatic vessels have no muscle in their walls. Additional immunohistochemical stains may be helpful in identifying lymphatic from venous invasion.

The rationale for reporting lymphatic invasion separately lies in its strong association with lymph node metastases. This is particularly relevant in early stage disease where local resection may be considered as definitive treatment. A meta-analysis demonstrating the significance of lymphatic invasion reported a 7-fold increased risk of lymph node metastases in the presence of lymphatic invasion in early colorectal cancer [231]. While the definition of early disease was not clear, it can be seen that the presence of lymphatic invasion conveys

additional relevant information on the risk of lymph node involvement which may be useful in guiding treatment decisions.

1.4.4.6 Perineural invasion

Perineural invasion is also a poor prognostic indicator which can be used to identify patients at high risk of recurrence and inferior survival in whom adjuvant chemotherapy may be of benefit. The involvement of nerves by tumour and spread within nerve sheaths is pathognomonic of perineural invasion. In a retrospective review of 249 patients who had undergone resection between 1995 and 2000, less than 1% had perineural invasion reported [232]. When the pathological specimens were reviewed, this rose to 22% and was associated with a fourfold decrease in disease-free survival [232]. In combination with other negative prognostic indicators, perineural invasion was found to influence disease-free survival in a prospective study of 448 patients with stage II colon cancer [233]. A further retrospective study in 255 patients from Korea with stage IIA colon cancer reported perineural invasion to be the only independent predictor of disease-free survival when adjusted for lymphovascular invasion, elevated preoperative CEA and features of obstruction [234].

1.4.4.7 Response to neoadjuvant therapy

A proportion of patients who undergo neoadjuvant therapy have a significant response to treatment, such that there is minimal or no viable tumour within the resection specimen. This is associated with an improved outlook when compared with those who experience minimal response to preoperative therapy [235–238]. Multiple tumour regression grades have been described but high rates of interobserver variability are commonly associated with such systems [239]. Moreover, variation in pathological complete response (pCR) rates across institutions may be attributable to differences in the thoroughness of pathological examination [240]. In the UK, the Royal College of Pathologists

recommend the use of a tumour regression grade modified from that reported by Ryan and colleagues [241] (Table 1.7).

Table 1-7 - Royal College of Pathologists' assessment of response to preoperative therapy.

Tumour regression grade	Description
0	No viable cancer cells (complete response)
1	Single cells or rare small groups of cancer cells (near-complete response)
2	Residual cancer with evident tumour regression, but more than single cells or rare small groups of cancer cells (partial response)
3	Extensive residual cancer with no evident tumour regression (poor or no response)

1.4.4.8 Peritoneal involvement

Similar to mesorectal involvement, tumours breaching the peritoneal layer confer a poor prognosis. Peritoneal involvement can be defined as tumour breaching the serosa with cells identifiable on the peritoneal surface, within the peritoneal cavity or separated from the serosa by a layer of inflammatory cells only [225]. Initially highlighted in the Gloucester Colorectal Cancer study, involvement of the peritoneum was found to be prognostic of local recurrence in rectal cancer [229] and later to be related to intra-peritoneal recurrence in colon cancer [224]. The presence of peritoneal involvement is therefore regarded as a high-risk feature for which adjuvant chemotherapy may be of some benefit.

1.4.4.9 Tumour perforation

The presence of a perforation within the tumour resulting in a communication between the bowel lumen and the external surface of the resection specimen is of extreme adverse prognostic significance. This is regarded as an entity distinct from a perforation occurring proximal to the site of an obstructing tumour. Intrinsic tumour perforation indicates T4 stage disease.

Patients with colon cancer and adverse features such as peritoneal involvement or tumour perforation are at high risk of peritoneal carcinomatosis [224,225,229,242]. This is the second most common cause of death after liver metastases in colorectal cancer [243].

1.4.4.10 Margin involvement

There are three margins in each specimen: proximal and distal (together referred to as longitudinal) and circumferential. Tumours with clear resection margins are categorised as R0 while those with microscopic involvement of any margin defined as tumour present within 1mm are categorised as R1; R2 status denotes macroscopic involvement of the resection margin. In rectal cancer, involvement of the circumferential resection margin is an independent predictor of local recurrence and survival [244-246]. Margin involvement is an independent predictor of poor prognosis and is particularly relevant in stage II colon cancer [225]. Tumours of the distal caecum and proximal ascending are at potential risk of retroperitoneal margin involvement through direct extension. The retroperitoneal margin can also be involved by a lymph node harbouring metastatic disease, venous invasion, tumour deposits or perineural invasion within 1mm of the margin.

1.4.4.11 Plane of surgery

Two prospective trials [247,248] examining the macroscopic appearance of the TME plane in rectal cancer resection specimens demonstrated its relevance to clinical

outcomes, in particular local recurrence. In 180 patients participating in the Dutch trial of radiotherapy plus TME, a quarter of patients were found to have an incomplete mesorectal specimen [249]. This was associated with an increased risk of local and distant recurrence of 36% versus 20% when compared with those who had a complete mesorectal specimen. In 1,130 patients involved in two trials (CR07 and NCIC-CTG CO16) run between 1998 and 2005 [160], Quirke and co-workers classified the plane of surgery as good (mesorectal), intermediate (intra-mesorectal) and poor (muscularis propria) and reported 3-year local recurrence rates in relation to this as 4%, 7% and 13% respectively [247]. Disease-free survival at 3 years was similarly related to the TME plane, with good, intermediate and poor resection specimens associated with survival of 79%, 75% and 70% respectively. Plane of surgery has subsequently become an important indicator not only of the quality of surgery, but of prognosis. Moreover, the data highlighted that preoperative radiotherapy and mesorectal plane of excision combined to produce an additive effect on improving recurrence and survival rates, with corresponding local recurrence rates of 1% in this trial.

Application of similar pathological standards to colon cancer specimens have been reported in retrospective studies. In 399 specimens from patients operated at one UK centre between 1997 and 2002, the plane of excision was intramesocolic in 44% and muscularis propria in 24% [250]. Improved survival was associated with an intact mesocolic plane of excision. This benefit was particularly marked in curative-intent stage III disease: among 154 patients, a 27% improvement in 5-year overall survival was noted (HR 0·39 [0·21–0·72], p<0·0001). Local recurrence is less frequent in colon cancer when compared with rectal cancer and it remains unclear whether a significant benefit would be derived from adoption of techniques such as complete mesocolic excision [251].

1.4.4.12 Lymph node status

Assessment of lymph node status enables accurate staging, prognostic stratification and guides provision of adjuvant chemotherapy. The minimum number of nodes recommended for evaluation in the pathological staging of colorectal cancer is 12 [252] and is a quality indicator in both colorectal cancer

surgery and its pathological reporting. However, in a population-based study of 111,730 patients in the USA, inadequate lymph node sampling occurred in 37% of patients who underwent resection with curative intent [253]. Several studies have shown that an increased number of nodes harvested and examined is associated with improved survival [254-256]. The survival benefit is seen in patients with node-positive disease, suggesting the effect is not limited to more accurate staging [257].

1.4.5 Adjuvant therapy

Adjuvant therapy following surgical resection of colorectal cancer aims to eliminate residual circulating tumour cells or micrometastatic disease. This is considered in patients with high risk pathological features or node-positive (stage III) disease. The concept of high-risk disease is broad and encompasses T4 tumours, those presenting with bowel obstruction or as an emergency, the presence of tumour perforation, lymphatic invasion or venous invasion.

The heterocyclic compound 5-fluorouracil (5FU) forms the backbone of chemotherapy for colorectal cancer. 5FU disrupts nucleoside metabolism and is incorporated into DNA and RNA in place of uracil or thymine, leading to cell death. It is administered intravenously while capecitabine is an oral precursor of 5-FU. Both are associated with cardiovascular side effects including coronary vasospasm, acute myocardial ischaemia, myocarditis, pericarditis and arrhythmias.

Chemotherapy regimens commonly combine 5FU with drugs such as oxaliplatin and irinotecan. Oxaliplatin is a water-soluble platinum-based cytotoxic drug that cross-links DNA, preventing replication and cell division. It can be associated with neurotoxic side effects, most commonly peripheral neuropathy. The pro-drug irinotecan is activated in vivo to SN-38, a cytotoxic metabolite that induces cell cycle arrest by inhibiting the enzyme topoisomerase.

Toxicities associated with these agents can limit treatment. In the acute setting, these range from diarrhoea, severe neutropenia and cardiotoxicity while

peripheral neuropathy can be chronic. The presence of adverse host characteristics is central to decision-making regarding adjuvant therapy.

1.4.5.1 Adjuvant chemotherapy and colon cancer

The National Surgical Adjuvant Breast and Bowel Project NSABP-C03 trial [258] was the first to demonstrate improved disease-free and overall survival in patients with resected Dukes B and C colon cancer receiving 5FU plus leucovorin (LV), a folinic acid derivative that enhances the activity of fluorouracil. The addition of levimasole, an anti-parasitic drug, to 5FU in patients with stage III disease colon cancer was associated with a 16% absolute reduction in both recurrence and mortality in a randomised trial comparing observation with 12 months levimasole plus 5FU or levimasole alone [259].

The value of adding leucovorin to 5FU alone and in combination with levimasole for high risk stage II and stage III disease was definitively assessed in the Intergroup-0089 trial. No statistically significant differences were reported in disease-free and overall survival between the treatment regimens (low dose leucovorin and 5FU, high dose leucovorin and 5FU, both with or without levimasole, and levimasole alone) [260]. It was evident that treatment courses of 6 to 8 months were as effective as 12 months and the addition of levimasole to 5FU/LV did not confer additional benefit. This was confirmed in five contemporary trials [254,261-264]. Subsequently, 5FU plus leucovorin was established as the standard of care.

1.4.5.2 Adjuvant therapy in stage II disease

Studies demonstrating a survival benefit from adjuvant 5FU/LV were not powered to detect differences in stage II disease [258,261,264]. To address this issue, pooled analyses were undertaken. Opposing conclusions were drawn from pooled analysis of the NASBP C-01 to C-04 trials [265] and the International Multicentre Pooled Analysis of B2 Colon Cancer (IMPACT B2) [266]. The NASBP results suggested additional benefit but of a smaller degree in stage II disease when compared with stage III disease. Among the caveats, the study design was highlighted: arms

using inferior and outmoded chemotherapy were included, as well as comparisons of contemporary standard of care regimens to no treatment.

The IMPACT B2 trial, however, compared 5FU/LV with no treatment but failed to demonstrate a statistically significant benefit in stage II disease. This trial included patients with B2 disease from 5 trials with evidence of significant heterogeneity. The comparatively low event rate when contrasted with stage III disease requires larger numbers to demonstrate a benefit in this group, a fact that highlights the small proportion of patients who derive benefit from adjuvant chemotherapy in earlier stage disease. The baseline prognosis of 80% survival at 5 years with observation alone necessitates a minimum estimated sample size of 5,000 patients to detect a difference in patients with stage II disease receiving adjuvant therapy compared with controls receiving observation only[267]. Furthermore, in patients with stage II disease without high risk features, it has been estimated that a sample size of over 9,000 would be required to detect a meaningful survival advantage [268]. Lack of benefit was confirmed in the QUASAR study where an absolute difference in survival of less than 3% was demonstrated, reinforcing that adjuvant therapy in stage II without high risk features is not warranted[209].

1.4.5.3 Double-agent chemotherapy

The addition of oxaliplatin to 5FU/LV was considered in the adjuvant setting following trials in patients with metastatic colorectal cancer [269-272]. In the Multicentre International Study of Oxaliplatin/5FU/Leucovorin in Adjuvant Treatment of Colon Cancer (MOSAIC), a 23% reduction in the relative risk of recurrence was reported in patients receiving oxaliplatin plus 5FU/LV [207].

Similar results were reported in the NASBP-C07 trial [273]. Comparing oxaliplatin/bolus 5FU/LV with standard bolus 5FU/LV, the relative risk reduction in recurrence adjusted for age and number of involved nodes was 19%. However, diarrhoea was a significant side effect of oxaliplatin, resulting in severe enteropathy requiring inpatient care [274]. XELOXA compared oxaliplatin in addition to oral 5FU (XELOX) with bolus 5FU/LV in stage III colon cancer [275]. On

multivariate analysis, a relative risk reduction of 22% in disease-free survival was associated with XELOX over bolus 5FU/LV. On the basis of these trials, 6 months of adjuvant therapy with oxaliplatin in addition to oral or IV 5FU and leucovorin was established as standard of care in in patients with resected colon cancer with high risk features.

1.4.5.4 Chemotherapy duration

The major side effect of oxaliplatin is peripheral neuropathy which can be long-lasting [276,277]. The SCOT trial (ISRCTN59757862) was designed to address whether 3 months of oxaliplatin-based adjuvant therapy was non-inferior to 6 months in terms of survival. The primary endpoint was disease-free survival at 3 years. Recruitment completed in 2013 and results are awaited. Notably, the final enrolment of 6,088 did not meet the target sample size of 6,144. The study may therefore be underpowered to detect a significant difference in its primary endpoint.

The SCOT trial forms one of six trials assigning patients to adjuvant FOLFOX or CAPOX as part of the International Duration Evaluation of Adjuvant Therapy (IDEA) collaboration. The patients, totalling 12,834, are to be pooled for a pre-planned analysis to assess the optimal duration of 5FU/LV/Oxaliplatin (FOLFOX) or capecitabine/oxaliplatin (CAPOX) for colon cancer in patients with stage III disease [278]. The pooled sample size provides greater numbers to determine whether the criteria for non-inferiority (not more than 12% reduction in benefit in 3 months treatment arm versus 6 months) are met. Full results are awaited.

1.4.5.5 Adjuvant chemotherapy and rectal cancer

The value of adjuvant chemotherapy in rectal cancer is challenging to interpret given the use of preoperative therapy and the wide variation across studies in sample size, chemotherapy regimens and use of combined modality adjuvant treatment. A Cochrane review pooling results from 20 trials including 8,530 patients [279] reported a statistically significant improvement in disease-free

survival (HR 0.75; 95% CI: 0.68–0.83). However, several trials took place before TME became standard of care, while two trials [209,280] enrolled patients who had received neoadjuvant therapy.

The EORTC 22921 examined pre or postoperative 5FU-based chemotherapy in addition to preoperative radiotherapy in 1,011 patients with T3 or T4 resectable rectal cancer [281]. No difference in overall or disease-free survival was reported in those receiving adjuvant chemotherapy after preoperative radiotherapy and surgery with or without chemotherapy compared to surveillance.

Two trials assessed the role of combination oxaliplatin-based adjuvant chemotherapy: ADORE [282] and R98 [283]. The ADORE trial demonstrated a statistically significant improvement in 3-year disease-free survival in patients receiving 8 cycles of FOLFOX compared with patients receiving 4 cycles of 5FU/LV (HR 0.657; 95% CI: 0.434–0.994), a benefit which was sustained on follow up at 6 years. In the French R98 trial, 69% of patients received preoperative chemoradiotherapy and 75% underwent TME. The patients were randomised to 6 or 12 cycles of 5FU or 12 cycles of 5FU/LV plus irinotecan. No difference was observed in disease-free survival at 5 years. A further trial comparing single-agent 5FU adjuvant therapy with combination oxaliplatin-based therapy, demonstrated improved disease-free survival in the oxaliplatin group (HR 0·79, 95% CI 0·64-0·98; p=0·03) [284]. However, administration of oxaliplatin during neoadjuvant treatment in the intervention arm was undertaken, making the true effect of adjuvant oxaliplatin difficult to discern.

Given the lack of robust evidence, the benefit of adjuvant therapy for rectal cancer in the context of neoadjuvant therapy remains unclear.

1.4.5.6 Targeted agents

In addition to standard cytotoxic therapy, novel agents targeting specific components of the carcinogenesis pathway have been developed. These include tumour-targeting monoclonal antibodies and antiangiogenic drugs. Bevacizumab is a monoclonal antibody targeting the vascular epidermal growth factor

receptor (VEGFR). Aberrant VEGF activity occurs in colorectal cancer [285] whereby endothelial cells express VEGF receptors that promote blood and lymphatic epithelial cell growth and avoidance of apoptosis when activated [286]. Bevacizumab is antiangiogenic and is thought to improve response to chemotherapy in metastatic setting by altering tumour vasculature and increasing chemotherapy delivery [287].

In the AVANT trial [288], bevacizumab combined with FOLFOX or XELOX failed to improve disease free survival over FOLFOX alone in patients with stage III colon cancer. It was in fact suggested to possibly be detrimental with inferior overall survival seen in patients receiving bevacizumab with conventional chemotherapy. In the NASBP C08 trial [289], the results were similarly negative, although no clear detrimental effect was evident. The QUASAR-2 trial [290] also found no improvement in disease free survival at 3 years when bevacizumab was combined with capecitabine.

Cetuximab targets the epidermal growth factor receptor (EGFR) and leads to cell cycle arrest, inducing apoptosis [291]. Epidermal growth factor plays a role in the epithelial to mesenchymal transition and reduction in cell to cell adherence that form key parts of the process by which tumour cells gain the ability to disseminate [292]. Thus its blockade has anti-tumour activity which has been effective in the treatment of metastatic colorectal cancer [293,294]. However, in both the PETACC8 [295] and N0147 [291] trials, FOLFOX combined with cetuximab did not confer an improvement in disease-free survival in patients with resected stage III colon cancer.

Resistance to EGFR inhibition is influenced by mutations in the KRAS oncogene. KRAS mediates the extra- and intracellular processes stimulated by EGFR receptor activation [296]. As such, only patients with wild-type KRAS respond to EGFR inhibition. Other genetic aberrations including PTEN loss and BRAF mutations can result in EGFR resistance, although these are not routinely screened for in the metastatic setting prior to commencing therapy, in contrast to KRAS status.

1.4.5.7 Immunotherapy

In 1891, surgeon William Coley injected streptococcus into patients with irresectable sarcoma and observed tumour regression in some cases [297]. This, the first example of active immunotherapy, much later inspired a series of trials of BCG admixed with autologous tumours cells from patients with resected colorectal cancer. It was demonstrated that systemic immunity to circulating tumour cells could be induced [298–301]. Initial results in patients with colon and rectal cancer randomised to surgery alone or surgery plus immunotherapy showed no difference in the rates of recurrence or survival. Later work focusing on patients with colon cancer demonstrated a significant reduction in recurrence rates and improved relapse-free survival among those with resected stage II colon cancer who were inoculated [301]. Vaccination as is described in these trials represents "active" stimulatory immunotherapy and while initially promising, has yet to translate into an established treatment.

1.4.5.8 Immune checkpoints

Modern immunotherapy evolved as a result of the discovery of immune checkpoints. These receptors and their ligands inhibit recognition of tumour cells by the immune system. Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) is expressed on activated and regulatory T cells and acts as negative regulator of cytotoxic T cells [302]. Inhibition by the monoclonal antibody ipilimumab results in reactivation and proliferation of cytotoxic T cells in addition to a reduction in the immunosuppressive regulatory T cell population [303]. Programmed cell death protein-1 is also an immune checkpoint that results in negative T cell regulation by a distinct mechanism. Expressed on the surface of T cells and monocytes, ligand binding induces PD-1 mediated down-regulation of T cell activation [304,305]. Therapeutic blockade can reactivate T cells and stimulate anti-tumour immunity. While durable responses have been reported in the metastatic setting in patients with metastatic colon cancer [306,307], these agents are most effective in immunogenic tumours with a high mutational burden such as MSI tumours. The small proportion of patients with such tumours (approximately 5% of all stage IV

colorectal cancer [308]) represents one of the current limitations in the scope of immunotherapy.

1.5 Host factors influencing surgical outcome

In patients with resectable non-metastatic colorectal cancer, surgery offers a potential cure and forms the central component of the treatment pathway. While mortality in the elective setting is low at approximately 2% , complications can affect as many as 50% of patients following colorectal resection [309], prolonging hospital stay, incurring additional cost and increasing the rates of functional compromise and impaired quality of life on discharge. Delayed or missed adjuvant therapy contributes to higher rates of recurrence in patients who experience complications [310,311]. Infective complications such as anastomotic leak or severe wound infection can impair cell-mediated immunity in the postoperative period [312], while common non-infective complications such as pulmonary embolism or atrial fibrillation require longer term treatment with drugs carrying a significant risk profile. Prevention of complications through pre-, intra- and postoperative strategies is therefore a vital component of surgical management of colorectal cancer.

Minimally invasive surgery and enhanced recovery protocols are established standards of care in the intra- and postoperative phases of the colorectal cancer treatment pathway. The preoperative component, however, is less standardised. Patients with adverse host characteristics experience higher rates of morbidity and mortality following curative colorectal cancer resection. Increasing age, comorbidity and lifestyle factors such as physical fitness, body composition and smoking are significant determinants of surgical outcome. It is likely that the impact of adverse host characteristics and the variable approach to host staging contributes to the variation in outcome experienced by patients with similar stage disease. A standard and reliable approach to host staging with a focus on modification of amenable adverse characteristics in the preoperative period may potentially improve surgical outcome.

1.5.1 Age

This major non-modifiable host factor is widely recognised to contribute to postoperative morbidity. In over 24,000 patients who underwent curative colon cancer resection over a 7 year period, patient age over 75 years was independently associated with increased odds of postoperative complications (OR=1.62, 95%CI=1.50–1.74) when patients under 65 were used as the comparator and adjustment made for patient factors including comorbidity [313]. The underlying mechanisms are multiple, reflecting the complex interaction between advancing age, declining physiological capacity and tolerance of surgical stress. Aside from the increased prevalence of comorbidity, factors including age-related loss of muscle mass [314], reduced cardiorespiratory fitness [315] and nutritional insufficiency [316] are likely major factors in the lower baseline functional capacity of a patient of advanced years undergoing colorectal cancer resection.

1.5.2 Comorbidity

The prevalence of comorbidity in patients with colorectal cancer has increased in recent years [317] and impacts on treatment received, completion, toxicity, quality of life and survival [318-320]. Cardiovascular disease in particular influences treatment selection, tolerance and response [321]. Despite this, formal assessment of comorbidity using validated indices such as those detailed in Table 1.8 is not routine in clinical practice. Current methods of preoperative assessment record the presence of comorbidity often in binary format, with stratification to reflect severity limited or absent.

Coding algorithms are frequently used to capture comorbidity scores in administrative datasets. Vital status is generally well recorded but more nuanced endpoints such as postoperative complications are less reliably captured. Therefore, the majority of scores assess its impact on survival, overlooking the key role comorbidity plays in the development and impact of postoperative complications.

Table 1-8 Comorbidity scores and indices

Measure	Population	Outcome(s)	Variables
Charlson comorbidity index	General medical patients	Death at 1 year	19
Elixhauser score	Adult acute care patients	LOS, cost and in-hospital mortality	30
Van Walraven-Elixhauser	Adult acute care patients	In-hospital mortality	21
National Institute of Ageing-National Cancer Institute Comorbidity Index	Patients with cancer	Early mortality	27
Adult Comorbidity Evaluation-27	Patients with cancer	Overall survival	27

1.5.2.1 Charlson Comorbidity Index (CCI)

First described in 1987, the Charlson Comorbidity Index (CCI) was developed to aid assessment of comorbidity in clinical trial participants at enrolment with the aim of improving the generalisability of trial results. It was later adapted for use with health claims data [322]. The adjusted relative risk of death at 1 year in relation to the presence of individual comorbidities was used to assign a weight to each comorbidity on a scale of 0 (least severe) to 6 (most severe). The weights are then summed to provide a summary score for an individual.

Initially developed in a mixed cohort of 604 patients admitted over a 1-month period to a New York hospital in 1984, its ability to predict mortality at 10 years was tested in an external cohort of patients treated for breast cancer (n=685).

The prevalence of comorbidity in this cohort was low: 86% scored 0. Despite this, the predicted and actuarial 10-year survival rates were similar but did differ more in those with the highest CCI scores (greatest comorbidity).

In a Danish national colorectal cancer registry study spanning 2000-2011 [323], CCI score 0 was present in 62%, CCI 1-2 in 29% and CCI 3+ was present in 9% of 5,777 patients with colon cancer. A similar distribution was noted among 2,964 patients with rectal cancer from the same registry. However, in a US-based sample of 7,803 patients with stage I-IV colorectal cancer diagnosed between 2008 and 2013, 41% had a CCI score of 0, 40% a CCI score of 1-3 and 19% a score of 4 or more [321]. It is clear that among the multitude of factors that influence comorbidity, it may also differ according to the geographical region. The relationship between all comorbidity measures, morbidity and mortality is difficult to interpret due to the inclusion of emergency and elective surgery, all stage disease and neoadjuvant and adjuvant therapy use. Moreover, national datasets enable large sample sizes but extrapolating data from administrative sources requires cautious interpretation.

1.5.2.2 Elixhauser Comorbidity Score

The Elixhauser comorbidity score [324] was developed in 1997 for use in assessing comorbidity associated with length of stay, cost and mortality. Like the CCI, it was also derived using regression estimates but in a dataset of over 1.7 million patients and specifically sought to reduce the influence of diagnoses related to the principal reason for admission and focus on pre-existing conditions. In total, 30 measures of comorbidity were found to be independently related to the outcomes of interest.

The van Walraven modification of the Elixhauser system was later proposed [325] using analysis of data collected from 12 years of admissions to a single centre. The original comorbidities were examined in relation to in-hospital death in 228,565 patients and validated in 117,230. The results for the 21 significant comorbidities were similar when compared with the original 30 but did not accurately predict in-hospital mortality. It did however capture pre-admission

morbidity in fewer variables than the original version. It was proposed that this be used to describe patient cohorts and adjust for the effect of patient comorbidity in administrative data.

When compared with the CCI, the Elixhauser score was found to be superior in predicting colorectal cancer survival in 574 patients with stage II-IV disease [326]. The base model using age, sex and stage variables had a c-statistic of over 0.8, classifying its discriminatory power to predict 2- and 3 year survival as excellent, but this was improved further with the addition of the Elixhauser comorbidities (2-year survival p=0.0051; 3-year survival, p=0.0017). This was not the case when CCI comorbidities were added. The study period spanned 2004 to 2006 and included 39% of patients with stage IV disease. Given that the significance of comorbidity is greatest when the prognostic impact of the tumour is small [327], and temporal changes in therapy have since occurred, re-examination in a contemporary cohort of patients with resectable disease is warranted.

1.5.2.3 National Institute on Ageing and National Cancer Institute comorbidity index

The NIA/NCI comorbidity index is a list of 27 comorbidities which was derived to assess the role of comorbidity in predicting the risk of early mortality in patients with cancer [328,329]. It resulted from a collaboration between the National Institute on Ageing Epidemiology, Demography and Biometry Program and the National Cancer Institute Surveillance, Epidemiology and End Results Program in which comorbidity burden, its correlation with disease stage and contribution to early mortality were assessed in 7 tumours including colon cancer.

Threat to life was assessed based on whether the comorbidity was a current problem or noted in the medical record but not considered an active issue. Categories of high, moderate, low and negligible impact were assigned using clinical judgement. TNM staging, date and cause of death were obtained.

In an age-stratified random sample of 1,610 patients diagnosed with stage I-IV colon cancer in 1992, 40% of patients were found to have 5 or more

comorbidities [329]. Total comorbidity and comorbidity with high impact life threat was related to increasing age. Regression models containing age, stage and gender confirmed those with more than 5 comorbidities to be at increased risk of early mortality. Individual comorbidities found to be independently prognostic included high-impact cardiovascular disease (angina, MI, congestive heart failure, valvular disorders), COPD, renal failure, liver disease and thyroid disease.

1.5.2.4 Adult Comorbidity Evaluation-27 (ACE-27)

The comorbidities in the ACE-27 were based on a modification of the Kaplan-Feinstein, Charlson and Elixhauser comorbidity indices [330]. Disease severity is accounted for by grading pre-specified conditions into one of three levels of comorbidity: mild (grade 1), moderate (grade 2) or severe (grade 3). The exception is obesity which is graded as 0 if body mass index (BMI) is less than 38 or 2 if BMI exceeds 38. Overall comorbidity score is determined based on the highest rank grade of the disease. If two or more comorbidities in different organ systems have moderate impact, the overall comorbidity burden is regarded as severe.

The ACE-27 was reported in 2004 in a multicentre cohort of 17,712 patients with breast, GI tract, urological, gynaecological and head and neck cancers. Of these, 1,914 patients had colorectal cancer. Comorbidity grading was reported for tumours of the GI tract as a whole without further classification. Since the prognosis of oesophagogastric cancers is generally poorer than that for colorectal, the true prognostic relevance of the ACE-27 categories is unclear. The hazard ratio for adjusted overall survival among patients with GI tract cancers graded as having mild comorbidity (n=840, 33%) was 1.13 (0.97 – 1.31) and for those with severe comorbidity (n=230, 9%) 1.73 (1.40 - 2.13) [330].

1.5.2.5 Summary

The comorbidity measures described above are among the most widely validated in patients with cancer. The majority of these were derived more than 20 years ago in mixed populations and therefore may not be representative of patients with colorectal cancer in the current era. The measures are comprehensive in their inclusion of multi-system comorbidity. However, the single most prevalent comorbidity among patients with colorectal cancer in these and subsequent studies is cardiovascular disease [321,326,329,331]. Both disease processes are influenced by lifestyle factors including smoking, physical inactivity and overweight and obesity. It is possible that in strategies aiming to both reduce the incidence of both diseases and minimise their impact in the perioperative period, interventions aimed at lifestyle factors have a key role. This, however, requires early identification of patients at risk. Comorbidity measures may be helpful in this regard but their labour-intensive nature and lack of routine use are potential barriers.

1.5.3 Cardiovascular disease and colorectal cancer

As the most common cause of death globally, cardiovascular disease (CVD) is frequently encountered in patients with colorectal cancer. CVD shares several risk factors with colorectal cancer and can limit suitability for both surgical and cytotoxic therapy. Its assessment and management is therefore a priority in optimising perioperative outcomes.

1.5.3.1 Atherosclerotic cardiovascular disease

CVD represents a spectrum of pathology including myocardial infarction (MI) and ischaemic heart disease, cerebrovascular disease, peripheral vascular disease and thromboembolic disease. However, MI and cerebrovascular disease account for 85% of deaths due to cardiovascular disease [332].

In health, the arterial endothelium does not express endothelial adhesion molecules. However, in disease states such as hypertension or dyslipidaemia, expression of endothelial adhesion molecules is upregulated, leucocytes including lipid-laden macrophages are attracted to the endothelium, permeability is increased and ultimately, intracellular cholesterol deposition occurs [333,334]. Progressive lipid accumulation results in plaque formation and luminal narrowing, referred to as atherosclerosis. Atherosclerotic disease can limit flow such that symptomatic ischaemia occurs when demand outstrips supply in the affected vascular territory. Acute cardiovascular events arise when plaque rupture and associated thrombosis occur, resulting in acute vascular occlusion.

Atherosclerosis is a diffuse process and can affect vascular beds anywhere in the arterial tree. It is common in areas of haemodynamic turbulence such as branching points [335]. The narrow calibre of the coronary arteries and the relative lack of collateral circulation [336] render the cardiovascular circulation vulnerable to myocardial ischaemia in the event of acute occlusion.

1.5.3.2 Traditional risk factors for cardiovascular disease

The individual components of metabolic syndrome - hypertension, smoking, overweight and obesity, diabetes and dyslipidaemia – are regarded as traditional risk factors for cardiovascular disease. Hypertension, defined as systolic blood pressure of 140 mm Hg or higher or diastolic blood pressure of 90 mm Hg or higher [337], leads to endothelial damage and increased lipid permeability, key components of atherosclerotic plaque formation. Cigarette smoking increases the production of free radicals, enhancing endothelial injury through systemic oxidative stress [338]. The chronic excess of free fatty acids and triglycerides in overweight and obese states saturates hepatic and myocyte lipid oxidation and storage pathways [339]. The subsequent accumulation of metabolites impairs insulin function [339] while the generation of adipokines by excess adipose tissue upregulates mediators of inflammation [340] and oxidative stress [341] that enhance endothelial dysfunction and lipid accumulation within the arterial wall. Diabetes mellitus (DM) is a metabolic disorder characterised by sustained hyperglycaemia due to defective insulin production (type I DM) or insulin resistance of target tissues (type II DM). DM is closely associated with atherosclerotic CVD. Chronic hyperglycaemia increases oxidative stress, alters lipoprotein levels and results in extracellular metabolic end products that activate the endothelium and induce chronic inflammation [342-344]. Finally, derangement of lipid levels, known as dyslipidaemia, is key to initiation and progression of atherosclerotic disease. Raised levels of low density lipoprotein and reduced levels of high density lipoprotein are closely related to MI and ischaemic stroke [345,346].

1.5.3.3 Non-traditional risk factors: Systemic inflammation

Systemic inflammation plays an integral role in the pathogenesis of cardiovascular disease and colorectal cancer. Pro-inflammatory cytokines such as interleukin-6 promote leucocyte infiltration to the arterial intima and alter smooth muscle cell function, contributing to atheromatous plaque formation [347,348]. Increased expression of endothelial adhesion molecules facilitating infiltration are common at arterial branching points [349]. While various inflammatory mediators are implicated in the development of atherosclerosis, C-

reactive protein (CRP), the downstream effector of the IL-6 pathway, is used as an indicator of future risk of cardiovascular events and represents a non-traditional cardiovascular risk factor. In the general population, a raised CRP is independently predictive of future cardiovascular events and related death as well as cancer mortality [350,351].

In colorectal cancer, systemic inflammation also holds prognostic value [40,352]. Tumour-associated inflammation is a complex phenomenon characterised by both the local immune response and systemic inflammatory response (SIR). Local inflammation is associated with improved outcome due to infiltration of the tumour by cytotoxic T cells, representing host immune recognition of the tumour and an attempt to suppress its development. However, systemic inflammation has consistently been associated with poor prognosis. In patients with operable colorectal cancer, the presence of a preoperative SIR measured using the modified Glasgow Prognostic Score (mGPS) is prognostic of inferior survival, independent of established determinants including tumour stage [353] and host comorbidity [354]. A clear link between carcinogenesis and the induction of systemic inflammation remains elusive. However, tumour necrosis has been associated with both the presence of systemic inflammation and reduced local lymphocytic infiltration of the primary tumour, translating to inferior survival [355]. Necrosis is thought to result from hypoxia and stimulates neo-angiogenesis, the process by which a tumour develops new blood vessels to enable continued growth [356,357]. It is conceivable that in patients with significant atherosclerotic disease of the vascular bed supplying the region of tumour development, flow restriction may predispose to a hypoxic tumour microenvironment, tumour necrosis and neoangiogenesis. This represents one possible interaction between atherosclerosis and tumour development that may influence outcome.

1.5.3.4 Non-traditional risk factors: Arterial calcification

A CRP level of >3 mg/L is established as a non-traditional risk factor used to improve risk stratification for future cardiovascular events in asymptomatic patients [358]. Similarly, the degree of coronary arterial calcification on computed tomography (CT) has been shown to be prognostic of future

cardiovascular events in asymptomatic patients, independent of traditional risk factors including smoking and hypertension [359-362]. The presence of calcification in any coronary vascular wall has been shown to be associated with four-fold increase in the risk of MI or cardiovascular death [363]. Subsequently, several software packages have been developed to quantify arterial calcification on non-contrast ECG-gated CT angiograms for the purpose of CVS risk prediction. The most common examples utilise the Agatston method which quantifies calcified lesions according to their number, area and peak Hounsfield units [359]. Aortic calcification (AC) visible on abdominal CT imaging has been shown to have similar prognostic value in predicting CVS risk [364-366].

Vascular calcification occurs as part of the process of atherosclerotic plaque development. Retained lipoprotein in the arterial intima stimulates influx of macrophages that become laden with lipid and form foam cells [367]. Apoptosis of foam cells with local release of inadequately-cleared toxins leads to formation of a necrotic plaque core [368] and depletion of calcification inhibitors [369]. The formation of calcium hydroxyapatite crystals in the intima is facilitated by an alteration in the phenotype of vascular smooth muscle cells stimulated by osteogenic proteins and inflammatory cytokines such as TNF-alpha and interleukin-6 [370].

Arterial calcification progresses from foci of microcalcification (0.5 - 15μm) in areas of severe inflammation such as the necrotic core [371]. Accumulation to larger calcified aggregates occurs with sustained inflammation. Areas of calcification smaller than 215 μm in diameter are not detectable on CT imaging using standard attenuation settings [372]. Calcification scores of zero therefore do not denote the absence of calcification but reflect the limits of detection.

1.5.3.5 Summary

A history of cardiovascular disease in symptomatic patients or the presence of traditional risk factors in asymptomatic patients may highlight increased vulnerability to cardiovascular complications of surgery. The use of non-traditional risk factors represents an alternative method of identifying patients

who may benefit from further assessment and optimisation of cardiovascular status prior to surgery. Characterising the relationship between such markers and short and long-term outcomes in patients undergoing colorectal cancer resection is required to evaluate their potential as preoperative markers of adverse outcome.

1.5.4 Physical activity

Low levels of physical activity are regarded as a risk factor for the development of colorectal cancer [373], while increased activity following diagnosis and treatment is associated with improved overall and cancer-specific survival [374–376]. To reflect intensity, physical activity is often described in terms of metabolic equivalent tasks (METs). One MET represents the oxygen consumption associated with resting quietly: 3.5 mL/kg/min [377]. The energy cost of common physical activities is shown in Table 1.9 [320].

The ability to perform 4 METs has been described as a threshold beneath which further investigation of a patient's cardiorespiratory status prior to surgery is recommended [378]. Ascending two flights of stairs or climbing a hill without symptomatic limitation are commonly used examples of activities requiring 4 METs. Inability to perform 4 METs is independently related to approximately double the risk of perioperative complications following non-cardiac surgery (OR 1.94, 95%CI 1.19-3.17) [379]. Self-reported measures of physical activity such as METs are limited by the potential for recall bias, as well as the influence of the subjective impression of the physician when assessing physical activity using this.

Table 1-9 Metabolic equivalent tasks

Activity	METs
Walking (<2mph)	2
Gardening (light)	2.3
Cleaning, walking briskly (3mph)	3.3
Leisure cycling (5.5mph)	3.5
Gardening (moderate), climbing stairs, cycling (<10mph)	4
Dancing	4.8
Mowing lawn, shoveling snow, hiking, rowing, doubles tennis	6
Climbing hills	6.3
Skiing	7
Carrying groceries upstairs	7.5
Push-ups, slow swimming, cycling (12-14mph), singles tennis	8
Running (5mph)	8

Adapted from the Compendium of Physical Activities

It has been demonstrated that objectively-measured physical fitness of patients prior to colorectal surgery correlates with postoperative morbidity. In a study of 136 patients having major elective colorectal resection, 48% experienced a complication. The median oxygen uptake at lactate threshold during cardiopulmonary exercise testing was 9.9ml/kg/min in the complications group, compared with 11.2ml/kg/min in those without complications ($p<0.01$) [309].

Complication rates were similar in a cohort of 95 patients undergoing rectal resection, although with a clearer difference in median oxygen uptake at lactate threshold: 9.4ml/kg/min in the group experiencing morbidity versus 12.7ml/kg/min in those with no morbidity [380]. Validation in a multicentre cohort of patients undergoing major colorectal surgery found a 37% morbidity rate with a median oxygen uptake at lactate threshold of 9.9mlkg/min in patients experiencing complications compared to 13ml/kg/min in those without (p=0.002) [381]. A cut-off for oxygen uptake at lactate threshold of 11.1ml/kg/min was derived in the whole cohort (n=703). Using a subgroup of 462 patients with complete data, this threshold was independently associated with an odds of complication of 7.56 (4.44-2.86), p<0.001.

Emerging evidence from a number of small randomised controlled trials supports the use of preoperative exercise to improve fitness prior to colorectal surgery (Table 1.12). However, to date, no such trial has been adequately powered to demonstrate an improvement in surgical or oncological outcome following an increase in physical activity or exercise prior to surgery. A trial aiming to address this evidence gap commenced in 2016 using hospital or home-based exercise or standard care in patients undergoing colorectal cancer resection [382]. The required sample size is 1,146 participants, powered to detect a change in the primary endpoint of postoperative morbidity at 30 days from 55% to 30%. If completed, this study would represent the largest RCT of preoperative exercise in patients undergoing elective colorectal cancer resection.

The variation in the prescribed frequency, intensity, duration and type of exercise among the trials is clear. Moreover, the use of different endpoints and variable timing of measurement have rendered unclear the potential contribution of exercise to pre and postoperative fitness. Adherence, particularly in unsupervised home-based regimens, is also difficult to ascertain, with rates of 16-94% for the RCTs conducted to date (Table 1.10).

Table 1-10 - Randomised controlled trials of preoperative exercise including patients with resectable colorectal cancer

Study (year)	Sample size (control)	Population	Exercise	Endpoint
Kim (2009) [383]	14 (7)	Benign and cancer	Home-based aerobic 4 x per week 20 – 30 mins	Change in CPET variables
Carli (2010) [384]	58 (54)	Benign and cancer	Cycling/resistance (intervention) vs. walking/breathing (control) 30-45 minutes daily	Distance covered in 6-minute walk test (preoperative)
Gillis (2014) [385]	38 (39)	Cancer	Home-based 3 x 50 mins per week aerobic and resistance	Distance covered in 6-minute walk test 8/52 after surgery

The long-term effect of a sustained change in exercise behaviour following completion of treatment has been demonstrated. In a meta-analysis of physical activity and colorectal cancer-specific mortality, an increase in 5-,10- or 15-MET hours per week post-diagnosis was associated with a reduction in cancer-specific mortality of 14, 25 and 35% respectively, suggesting a dose-response relationship [374]. Exercising for 10-MET-h is equivalent to 150 minutes of moderate intensity exercise per week, the current recommendation of the American Cancer Society for cancer survivors [386]. Compared to the more modest improvements in survival gained through the use of chemotherapy and its attendant side-effects, the potential for exercise to improve outcome throughout the continuum of cancer care is promising.

1.5.5 Adverse body composition

Obesity is a risk factor for surgical complications. The technical challenges posed by excess body fat and increased risk of pre-existing or subclinical cardiometabolic disorders influence the development of related complications. Use of BMI categories to define obesity can be misleading, as highlighted by the paradoxical association between increasing BMI and improved colorectal cancer-specific survival [387]. Patients with a high BMI secondary to a high skeletal muscle mass may contribute to the obesity paradox, while those with excess adiposity have been suggested to have sufficient reserve to tolerate the catabolic effects of colorectal cancer and its treatment [388]. In response, body composition analysis techniques were developed to assess of the distribution of adipose tissue and the quality and quantity of skeletal muscle on CT imaging.

The majority of studies have focused on the relationship between low muscle mass (sarcopenia) and survival. However, in a UK cohort of 805 patients with stage I-IV colorectal cancer undergoing elective resection, sarcopenic obesity defined as reduced L3 skeletal muscle index and BMI>30kg/m^2 was present in 10% and associated with major complications (22% vs 13%, p=0.019) and death within 30 days [389]. A similar incidence of sarcopenic obesity and association with higher rates of total and surgical complications was reported by Chen and coworkers in a cohort of 376 Chinese patients [390]. Comparable cohorts examining morbidity are limited. Some have included emergency patients with perforation and obstruction while examining infective complications [391], while others use small sample sizes [392] and non-standard methods of body composition analysis [393]. The potential to use body composition metrics as a phenotypic marker of the patient at high risk of postoperative morbidity is attractive. Studies in large, homogenous cohorts with a standardised approach to measurement and validated definitions of adverse body composition are first required. In parallel, trials examining dietary and physical activity interventions aimed at optimising body composition prior to surgery are warranted.

1.5.6 Smoking

Smoking produces toxins and reactive oxygen species that in turn induce pro-inflammatory mediators [394], impair neutrophil function [394], increase tissue degradation by altering proteinase levels [395] and decrease tissue oxygenation through the action of nitric oxide-induced vasoconstriction and carbon monoxide [396]. The impact of smoking on infective postoperative complications and impaired wound healing is well documented. Among 26,333 patients undergoing elective surgery for colorectal cancer, ex or current smokers had a 10-30% increased risk of infective or major complications, independent of age, gender, obesity, comorbidity, steroid use and ASA grade [397]. In serious complications such as anastomotic leak, the decrease in tissue oxygenation is thought to play a role in impairing anastomotic healing [398,399].

Minimising the risk of postoperative complications through smoking cessation has been explored in several trials. A Cochrane review published in 2014 demonstrated that smoking cessation has been associated with reduced complication rates, but requires intensive interventions over a minimum of 8 weeks to produce a beneficial effect on surgical outcome, limiting its utility in patients managed within time-targeted cancer pathways [400]. However, abnormalities of platelet aggregation and neutrophil function decrease within two weeks [401,402]. Following 4 weeks of abstinence, circulating levels of endothelial progenitors are also restored, suggesting bone marrow adaptation and reduced endothelial injury [403,404]. Therefore, smoking cessation at any point in the preoperative period is likely to have a positive effect at cellular level that may attenuate the risk of adverse outcome.

1.6 Preoperative evaluation

Identifying and attempting to optimise patients with adverse host factors prior to surgery is a key step in reducing perioperative complications. As standard, all patients undergo nurse-led preoperative assessment using a pro-forma approximately 2 weeks prior to surgery. High-risk anaesthetic clinics are available in some hospitals and provide a service aimed at identifying modifiable risk factors in high risk patients while improving communication of risk and shared decision-making. However, the Scottish Government 31-day Time-to-Treatment guarantee covers the period between diagnosis and surgery, significantly limiting the time available for meaningful further assessment and risk reduction strategies.

The current ACPGBI recommendations on preoperative assessment of patients undergoing colorectal cancer surgery support the use of subjective methods, morbidity risk prediction models and objective measures such as cardiopulmonary exercise testing (CPET) [170]. The American Society of Anaesthesiologists' (ASA) grade continues to be endorsed, despite its subjective and non-specific nature. Recent data from 1,400 patients undergoing major surgery demonstrated that physician-led subjective assessment had a sensitivity of 19.2% to detect patients with severely limited exercise tolerance [405]. Moreover, subjective assessment was not predictive of any short- or long-term adverse outcomes (myocardial injury, postoperative morbidity, death within 30 days or survival at 1 year).

Models such the Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity (POSSUM) are suggested for assessment of operative risk, while patients perceived as high risk are recommended to undergo cardiopulmonary exercise testing (CPET). The guidance does not address the issue of how to identify the high-risk patient or manage their adverse risk profile. Preoperative optimisation is described but limited to the treatment of anaemia and medical management of comorbidity.

1.6.1.1 ASA

The ASA classification system (Table 1.11) was introduced in 1941 to standardise assessment of physical status prior to surgery [406]. ASA alone is not predictive of perioperative risk but in conjunction with factors such as the functional capacity of the patient and type of surgery can assist in risk prediction. ASA grade is generally assigned in the immediate preoperative period by the anaesthetist, limiting its use in the identification of patients who may benefit from preoperative optimisation.

Despite widespread use, reliability was not assessed until 30 years after its inception [407]. Among 235 anaesthetists who classified 10 test patients, agreement was reached for 6. More recently, Sankar and colleagues demonstrated moderate reliability and correlation with validated comorbidity indices including the CCI and the Revised Cardiac Risk Index (RCRI) for in-hospital mortality and cardiovascular complications in a cohort of 10,864 patients [408].

Table 1-11 - ASA Physical Status Classification System.

ASA grade	Definition	Example
ASA I	A healthy patient	Healthy, non-smoking, no/minimal alcohol use
ASA II	A patient with mild systemic disease	Mild disease without functional limitation e.g. current smoker, social alcohol drinker, pregnancy, obesity, well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	One or more moderate to severe diseases e.g. poorly controlled DM or HTN, COPD, morbid obesity, active hepatitis, alcohol dependence, pacemaker, moderate reduction of ejection

		fraction, ESRD undergoing regular dialysis, MI more 3 months ago, CVA, TIA or CAD/stents
ASA IV	A patient with severe systemic disease that is a constant threat to life	Recent (within last 3 months) MI, CVA, TIA, CAD/stents, ongoing cardiac ischaemia or severe valve dysfunction, severe reduction of ejection fraction, sepsis, DIC, ARD, ESRD not undergoing regular dialysis
ASA V	A moribund patient who is not expected to survive without operation	Ruptured abdominal or thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischaemic bowel in a patient with significant cardiac pathology, multi-organ dysfunction

Abbreviations: ARD, acute respiratory distress; BMI, body mass index; CAD, coronary artery disease; CVA, cerebrovascular accident; DIC, disseminated intravascular coagulation; DM, diabetes mellitus; ESRD, end-stage renal disease; HTN, hypertension; MI, myocardial infarction; TIA, transient cerebral ischaemic attack.

1.6.1.2 POSSUM

The POSSUM scoring system was developed to predict the risk of postoperative mortality based on the degree of preoperative physiological derangement combined with the operative risk. It was later adapted for use in colorectal surgery (CR-POSSUM) [409]. Age, cardiac failure, preoperative systolic blood pressure, pulse and urea and haemoglobin values are incorporated in the physiological score. Operative severity, urgency, degree of contamination and presence of cancer determine the operative risk. Multiple prospective and retrospective studies of CR-POSSUM in patients undergoing colorectal cancer resection have highlighted wide variability in the observed (0.7%-10.7%) and predicted (2.1-13.1%) mortality rates, with both under- and overestimation evident [410]. In the original study, the CR-POSSUM model was derived in a

population where over 50% of the procedures included were minor or non-cancer surgeries. Moreover, death within 30 days of elective colorectal cancer resection in Scotland is low at 2% [411], suggesting models predicting more common outcomes such as postoperative morbidity may better inform shared decision-making between the patient and multidisciplinary team.

1.6.1.3 Cardiopulmonary exercise testing

The use of CPET to assess fitness prior to elective colorectal cancer resection is increasing [412]. CPET is an exercise stress test during which oxygen uptake and carbon dioxide production are determined using expired gas and ventilatory flow. This is achieved using a fitted face mask and is measured breath by breath throughout the test, enabling assessment as the workload incrementally rises. A CPET system consists of a metabolic cart with in-built oxygen and carbon dioxide gas analysers and a cycle ergometer or treadmill. Electromagnetically-braked ergometers are recommended and most commonly used as software can then be used to control the work rate [412].

CPET directly assesses the integrated response of the pulmonary and cardiovascular systems and indirectly, the metabolic and haematologic systems, to the stress of exercise. Its use in the preoperative evaluation of patients marked an attempt to move from subjective clinical assessment or investigations performed at rest. The increasing work rate is designed to mimic conditions of stress such as perioperative period, where oxygen demand exceeds that at rest. As a consequence, an increase in cardiac output is required.

Of the multiple parameters generated, oxygen uptake at lactate threshold is considered clinically relevant as it can be used to stratify patients according to their risk of postoperative morbidity and mortality. This marks the point of transition from aerobic to anaerobic metabolism, reflected in rising CO_2 levels as a result of increasing metabolic acidosis [413]. This shift is independent of patient effort and is influenced by factors limiting oxygen delivery. In the seminal work of Older and colleagues, values below 11 ml/min/kg in patients aged over 60 undergoing major abdominal surgery were significantly associated with

perioperative death due to cardiovascular causes [414]. Subsequent studies have derived and validated thresholds for the prediction of postoperative morbidity in patients undergoing colorectal surgery [309,380,381].

In a proportion of hospitals, all patients scheduled for colorectal resection undergo preoperative CPET. However, the initial cost of CPET systems and need for specialist staff to execute and interpret tests have prevented widespread uptake. Moreover, consensus on the identification of patients who require CPET is lacking. Defined criteria guiding access to CPET was used in only 22% of hospitals providing a CPET service in 2013 [415]. Over 75% of units used surgery type as the main indication for testing, with age, risk score or screening questionnaires used as supplementary factors to support testing in a further 5 – 16% [416].

In the recent METS study, a global randomised control trial, methods of functional assessment prior to major non-cardiac surgery were compared in over 1300 participants [405]. The primary outcome was death or myocardial infarction within 30 days of surgery. CPET was predictive of in-hospital moderate to severe morbidity but was not associated with the primary outcome. This contrasts older literature in smaller sample sizes but suggests CPET variables including peak oxygen consumption and anaerobic threshold may not be the optimal indicator of myocardial ischaemia in the perioperative period. Of note, self-reported functional capacity scores measured by the Dukes' Activity Status Index (DASI) were the only variable associated with the primary outcome of myocardial injury or death within 30 days.

1.6.1.4 Management of preoperative anaemia

Anaemia is present in up to 40% of preoperative patients with colorectal cancer [417]. It is defined in males as a haemoglobin concentration below 130 grams per litre (g/L) and in females as haemoglobin below 120 g/L [418]. Preoperative management is aimed at optimising oxygen-carrying capacity in anticipation of the perioperative increase in oxygen demand associated with the surgical stress response. Further objectives include reducing the need for blood products in the

perioperative period. Aside from the risk of transfusion reactions, concerns remain regarding the immunogenic effects of perioperative transfusion and increased risk of recurrence and cancer-related mortality [419]. In a recent randomised trial of preoperative oral versus intravenous iron in 116 patients undergoing curative colorectal cancer resection, neither treatment reduced perioperative transfusion but intravenous iron led to higher haemoglobin and ferritin levels at the time of surgery [420]. The trial also highlighted an ideal duration of 3 weeks' therapy preoperatively, suggesting iron replacement should be started as early as the time of GP referral.

1.6.1.5 Medical management of comorbidity

Control of hypertension and diabetes are recommended as part of the preoperative process [170]. Observational data supports the hypothesis that preoperative hypertension is associated with increased risk of adverse perioperative events [421]. Diastolic pressure exceeding 84mmHg is independently associated with an increased risk of death within 30 days of non-cardiac surgery, possibly mediated through the effect of perioperative hypotension on coronary perfusion during diastole. This was defined in a cohort study of over 250,000 UK participants in which systolic hypertension was not associated with postoperative mortality [421]. This contradicts previous work reporting a linear association between preoperative hypertension and silent myocardial injury as captured by continuous ECG monitoring in perioperative period [422]. This study recruited 140 participants and in association with similar sized studies published by the same group has underpinned the guidance on the management of preoperative hypertension and influenced the postponement of surgery in patients deemed to require optimisation [423].

Perioperative hyperglycaemia is recognised as a risk factor for infective complications following colorectal surgery among diabetic and non-diabetic patients [424-426]. RCTs addressing glycaemic control focus exclusively on interventions in the intra- and postoperative period in ICU and cardiac surgery populations [427]. Preoperative optimisation of glycaemic control in diabetic patients undergoing surgery is recommended by the British Diabetes Societies for Inpatient Care and The Association of Anaesthetists of Great Britain and Ireland [428]. Measurement of glycated haemoglobin (HbA1c) reflects glycaemic control

over the preceding 12 weeks. A HbA1c threshold of greater than 69mmol/mol is used to identify preoperative patients who should undergo optimisation of glycaemic control [428]. Beyond this, guidance on the goals and methods of optimising control is absent aside from recommendations to follow the local protocol for referral to the diabetes specialist team. With each 10-unit increase in postoperative glucose in the 48 hours following surgery corresponding to a 7% increase in the risk of infective complications [425], enabling better glycaemic control preoperatively through clearer guidance is an active priority.

1.6.2 Preoperative cardiovascular risk assessment

While the interplay between the shared risk factors of CVD and colorectal cancer may be implicated in the development and progression of both diseases, their co-existence holds particular relevance in the perioperative period. Major adverse cardiovascular events (MACE) occur in 1 in 33 patients following major non-cardiac surgery [429] and are associated with a four-fold increase in death within 30 days [430]. Asymptomatic myocardial injury as quantified by elevated troponin in the postoperative period is similarly related to postoperative death and independently associated with increased risk of cardiovascular complications and death within 2 years [430-433]. Since the risk applies to patients with pre-existing CVD and those who are asymptomatic, a high index of suspicion is required to identify all those at risk.

There are two key domains of preoperative cardiovascular risk assessment: the risk associated with the procedure and the individual patient risk. Intra-peritoneal surgery is associated with intermediate cardiac risk, that is a 1-5% risk of MACE [434]. Individual risk is multi-faceted but broadly constitutes the patient's clinical characteristics and functional capacity. The latter can be assessed subjectively using self-reported methods such as METs, field tests or objectively using CPET. Additional tools including cardiovascular-specific risk calculators such as the RCRI can be used as adjuncts. Perioperative interventions aimed at risk reduction have been described but have not translated to a change in practice.

1.6.2.1 Clinical assessment

History and examination in those with pre-existing stable CVD often provide sufficient information to enable cardiac risk stratification. Active cardiac conditions that preclude elective surgery include decompensated heart failure, significant arrhythmias (AF rate>100, symptomatic bradycardia and ventricular arrhythmias or severe valvular disease (severe aortic stenosis or symptomatic mitral stenosis). In patients with recent MI or the presence of unstable angina, postponement of surgery to enable further assessment is indicated.

Joint guidance issued in 2014 by the European Society of Cardiology and European Society of Anaesthesiology recommends delaying surgery in the setting of recent percutaneous stent placement until dual anti-platelet therapy (DAPT) can be stopped (30-45 days for bare metal stents and 1 year for drug-eluting stents) [435]. A delay of 1 year for colorectal cancer surgery carries the potential for interim progression and development of metastatic disease. Next generation stents have become available and require a shorter duration of DAPT, in some cases of 3 months [436]. In this situation, balancing the risk of postoperative MACE if DAPT is stopped prematurely with the potential for tumour progression if surgery is delayed requires a coordinated approach between cardiologist, anaesthetist, surgeon and patient.

1.6.2.2 Non-invasive testing of cardiac function

A 12-lead electrocardiograph (ECG) is routinely performed prior to colorectal resection. It is recommended for all patients with cardiovascular risk factors undergoing intermediate or high risk surgery[437]. The assessment of resting left ventricular function using echocardiogram is not recommended by the ESC/ESA in the preoperative period unless the risk associated with surgery is high (vascular, hepatopancreaticobiliary, upper gastrointestinal procedures). Its limited value in predicting perioperative MACE is presumed to relate to the inability to detect atherosclerotic disease [437].

Similarly, preoperative myocardial perfusion imaging with pharmacologic stress can be considered in patients subjectively determined to be incapable of performing 4 METs and undergoing high-risk surgery. The guidance on the use of CPET in patients undergoing intermediate surgery such as colorectal resection is ill-defined. The opportunity to assess the degree of exercise-induced ischaemia is attractive but is not substantiated by randomised controlled data to suggest that it influences perioperative management and merits delaying surgery [437]. Together with the evidence regarding the low sensitivity of physician's assessment of METs, the lack of clarity on how to identify and assess patients with established cardiovascular disease or related risk factors represents a clinical challenge.

1.6.2.3 Revised cardiac risk index

As the leading cause of death after non-cardiac surgery [438], identifying those at risk of cardiac complications is a prime concern for surgeons and anaesthetists in the preoperative period. To this end, the Cardiac Risk Index was originally reported in a cohort of 1,001 patients undergoing major non-cardiac surgery in 1977 [439]. It was later modified following prospective derivation (n=2,893) and validation (n=1,422) in 1999 [440]. The six independent predictors of cardiac complications included high-risk surgery (intra-peritoneal, intra-thoracic or supra-inguinal vascular surgery), history of ischaemic heart disease, congestive heart failure or cerebrovascular disease, preoperative treatment with insulin and preoperative serum creatinine >2.0mg/dL. The presence of 2 or more of these factors is associated with moderate to high cardiac complication rates. The main benefit of the Revised Cardiac Risk Index (RCRI) lies in its ease of use: the RCRI does not require a risk calculator. However, the original risk estimates are lower than the observed events in subsequent cohort studies [441,442]. Subgroup analysis suggested that the increased risk may extend to 6 months post-procedure in patients with a history of ischaemic heart disease and history of congestive heart failure [443].

1.6.2.4 Serum markers of cardiac dysfunction

Enzymes and peptides released from myocytes as a result of myocardial injury have been proposed as biomarkers of cardiac dysfunction in the perioperative period. These include troponins I and T as well as B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP). The production of the latter is triggered by increased myocardial wall stretch and is used to represent the degree of myocardial wall stress. It is independent of ischaemia and therefore a useful marker in patients with heart failure.

While monitoring of troponins for the detection of MACE in the immediate perioperative period in high risk patients, defined as inability to perform 4 METs or RCRI>2, is recognised, it is not endorsed by randomised trial data [437]. Similarly, the use of BNP and NT-proBNP in the prediction of MACE following

vascular surgery is well established, but in major abdominal surgery, there is insufficient data to support its routine use. In a mixed cohort of patients undergoing elective major surgery, of whom 464 (33%) had intra- or retroperitoneal procedures, NT-proBNP was associated with myocardial injury or death at 30 days and 1-year [405]. Interestingly, neither peak oxygen consumption nor anaerobic threshold during CPET was associated with any of the short or long-term outcomes.

1.6.2.5 Interventions to reduce perioperative cardiovascular risk

The Coronary Artery Revascularization Prophylaxis trial randomised patients with stable CVD to revascularisation (percutaneous coronary intervention or coronary artery bypass grafting) or optimal medical therapy prior to elective surgery for abdominal aortic aneurysm or peripheral arterial disease [444]. Neither intervention resulted in a significant difference in postoperative MI rates (12% vs 14% in revascularised versus control, p = 0.37) or death within 2.7 years following randomisation. It is therefore not routinely recommended in cardiac-stable patients undergoing non-cardiac surgery.

The influence of beta-blockade on perioperative MACE in non-cardiac surgery was examined in the PeriOperative ISchemic Evaluation (POISE) trial. Extended release metoprolol commenced on the day of surgery was associated with a reduction in MACE (5.8% vs. 6.9%, hazard ratio [HR] 0.84, 95% CI 0.70-0.99, p = 0.04). However, increased total mortality (3.1% vs. 2.3%, HR 1.33, p = 0.032) and ischaemic stroke (1.0% vs. 0.5%, HR 2.17, p = 0.0053) were evident in the intervention arm, potentially mediated through increased hypotension and bradycardia [445]. The use of beta-blockade in previously naïve patients in the perioperative period is therefore not recommended.

The administration of aspirin prior to major non-cardiac surgery as a method of reducing MACE was examined in the POISE-2 trial [446]. There was no difference in the primary endpoint of death or myocardial infarction within 30 days of surgery between those in the intervention arm receiving 200mg of aspirin prior to

surgery and continuing it at 100mg daily for 30 days postoperatively (7%) to those receiving placebo (7%).

1.6.2.6 Summary

The identification of patients with cardiovascular disease is largely based on clinical assessment. In patients with related risk factors but no cardiac history, the risk of perioperative adverse events is likely underestimated. The need for a biomarker of cardiovascular risk that relates to treatment outcomes and survival and could guide shared decision-making during colorectal cancer management is highly desirable. Developing low risk, effective strategies to reduce cardiac risk in the perioperative period may then be feasible.

1.6.3 Cardiovascular disease and cytotoxic therapy

Chemo- and radiotherapy form key components of colorectal cancer management. Suitability for treatment with cytotoxic therapy is influenced by host factors such as age, degree of comorbidity and fitness. Cardiovascular comorbidity is particularly relevant as some chemotherapy drugs have toxic effects on myocardial function. Radiotherapy also depends on adequate oxygen delivery to the tumour. In the presence of factors such as anaemia and atherosclerotic disease, the response to radiotherapy may be suboptimal.

1.6.3.1 Anaemia and response to radiotherapy

Several studies have reported lower rates of tumour regression [447-449] and complete pathological response [450,451] in patients with anaemia compared to those without. It has been postulated that anaemia impairs the response to NACRT by contributing to tumour hypoxia. Defined as areas of reduced oxygen tension (typically <20 mmHg) [452], tumour hypoxia results from the abnormal vasculature characteristic of malignancy and is often present as a gradient across the tumour. The presence of oxygen is required for free radical production that in turn enhances radiotherapy-induced DNA damage [453]. The 'oxygen effect' was first observed in 1909 when temporary occlusion of the cutaneous blood supply was found to diminish the skin reaction induced by radiation. However, the therapeutic implications were not clarified until 1955 when Thomlinson and Gray observed that tumour cells peripheral to the tumour blood supply were able to survive in areas of lower oxygen tension than those located more closely to the vasculature; critically, the peripherally-located hypoxic cells were resistant to radiotherapy [454].

Despite recognition of the negative influence of anaemia on outcome, the impact of treatment of anaemia prior to or during neoadjuvant therapy has not been assessed. Two randomised controlled trials examining the role of IV iron in patients with colorectal cancer have focused on the impact on perioperative transfusion requirements [420,455]. Furthermore, the role of other host factors that may influence oxygen delivery such as the presence of atherosclerotic disease in

the related arterial tree have been overlooked. This is in spite of cardiovascular disease forming the largest proportion of comorbidity in patients undergoing preoperative radiotherapy for rectal cancer [456]. The reasons for this paucity of data are likely multifactorial: exclusions of comorbid patients from clinical trials [457], greater focus on pathological factors as determinants of treatment suitability and outcome in clinical guidelines [458] and absence of formal comorbidity evaluation in routine clinical practice. Atherosclerosis affects multiple vascular beds and by means of diminished vessel pliability, restricted pulsatility and reduction of vessel diameter, could limit flow of oxygenated blood to the tumour site. This may be compounded in colorectal cancer by iron-deficiency anaemia secondary to blood loss.

1.6.3.2 Cardiovascular considerations in adjuvant chemotherapy

Cardiotoxicity, defined as a reduction in baseline left ventricular ejection fraction (LVEF) induced by cancer therapy, is a recognised complication of chemotherapy in patients with colorectal cancer. Formal diagnosis relies on echocardiography with LVEF below 50% [459]. However, any myocardial injury that results in either cardiac symptoms or signs represents cardiotoxicity. The fluoropyrimidines capecitabine and 5-fluorouracil are associated with cardiotoxic potential. The reported incidence is variable but is approximately 5%. The most common manifestations include acute coronary syndromes, heart failure and arrhythmia, although cardiogenic shock and sudden death have been reported [460].

For this reason, assessment of patients prior to chemotherapy to identify cardiovascular risk factors including smoking, hypertension, diabetes, dyslipidaemia and obesity is recommended. In patients with significant risk or established cardiovascular disease, a baseline echo to document pre-existing LV impairment is advocated [461]. Aside from identifying and treating risk factors and comorbidity, no specific guidance is available [459]. Cardioprotective strategies such as the prophylactic use of ACE-inhibitors and beta-blockers to minimise the impact on cardiac function are not routinely recommended for patients taking fluoropyrimidines.

The chemotherapy agents used to treat colorectal cancer are less cardiotoxic than those used to treat other cancers. Radiotherapy for rectal cancer carries the benefit of not involving the cardiac contours within its treatment field, a risk factor for cardiotoxicity. However, over 50% of patients treated for stage I-III colorectal cancer above the age of 65 are diagnosed with CVD within 10 years [462]. Indeed, rates of CVD in the 10 years after diagnosis of colorectal cancer are higher than in the general population. This is not solely attributable to adjuvant therapy and instead reflects the complex and interrelated nature of cardiovascular disease and colorectal cancer. To address the adverse impact of cardiovascular disease, early identification of patients at risk, careful monitoring and lifestyle modification throughout the colorectal cancer treatment pathway are required.

1.6.3.3 ECOG performance status

Administration of chemotherapy requires an assessment of the functional capacity of the patient. Tools developed specifically for this purpose include the Performance Status (PS) scale, developed in 1982 by the Eastern Cooperative Oncology Group to give an overall assessment of patient status in oncology trials (Table 1.12)[463]. The ECOG PS is comprised of descriptors of functional capacity relating to the ability to self-care, perform activities of daily living and physical tasks such as walking and work. These domains can be influenced by comorbidity and cancer. The only measurable aspect is the amount of time spent in bed or a chair in grades 2 and 3 (threshold 50%). A PS of 2 or greater is considered a relative contraindication to chemotherapy [464]. Many clinical trials restrict eligibility for inclusion to patients with a performance status of 0 or 1. The evidence base for chemotherapy in patients with higher PS grades is therefore comparatively small [465].

Table 1-12 - ECOG performance status descriptors

Grade	ECOG
0	Fully active, able to carry out all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair
5	Dead

Among 4,819 patients pooled from three RCTs of oxaliplatin-containing chemotherapy to examine the impact of age and comorbidity, 644 (20%) patients had a CCI score of 1 or more; PS 1 was present in 19% [466]. When compared with non-trial patients, a similar comorbidity profile was observed: 16-25% of 4,060 patients had a CCI of 1 or more [467]. A CCI of 1 is likely to represent low impact comorbidity such as hypertension in patients with a good PS. The paucity of data in patients with a representative spectrum of comorbidity persists. Moreover, trials focus on survival outcomes. The association between comorbidity and short-term endpoints including chemotherapy tolerance, dose modification and discontinuation is unclear.

1.6.4 Evaluation of a cardiovascular risk factor as a marker of outcome in the treatment of colorectal cancer

Patients with existing cardiovascular disease form a cohort of patients for whom tailored risk assessment is required to balance the risks associated with treatment and the benefits. As described, the methods used to evaluate such patients are largely generic and currently available strategies for optimisation are limited. The shared risk factors for cardiovascular disease and colorectal cancer highlight that a proportion of patients undergoing treatment will have subclinical evidence of cardiovascular disease in addition to those with established disease. In such patients, surgical and cytotoxic therapy may be adversely impacted by inability to tolerate the accompanying physiological stress. Moreover, surgical and cytotoxic treatment may unmask or accelerate subclinical cardiovascular disease.

As a radiological biomarker of cardiovascular disease, the burden of arterial calcification evident on CT imaging is used to identify asymptomatic patients at risk of future cardiovascular events and related mortality [363]. In recent years, it has been associated with increased morbidity following abdominal surgery. Arterial calcification is an integral component of the process of atherosclerosis and can be assessed on CT by the non-expert. An imaging-based risk factor with relevance to cardiovascular and surgical endpoints may improve existing methods of identifying patients at higher risk of complications arising from treatment. However, multiple aspects require evaluation prior to its use, including the optimal method of calcification assessment, and its relation to surgical outcome.

1.6.4.1 Aortic calcification and postoperative complications

In patients undergoing abdominal surgery, the degree of abdominal aortic calcification (AC) has been suggested to be a risk factor for the development of postoperative complications. Using the Michigan Surgical Quality Collaborative database, Harbaugh and colleagues used software assessment to determine the percentage calcification of the abdominal aortic wall in 1,180 patients who had

undergone elective general or vascular surgery [468]. Minor abdominal and unspecified procedures were most common (61%). AC was absent in 54%. The study cohort was stratified by the presence or absence of clinical CV risk factors. Those with no CV risk factors (60%) formed the study sample in which the association between aortic calcification (AC) and postoperative morbidity was derived. The mean percentage of AC among those with CV risk factors was 9.4% ± 14.5% and in those with significant AC but no CV risk factors was 20.8% ±13.6%.

Infective complications developed in 191 patients (16.2%). These were not described in further detail or classified using a validated system. AC was reported to be an independent risk factor for the development of infective complications in those with no CV risk factors. Its relevance in patients with existing CV risk factors was not reported. Furthermore, the univariate associations between recognised adverse host factors such as advanced age, high BMI and smoking status were not presented. Covariates in the multivariate analysis were restricted to preoperative albumin, preoperative sepsis, case complexity and AC. Despite the large sample size, the generalisability of the study results is unclear. The heterogenous nature of the study cohort and low rates of both AC and morbidity increase the likelihood of a type II error. The study does, however, suggest that the use of subclinical markers of cardiovascular disease may have a role to play in risk stratifying patients prior to surgery. Their relevance to morbidity, including cardiovascular-related complications, requires adequately powered studies and adjustment to minimise confounding.

1.6.4.2 Aortic calcification and anastomotic leak following gastrointestinal resection

The development of anastomotic leak (AL) following gastrointestinal resection remains an important arbiter of long-term outcome. Reported rates vary by site but are generally accepted to be up to 10% for colorectal resection [398]. Cardiovascular comorbidity is a recognised risk factor [398,469]. Several risk factors predispose to AL, although it is well-established that inadequate perfusion resulting in anastomotic ischaemia is a critical component of the pathogenesis.

Systemic factors such as global hypoperfusion can predispose to poor perfusion of the apposed bowel ends, while local factors including tension across the anastomosis can restrict adequate flow and result in ischaemia. Limitation of blood flow to the vessels supplying the anastomotic region can compound both systemic and local factors. In patients with a high burden of aortic calcification, flow limitation could be a critical aetiological element in the development of AL.

In order to evaluate the current literature on the relationship between aortic calcification and anastomotic leak following gastrointestinal (GI) resection, a systematic review was undertaken. The search strategy used is demonstrated in Appendix 1.0. The protocol was registered prospectively with PROSPERO, the international register for systematic reviews (reference CRD42018081128).

Several single-centre cohort studies have reported an association between AC and AL in patients undergoing gastrointestinal (GI) surgery. Initially reported in 246 patients undergoing oesophageal resection, an increasing degree of AC measured by visual means was associated with an increased risk of anastomotic leak (OR 2.87, 95%CI 1.22-6.72, p=0.015), independent of gender and the presence of COPD [470]. The multivariate analysis did not include relevant factors such as the presence of cardiovascular disease or other measures of preoperative comorbidity that are recognised to influence the risk of anastomotic leak. However, this finding was validated in an external cohort of 167 patients and found to be independent of age and coronary artery disease [471]. Compared to absent thoracic AC, the odds ratio associated with anastomotic leak in patients with minor calcification was 5.35 (95% CI 1.73-16.55, p=0.004) and 7.01 (95% CI 1.86-26.44, p=0.004) in patients with major calcification. Relevant to these findings is the fact that thoracic aortic branches are end arteries and AC here is more likely to impact on anastomotic healing than in the lower GI tract, where the colon and rectum are supplied by a highly collateralised network of vessels arising from the superior and inferior mesenteric arteries (Figure 1.1).

1.6.4.3 Aortic calcification and anastomotic leak following colorectal resection

The literature examining the relationship between AC and postoperative morbidity in patients undergoing colorectal resection has similarly focused on the risk of AL. Five studies [472–476] performed in predominantly European cohorts included patients with benign disease or cancer (n=556) (Table 1.13). Software was used to determine the calcium score of the aorta in four of five studies [472,474–476], in contrast to similar studies assessing thoracic AC in patients undergoing oesophageal resection, where visual methods were used [470,477–479]. It was not possible to compare the prevalence of AC among patients undergoing colorectal resection as all studies using software scores reported only the mean or median, overlooking the patients with no evidence of AC. In a cohort of 60 patients, Eveno and colleagues reported a prevalence of AC of 82% using visual scoring [473], contrasting the rate of 46% reported by Harbaugh and colleagues [468].

Table 1-13 - Characteristics of studies examining AC and AL following colorectal resection

Study & Year	Country	Study design	Cohort size	Pathology
Boersema 2016	The Netherlands	Case-control	30 cases: 105 controls	Benign & cancer
Eveno 2016	France	Cohort	60	Benign & cancer
Komen 2011	The Netherlands	Cohort	122	Benign & cancer
Norooz 2016	Iran	Cohort	100	Cancer
Pochhammer 2018	Germany	Cohort	139	Benign & cancer

The cumulative incidence of AL across these studies appeared representative, with 59 events in the 421 patients (14%) undergoing colorectal resection. However, AL rates ranged from 8 to 22% [474,475,480,481]. One study (n=135) was

excluded as the leak rate could not be clearly defined due to the case-control design [472]. The same study included only AL confirmed radiologically while in the remainder, AL was diagnosed on imaging and/or at re-operation. The potential to mis-classify patients who were diagnosed clinically with AL and treated conservatively was therefore a source of bias across all studies. Of note, four studies examining AC and AL included benign disease (e.g. diverticular disease) and emergency operations, both of which can influence complication rates [472,474,480,481]. In the single study of patients undergoing colorectal cancer resection, a leak rate of 20% was reported, higher than that generally expected [398], and this included only patients with ASA grades 1 or 2 in whom CVD risk is likely to be low [475].

It is likely that the effect of confounding factors has contributed to the differing findings of studies assessing the interplay between AC and AL in patients following colorectal surgery (Table 1.14). An increasing degree of AC was associated with AL in two studies [473,475] while a further study reported found no association between the calcium score of the aortoiliac tract and AL [472] and another two reported associations between higher calcium scores in the iliac arteries and AL [474,476].

The pre- and intraoperative factors considered in the analysis of AC and AL for each study are summarised in Table 1.15. None of the included studies reported all the recognised preoperative risk factors. Technical factors including drain use [472–474] and formation of diverting stomas [472,473,476] were each reported in three studies. There was no evidence of an association with either drain use or stoma formation and the development of AL. Urgency of surgery (emergency versus elective) was reported in four of five studies [473–476] but no association was found with AL. Anastomotic site and urgency of surgery were associated with AL on univariate analysis in one study [473]. There was no association with other intraoperative risk factors and AL in the remaining colorectal studies.

Assessment of the relationship between risk factors and AL was limited to univariate analysis in all studies. It was therefore not possible to fully assess the role of aortic calcification in colorectal AL given that adjustment for relevant confounders such as cardiovascular disease was not performed.

Other relevant methodological considerations include the use of software AC assessment on contrast-enhanced preoperative CTs obtained for purposes other than risk prediction. Commercially available calcium-scoring software is designed for use on cardiac imaging. Its use on non-gated, contrast-enhanced imaging is not recommended due to the risk of overestimation [482]. Validated visual methods adapted from coronary calcification scoring systems have been described [483–485]. Mesenteric vascular abnormalities including stenosis or occlusion are not assessed directly by either visual or software methods of AC assessment. However, asymptomatic stenosis (<50% or >50%) or occlusion has previously been reported not to correlate with adverse outcome [175]. Whether macrovascular calcification relates to microvascular disease of the mesenteric circulation and impacts colorectal perfusion also remains unclear.

Table 1-14 - Overview of studies assessing degree of aortic calcification in patients undergoing colorectal surgery.

Study Participants	Outcome parameter	Event rate (AL)	Method of calcification assessment	Vessel(s) assessed	Prevalence (%) of AC or mean calcium score (SD) in patients with AL†	Prevalence (%) of AC or mean calcium score (SD) in patients with no AL†	Relationship between AC and AL?
Eveno, 2016 n=60	Mortality Major morbidity (including AL)	13 (21.7%)	Visual grading system (0/1/2)	Abdominal aorta	0 – 0 (-)	0 – 11 (23%)	Yes
					1 – 6 (46%)	1 – 27 (57%)	
					2 – 7 (54%)	2 – 9 (19%)	
Boersema, 2016 n=135	Anastomotic leakage (AL)	30 cases: 105 controls	Software-derived calcium score	Aorta Iliac arteries	4.93 (2.93)	4.7 (3.1)	No
Komen, 2011 n=122	Anastomotic leakage	11 (9%)	Software-derived calcium score	Aorta Iliac arteries	1489 (SD 2054)	618 (SD 1248)	No (Iliac calcification associated with AL)

Study	Outcome parameter	Event rate (AL)	Method of calcification assessment	Vessel(s) assessed	Prevalence (%) of AC or mean calcium score (SD) in patients with AL†	Prevalence of AC by grade or mean calcium score (SD) in patients with no AL (%)	Relationship between AC and AL?
Norooz, 2016 n=100	Anastomotic leakage	20 (20%)	Software-derived calcium score	Descending aorta Iliac arteries	792 (SD 39)	405 (SD 45)	Yes
Pochhammer, 2018 n=139	Anastomotic leakage	15 (11%)	Software-derived calcium score (Median + range)	Infrarenal aorta Iliac arteries	250 (Range 0 – 659)	45 (Range 0 – 2572)	No (Iliac calcification associated with AL)

Abbreviations: AC aortic calcification, AL anastomotic leak, CIA common iliac artery, EIA external iliac artery, SD standard deviation, SMA superior mesenteric artery, IMA inferior mesenteric artery, LIIA left internal iliac artery, RIIA right internal iliac artery.

† Differences in prevalence of AC arises due to use of categorical approaches to AC quantification by some studies and use of continuous data in others.

Table 1-15 - Reported risk factors for AL and relationship with AL in patients undergoing gastrointestinal resection.

Study	Patient factors	Comorbidity	Tumour factors	Anastomotic site	Technical factors	Statistical analysis	Relationship with AL
Boersema	Age Gender BMI Smoking status Alcohol use	ASA CVD PVD DM Medication (anti-hypertensives, steroids, statins)	-	Left colonic and rectal	Operation type and approach Anastomotic configuration Stapled vs. hand-sewn Operating surgeon Drain use Stoma formation	Univariate	Cardiac comorbidity associated with AL on univariate analysis Results of multivariate analysis presented for calcium score only
Eveno	Age Gender BMI	ASA	-	Left colonic and rectal	Emergency/elective surgery Operation type Drain use Stoma formation Anastomosis type (e.g. colocolic) Preservation of left colic artery	Univariate	Surgery and anastomosis type associated with AL No association with patient factors or comorbidity
Komen	Age Gender	ASA CAD	NT use	Colonic or rectal	Emergency/elective surgery Operation type	Univariate	No association

	BMI Smoking status	PVD DM Medication			Operative approach Anastomotic configuration (side-to-side vs. end-to-side) Stapled vs. hand-sewn Drain use		
Norooz	Age Gender BMI Smoking status	Hypertension PVD Medication (steroids, NSAIDs)	NT use	Colonic or rectal	Emergency/elective surgery Stapled vs. handsewn anastomosis Operative time	Univariate	Male gender, DM, smoking associated with AL
Pochhammer	Age Gender BMI	ASA ≥ 3 Cardiac Renal Vascular Pulmonary	-	Rectal anastomosis	Emergency surgery Stoma formation Operative approach	Univariate	Age, renal disease, vascular disease, DM and ASA ≥ 3 associated with AL

Abbreviations: AL anastomotic leak, ASA American Society of Anaesthesiologists grade, BMI body mass index, CAD coronary arterial disease, COPD chronic obstructive pulmonary disease, DM diabetes mellitus, NT neoadjuvant therapy, NSAIDs non-steroidal anti-inflammatory drugs, PVD peripheral vascular disease, TNM tumour node metastases.

1.6.4.4 AC and outcomes following colorectal resection – evidence gaps

The current literature addressing the relationship between AC and AL following colorectal surgery is restricted to single centre studies originating mainly within western Europe. The evidence base requires prospective studies with adequate control in the analysis for common confounders but points towards a potential role as a risk factor for postoperative morbidity and in particular, AL. Comprehensive assessment of the complication spectrum (all, major, infective and non-infective complications) is required.

AC varies throughout the length of the aorta [370] with regions of increased shear stress at anatomical branching points such as the origin of mesenteric arteries and the bifurcation [486,487]. The anatomical site in addition to the burden of AC may be relevant to clinical endpoints but has yet to be considered. The limited availability of clinical methods to assess colorectal perfusion is a barrier to understanding the effects of AC on the haemodynamics of the collateral mesenteric circulation. The development of non-invasive methods of reliably assessing mesenteric flow would represent a significant advance with potential to avoid the morbidity of AL in the patients most at risk.

It is possible that AC is simply a surrogate marker of cardiovascular disease. Exploring the association between AC, cardiovascular disease and related pathophysiological processes such as systemic inflammation is required to contextualise its clinical relevance. Additional insight may also be gained from investigation of the relationship between AC and dynamic tests of cardiovascular function such as cardiopulmonary exercise testing in patients prior to GI surgery.

The findings generated from such studies would enable a more comprehensive understanding of AC in the context of adverse outcome following colorectal cancer resection.

2 Summary and Aims

2.1 Summary

In 2016, cardiovascular disease and colorectal cancer ranked among the top 10 causes of death in high-income countries. As such, a significant proportion of patients in the UK presenting with colorectal cancer have co-existing cardiovascular disease. Lifestyle risk factors including a diet rich in processed foods, cigarette smoking, obesity and physical inactivity are common to both cardiovascular disease and colorectal cancer. Both diseases also share a pro-inflammatory aetiology. However, the effect of cardiovascular disease on outcome following colorectal cancer resection has been largely unexamined.

Calcification of the coronary arteries assessed on computed tomography (CT) imaging is associated with a four-fold increase in the risk of future cardiovascular events. Aortic calcification has similar prognostic utility. Studies in patients undergoing major abdominal surgery and more recently, oesophageal and colorectal resection, have suggested a relationship between an increasing burden of aortic calcification and the development of postoperative complications. Complications, particularly infective, following colorectal cancer resection are associated with delayed or missed adjuvant therapy and later recurrence and impaired survival. Prevention is key to improving outcome.

While aortic calcification is likely a surrogate marker of cardiovascular health, its influence may be more multifaceted in patients undergoing colorectal cancer resection. Calcified mesenteric vessels may restrict flow to healing tissues at anastomotic sites resulting in higher rates of anastomotic leak. Perfusion to surgical wounds may be similarly affected, predisposing to hypoxia and infection. Resolution of infection may be limited by reduced penetrance of immune and inflammatory cells to hypoxic tissues, as well as impaired delivery of drugs such as antibiotics. This may also influence the efficacy of adjuvant chemotherapy, while restricted oxygen delivery as a result of local atheromatous disease may impair oxygen-dependent modalities such as radiotherapy. In this

manner, the degree of AC may play an important role in determining response to radiotherapy and chemotherapy. Moreover, a significant degree of vascular calcification in the vessels supplying the colon and rectum may predispose to low flow states and the development of tumour on a background of relative ischaemia. Indeed, hypoxic tumour microenvironments have been associated with increased rates of tumour dissemination and recurrence.

Widespread atheromatous disease is also associated with low-grade systemic inflammation. In this way, the presence of significant vascular calcification may drive or at least contribute to the poorer prognosis associated with colorectal cancer in the presence of systemic inflammation. Systematically examining the influence of aortic calcification on clinical and cancer-specific endpoints is warranted to investigate these potential inter-relationships. Furthermore, comorbidity is a well-recognised but poorly characterised host factor strongly linked with postoperative complications and poorer cancer outcomes. Comprehensive comorbidity indices and scores to identify patients at risk of morbidity and mortality have been described, but their translation to clinical use has been limited by the labour-intensive case-note review required. It is possible that aortic calcification, a potentially standardisable measure assessable on standard of care imaging, may outperform existing comorbidity measures and risk scores as an indicator of postoperative morbidity and inferior survival.

The preoperative period is increasingly recognised as an opportunity to optimise host characteristics such as poor cardiorespiratory fitness that are associated with postoperative morbidity. Identifying patients who may benefit remains a primary source of contention. Some advocate use of formal cardiopulmonary exercise testing, a resource with significant cost and expertise implications. The degree of aortic calcification visible on CT may relate to cardiorespiratory fitness variables, with utility as an objective radiological marker that could aid preoperative identification and trigger further investigation and intervention of high-risk patients. Assessment of calcification does not entail additional testing and associated further cost. It can be assessed on standard of care imaging and is likely to require less training and specialist staff than methods such as CPET, reducing the burden on the patient and the healthcare system. Moreover, it may have a role in selection of patients for optimisation with exercise and lifestyle modification prior to surgery, known as prehabilitation. The association between

prehabilitation and lower rates of postoperative morbidity suggests there is a potential short-term benefit that could translate to shortened hospital stay and improved patient flow, as well as enhanced functional recovery following surgery.

It should be acknowledged that the degree of aortic calcification represents a static marker of a dynamic disease process affecting the macro- and microcirculation. Currently, there is no routinely available non-invasive dynamic assessment of the abdominal vasculature to enable preoperative assessment of the mesenteric circulation. Magnetic resonance imaging with dynamic flow assessment sequences is currently being developed. The practicality of measuring mesenteric flow using this form of imaging is unknown. This represents a potentially useful tool in the preoperative assessment of selected patients with significant vascular calcification who may benefit from techniques such as stoma formation to mitigate the risk and impact of anastomotic leak. Therefore, exploratory work is urgently required.

Several methods of estimating aortic calcification have been described, including the use of software and visual approaches. However, some methods have been transposed directly from the field of cardiovascular risk prediction, where dedicated CT imaging of the coronary arteries is undertaken. The applicability and reproducibility of these methods in non-dedicated CT imaging is unclear. To date, a standardised approach to the assessment of abdominal aortic calcification in patients undergoing surgery has not been developed. This is required to enable further meaningful evaluation of the clinical relevance of AC in patients undergoing colorectal cancer resection. A simple, non-invasive radiographic marker that relates to clinical outcome and requires no additional testing or cost represents a valuable tool, meriting systematic evaluation of its potential.

2.2 Aims

The theories outlined above make a compelling case for detailed analysis of the clinical relevance of aortic calcification in the management of patients with

operable colorectal cancer. In order to investigate these hypotheses, studies were conducted with the following aims:

1. To develop a robust measure of calcification in the abdominal aorta on preoperative CT imaging with relevance to colorectal surgical outcomes

2. To compare the reproducibility of different approaches to the quantification of aortic calcification and validate its use in an external cohort

3. To assess the relationship between the burden of aortic calcification and the full spectrum of postoperative complications (infective, non-infective and major) in patients undergoing resection of colorectal cancer with curative intent

4. To investigate the survival of patients undergoing curative colorectal cancer resection in relation to the burden of calcification present preoperatively alongside established determinants including age and disease stage

5. To assess the influence of aortic calcification on tolerance and response to neoadjuvant and adjuvant therapies for colorectal cancer

6. To evaluate the tumour microenvironment in colorectal cancer for evidence of hypoxia and its association with the degree of aortic calcification present in the host

7. To establish whether cancer patients exhibiting systemic inflammation prior to surgery have higher rates of aortic calcification and to examine the potential inter-relationships in relation to site and stage of disease

8. To determine the predictive capacity of AC for short- and long-term outcome in comparison to validated comorbidity measures

9. To correlate the burden of aortic calcification with the cardiopulmonary exercise test (CPET) variables of patients undergoing CPET prior to curative resection of non-metastatic colorectal cancer

10. To evaluate a novel imaging technique to assess mesenteric flow in patients awaiting surgery as a potential preoperative investigation of patients with significant aortic calcification prior to resection.

3 A comparison of visual and software scoring methods for the evaluation of aortoiliac calcification.

3.1 Introduction

Calcification of the coronary arteries on computed tomography (CT) imaging has become a well-established indicator of the risk of future cardiovascular (CVS) events in asymptomatic patients[359,361,488]. Subsequently, several software packages have been developed to enable quantification of arterial calcification for the purpose of CVS risk prediction. The most common examples utilise the Agatston method which quantifies calcified lesions within the coronary arteries according to their number, area and peak Hounsfield units[359]. Aortic calcification (AC) visible on abdominal CT imaging has been shown to have similar prognostic value in predicting CVS risk[364–366]. It has been suggested that AC may hold predictive value beyond the cardiovascular domain, with studies demonstrating an association between increased levels of AC on the preoperative CT scan and postoperative complication rates in patients undergoing major abdominal surgery[489]. Moreover, AC is also significantly associated with all-cause mortality[490,491], suggesting it may be prognostic of both short and long-term outcomes.

Calcium scoring software was originally designed for use on ECG-gated, non-contrast dedicated coronary imaging. Therefore, its applicability when used on contrast-enhanced abdomino-pelvic CTs is uncertain. The degree of arterial contrast enhancement varies with the volume and rate of contrast injection, as well as patient factors such as BMI and cardiac output[492]. As contrast enhancement is flow-limited, patients with reduced cardiac output have slower clearance, resulting in prolonged aortic enhancement[492]. In portal-venous phase abdominal CTs, this delay can result in difficulty distinguishing calcified lesions of a similar or lower radio-opacity to the contrast agent, with consequent overestimation of calcification burden by software[482].

Visual approaches to the assessment of arterial calcification have been adapted from those used in CVS risk prediction and proposed as an alternative to

software quantification[483–485]. A visual approach enables identification of scans which are not suitable for calcium assessment due to a high degree of arterial contrast enhancement and dispenses with the need for specific scan protocol requirements such as ECG gating. In patients undergoing oesophageal cancer resection, a relationship between a higher burden of visually-assessed thoracic AC and anastomotic leak (AL) has been reported and subsequently validated[470,478].

The association between AC and AL in studies of patients undergoing colorectal resection is less clear. In a case-control study of 135 patients undergoing left-sided colorectal resection, software-derived calcium score and volume were not associated with the development of AL[472]. By contrast, the software-generated mean calcium score in the aortoiliac region was found to be significantly higher in 20 patients who developed AL in a cohort of 100 following CRC resection in a separate cohort[475]. In a further study, calcium scores in the iliac arteries assessed using software were elevated in 11 patients who developed AL in a cohort of 122 patients undergoing colorectal resection[474]. Using visual quantification, a correlation between increased AC burden and AL was reported in 60 patients undergoing left colonic or rectal anastomosis formation[481].

It is clear that the nature of the relationship between AC and AL following colorectal resection requires further evaluation. A robust, standardised and reproducible method of AC measurement is a pre-requisite to systematic exploration of the relationship between AC and outcomes following colorectal cancer treatment. Currently, no dedicated proprietary software for assessment of calcification within the abdominal aorta is available. It is also unclear whether visual and software assessment of the burden of calcification differs in terms of reproducibility. The aim of this chapter was therefore to assess the reproducibility of visual and non-proprietary software-based methods of AC quantification in a cohort of patients undergoing rectal cancer resection.

3.2 Methods

Consecutive patients who underwent anterior resection for histologically-proven rectal cancer at Glasgow Royal Infirmary (GRI) between 2008 and 2015 were identified from a prospectively-maintained colorectal cancer database. The database has been collected prospectively by consecutive clinical research fellows since 1997. Patients over the age of 18 undergoing elective or emergency colorectal cancer resection as either a curative or palliative procedure are included. Cases are identified from weekly MDT outcome letters. Basic demographics, clinical and pathological characteristics as well as treatment characteristics, postoperative complications and vital status are routinely collected. Date and cause of death between 1997 and 2008 were obtained from the National Records of Scotland. Since 2008, these data have been collected from patient electronic records via Clinical Portal. Currently, data from over 2000 patients who have undergone elective or emergency colorectal cancer resection at GRI are stored within the database. General ethical approval provided by the West of Scotland Research Ethics Committee is held for data storage. Additional ethical approval is gained for individual studies where routinely collected and additional data are used. Patients are pseudo-anonymised using a database identification number. A separate password-protected master file linking the database identification number to the patient identifier is stored securely on an NHS computer. An annual survival update is undertaken by a single Clinical Research Fellow in July of each year. Date and cause of death is recorded as 0 for alive, 1 for cancer-related death and 2 for non-cancer death. In cases where cause of death is unclear, review is undertaken by a Senior Clinical Lecturer to ensure accuracy of recording. A complete case analysis approach is taken when utilising variables from database cases i.e. variables with missing data are identified as system missing and not included in analysis.

Three methods of AC quantification were used: non-proprietary software assessment, visual assessment as described by van Rossum[470] and a semi-quantitative visual method developed in-house (Table 3.1).

Software analysis was undertaken using ImageJ [NIH ImageJ, version 1.8.0_112, https://imagej.nih.gov/ij/index.html]. Single axial slices of the preoperative staging CT were used to assess the presence of aortic calcification at four levels: the coeliac axis, superior mesenteric artery (SMA) origin, the inferior mesenteric artery (IMA) origin and the aortic bifurcation with a Hounsfield threshold segmentation technique. Following selection of the aortic level corresponding to the region of interest, a lower limit of 200 Hounsfield units (HU) was applied and the calcium area automatically calculated.

Visual analysis using the system proposed by van Rossum et al quantifies the number of calcified foci over an aortic trajectory and groups calcification in to three categories (0 = absent, 1 = <9 calcified foci or 2 = >9 calcified foci)[470]. This was applied to the abdominal aorta extending from the coeliac axis to the bifurcation. In addition, a semi-quantitative method developed by a radiologist and surgeon at our institution which assessed the burden of circumferential aortic calcification by quadrant was used. Single transverse slices from the preoperative staging CT of the aorta at the level of the SMA origin (proximal AC) and at the bifurcation (distal AC) were assessed. The inferior mesenteric artery (IMA) is routinely ligated in left-sided colonic and rectal resections; AC at the level of the IMA origin was therefore not assessed.

The proximal aorta at the level of the SMA origin was located, its circumference divided into quadrants and a point assigned for each calcified quadrant. A slice showing the distal aortic circumference in its entirety immediately proximal to the bifurcation was then selected and the same scoring methodology applied. A slice immediately distal to the bifurcation was used to score the circumference of both common iliac arteries. Examples are displayed in Figures 3.1 - 3.3.

A score of 0 to 4 was assigned according to the number of calcified quadrants visible. A maximum score of 4 was possible for the proximal aorta at the level of the SMA. For calcification at the bifurcation, a score of 0 to 4 was possible for each of the three vessels - the distal aorta immediately proximal to the bifurcation and each common iliac artery at its origin. These were summed to provide a combined distal AC score with a maximum of 12 possible.

Three factors prompted derivation of the latter technique. Firstly, to negate the effect of CT slice thickness when applying a technique which quantifies calcification burden in a volumetric manner as in the van Rossum method. The potential to overestimate calcification burden arises when using CT images acquired with lower slice thickness as foci appear to extend over a greater number of slices when compared with images of larger slice thickness. Secondly, to prioritise ease of use and time-efficiency with previous work highlighting that simple visual assessment of coronary calcification was preferred over software assessment by reporting radiologists[483]. Finally, to incorporate assessment of the iliac arteries on the basis of existing literature suggesting iliac arterial calcification held prognostic significance for AL in patients undergoing colorectal surgery[474–476].

All scans were assessed using ImageJ software by a single radiologist (DHB) with previous experience in quantification of vascular calcification. Visual assessment of the same scans was performed by a separate individual (KK). Three further raters (AG, DD, KB) assessed a random sample of 30 scans using all three methods to assess inter-observer reliability. Intra-observer reliability for each method was assessed by one individual (KK) on the same sample of scans following a two-week wash-out period. All assessors were blinded to clinicopathological characteristics at the time of scan assessment.

3.2.1 Statistical analysis

Descriptive statistics were used to summarise the baseline demographics and clinicopathological characteristics of the study cohort. Using previously employed methodology[468], total calcium software scores were stratified with reference to the median into no calcification (total score 0 mm^2 = 0), minor calcification (greater than 0 but less than median value) and major calcification (greater than median). Semi-quantitative scores (proximal and distal AC) were similarly stratified with reference to the median and grouped into no, minor and major calcification categories. Tertile values for continuous proximal and distal AC scores were also derived and found to closely align with the median values. The median value was therefore used to derive calcification categories.

Correlation between scores was assessed using Spearman's correlation coefficient. A p-value of less than 0.05 was considered significant. The intra-class correlation coefficient (ICC) was used to assess intra- and inter-observer reliability of each scoring method. ICC estimates and their 95% confidence intervals were calculated based on a mean rating (k = 4), absolute agreement, 2-way mixed-effects model. An ICC less than 0.5 was considered poor, between 0.5 and 0.75 moderate, 0.75 to 0.9 good and greater than 0.9 excellent reliability[493]. Statistical analysis was undertaken using SPSS version 24.0 (SPSS Inc, Chicago, IL).

3.3 Results

During the study period, 167 patients underwent anterior resection for rectal cancer. Of these, 16 were excluded due to lack of available CT imaging in 8 patients and imaging that was not suitable for analysis for technical reasons in 8 patients including high aortic contrast concentration (6) and aberrant vascular anatomy (2). In total, 151 scans were included.

The degree of calcification identified varied according to method of scoring (Table 3.2). Using the software-generated calcium score, 37 patients (24%) had no detectable calcification. Among those with evidence of calcification, the median calcium score was 69.4 (maximum 503). Stratifying patients with reference to the median, patients with minor calcification comprised 38% (n=57) and patients with major calcification 38% (n=57).

Using the van Rossum score, 32 patients (21%) had no calcification. The presence of minor calcification (9 or fewer foci) was evident in 66 patients (44%) and major calcification (>9 calcified foci) was present in 53 patients (35%).

Using the semi-quantitative AC score, 48 patients (32%) had no calcification. In patients with calcification, the median score was 3 (maximum 10). Patients were classified as having minor calcification if the total score was 3 or less (58 patients, 38%) and major calcification if the total score was 4 or greater (45 patients, 30%).

Correlation between scores was then assessed. A statistically significant, strong positive correlation between the software calcium score and van Rossum score (r_s 0.854, $p < 0.001$) and the software score and semi-quantitative score (r_s 0.824, $p < 0.001$) was found. When assessing the semi-quantitative and van Rossum scores, Spearman's correlation coefficient was 0.797, $p < 0.001$.

The inter-relationships between software and visual scores are shown in Tables 3.3-3.5. Significant differences in the classification of calcification were evident when comparing scores. Good agreement between the scores was evident in patients with no calcification: 94% were classified as scoring 0 when comparing the software and the semi-quantitative scores to the van Rossum score.

However, only 73% of patients classified as having no calcification using the software method were also classified as having no calcification using the semi-quantitative score. Good agreement was also seen for major calcification when comparing the software and van Rossum scores for major calcification and software and semi-quantitative scores. However, when the visual scores were directly compared in this category, only 74% were classified as having major calcification by both the semi-quantitative score and van Rossum scores.

Intra-rater reliability was performed on a sample of 30 scans analysed by one individual with an interval of 2 weeks (Table 3.6). Reliability was excellent with an ICC of 0.98 (95% CI 0.98 – 0.99, $p < 0.001$) for the software score and 0.97 (95% CI 0.96-0.98, $p<0.001$) for the semi-quantitative score. Reliability was moderate with an ICC of 0.87 (95% CI 0.72 – 0.94, $p < 0.001$) for the van Rossum score.

Inter-rater reliability was assessed on a sample of 30 scans scored by four individuals (KK/AG/KB/DD) (Table 3.7). Reliability was excellent with an ICC of 0.99 (95% CI 0.96 – 0.72, $p < 0.001$) for the software score and 0.92 (95% CI 0.87 – 0.96, $p < 0.001$) for the semi-quantitative score. Inter-rater reliability for the van Rossum score was moderate at 0.69 (95% CI 0.52 – 0.82, $p < 0.001$).

3.4 Discussion

This study examined visual and software-based methods of AC quantification in a cohort of patients undergoing rectal cancer resection. There were significant differences between scores in the classification of the degree of calcification. In particular, the proportion of patients classified as having no calcification was highest using the semi-quantitative score (32% versus 21% for van Rossum score and 24% for software score). Correlation between scores was positive and intra- and inter-rater reliability were excellent for both the semi-quantitative and software scores. The van Rossum score had satisfactory reproducibility with good agreement for intra-rater reliability and moderate inter-rater reliability. The encouraging reproducibility and ease of application of the semi-quantitative score suggest that this method is an acceptable and robust approach to the quantification of AC.

While not assessed quantitatively in this study, the time taken for analysis was less for the visual scoring methods than that required for software analysis. The latter requires selection of a slice at each aortic level which is then exported as a dicom file to a dedicated image analysis platform. Manual setting of a lower threshold of Hounsfield units is also required for each scan slice prior to calculating the calcium area at each level. This process can take several minutes. By contrast, the visual scoring methods assess calcification within the imaging system used for clinical purposes, without the need to export files or have access to dedicated calcium-scoring software. Thresholds do not need to be selected for the visual scores, further maximising efficiency.

The optimum threshold for software analysis of AC on contrast-enhanced scans is also unclear. The reference standard for non-contrast scans is 130 Hounsfield units (HU)[494]. Previous studies have used a variety of thresholds. In cohorts of patients undergoing colorectal resection, a lower threshold (LT) of 500HU was selected[472,474], while a further study used 300HU[476]. In a mixed cohort of patients who underwent abdominal surgery, Harbaugh and co-workers set a LT of 25% greater than the maximum HU within the aortic lumen[489]. In a study examining the effect of different LTs in 15 non-contrast and contrast-enhanced CT scans, Komen and colleagues reported an inverse relationship between increasing LT and calcium score[474]. The LT of 200HU used to calculate the software score in

this study is therefore unlikely to have influenced the resulting calcium scores. It is however a feature of all currently available calcium-scoring software that homogeneity in the radiodensity of opacified lesions and intra-vascular contrast in contrast-enhanced CTs can result in overestimation of calcium burden[482].

The aortic trajectory assessed by each score also requires consideration. The score devised by van Rossum visually assesses the number and distribution of calcified plaques over an aortic trajectory, in this case extending from the coeliac axis to the bifurcation. This offers the advantage of providing a global overview of atherosclerotic disease within the segment. However, problems arise with over-estimation of calcification in scans with smaller slice thickness, where calcification may be seen to project over multiple slices, falsely inflating the calcification grade. By contrast, visual scores limited to a particular region such as the semi-quantitative score mitigate the variability in slice thickness but provide only a snapshot of the overall burden. This could account for the difference in prevalence of calcification between the visual scores in this study. Previous work has demonstrated that atherosclerotic disease is most evident in regions of increased shear stress such as the bifurcation[487], suggesting the burden of calcification captured by the semi-quantitative score is likely to reflect the burden of aortic calcification in general.

Limitations of this study include the small sample of scans used to determine intra- and inter-rater reliability. However, given that the main aim was to assess the reproducibility of each method, this represents sufficient volume[493]. The use of an open source software package for calcification assessment rather than proprietary software may be considered a limitation, although previous studies have used a variety of both proprietary and non-proprietary software for calcification assessment[474–476,489]. Exclusion of 16 patients from the study population may also have resulted in a selection bias but is thought unlikely to impact on the measures of reliability being assessed.

The intra- and inter-rater reliability was excellent for both the software and semi-quantitative scores, as well as comparable. Given the need for dedicated calcium-scoring software and the effect of different LTs on the calcium score, visual assessment using the semi-quantitative score represents an efficient and reproducible approach to the assessment of aortic calcification on contrast-

enhanced CT imaging. Its relation to clinical endpoints such as anastomotic leak and other postoperative morbidity, as well as longer-term outcomes such as survival, remains to be determined.

In summary, the findings of this study support the use of semi-quantitative visual AC assessment as a robust and reproducible measure of abdominal aortic atherosclerotic burden. This technique will be applied to investigate the relationship between AC and clinical outcomes in patients undergoing curative colorectal cancer treatment in subsequent studies as outlined in the aims of this thesis.

Table 3-1 - Description of calcification scores.

	Trajectory	**No calcification (score 0)**	**Minor calcification (score 1)**	**Major calcification (score 2)**
Software score	4 aortic levels: Celiac axis SMA IMA Bifurcation	0 mm^2	≤69.4$mm^{2(a)}$	≥69.4$mm^{2(a)}$
van Rossum score (visual)	Abdominal aorta from celiac axis to bifurcation	None	≤9 calcified foci or ≤3 foci extending over three or more sections	>9 calcified foci or >3 foci extending over three or more sections
Semi-quantitative score (visual)	Proximal aorta at level of SMA Distal aorta and iliac arteries at bifurcation	No calcified aortic or iliac quadrants	≤3 calcified quadrants	≥ 4 calcified quadrants

(a) Value denotes median.

Abbreviations: SMA superior mesenteric artery, IMA inferior mesenteric artery.

Table 3-2 - Prevalence of calcification according to AC scoring method.

	No calcification (score 0)	**Minor calcification (score 1)**	**Major calcification (score 2)**
Software score	37 (24%)	57 (38%)	57 (38%)
van Rossum score	32 (21%)	66 (44%)	53 (35%)
Semi-quantitative score	48 (32%)	58 (38%)	45 (30%)

Table 3-3 - Associations between van Rossum score and software scores (Chi-square test, p<0.001).

	van Rossum = 0 n = 32 (%)	**van Rossum = 1 n = 66 (%)**	**van Rossum = 2 n = 53 (%)**
Software score = 0 (n=36)	30 (94)	6 (9)	0 (0)
Software score = 1 (n=59)	2 (6)	50 (76)	6 (11)
Software score = 2 (n=58)	0 (0)	10 (15)	47 (89)

Table 3-4 - Associations between van Rossum score and semi-quantitative score (Chi-square test, p<0.001).

	van Rossum = 0 n = 32 (%)	**van Rossum = 1 n = 66 (%)**	**van Rossum = 2 n = 53 (%)**
Semi-quantitative score = 0 (n=48)	30 (94)	18 (27)	0 (0)
Semi-quantitative score = 1 (n=58)	2 (6)	42 (64)	14 (26)
Semi-quantitative = 2 (n=57)	0 (0)	6 (9)	39 (74)

Table 3-5 - Associations between semi-quantitative AC score and software scores (Chi-square test, p<0.001).

	Semi-quantitative score = 0 (n=48)	**Semi-quantitative score = 1 (n=58)**	**Semi-quantitative score = 2 (n=57)**
Software score = 0 (n=36)	35 (73)	1 (2)	0 (0)
Software score = 1 (n=59)	13 (27)	40 (70)	5 (11)
Software score = 2 (n=58)	0 (0)	17 (28)	40 (89)

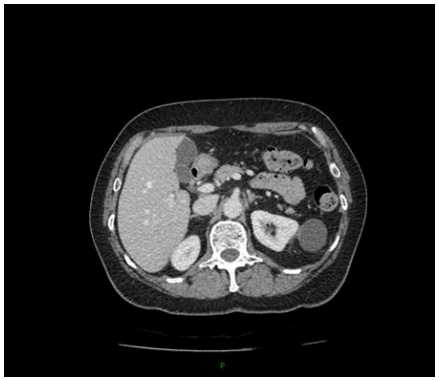
Table 3-6 - Intra-class correlation coefficient for intra-observer agreement (single measures, absolute agreement).

	Software score	**Semi-quantitative score**	**Van Rossum score**
Intra-class correlation coefficient	0.96	0.97	0.87
95% Confidence Interval	0.92 – 0.98	0.96 – 0.98	0.72 – 0.94
p-value	<0.001	<0.001	<0.001

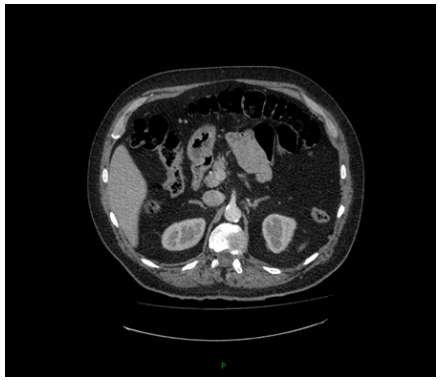
Table 3-7 - Inter-class correlation coefficient for inter-observer agreement between 4 raters (single measures, absolute agreement).

	Software score	**Semi-quantitative score**	**Van Rossum score**
Intra-class correlation coefficient	0.99	0.92	0.69
95% Confidence Interval	0.98 – 0.99	0.87 – 0.96	0.52 – 0.82
p-value	<0.001	<0.001	<0.001

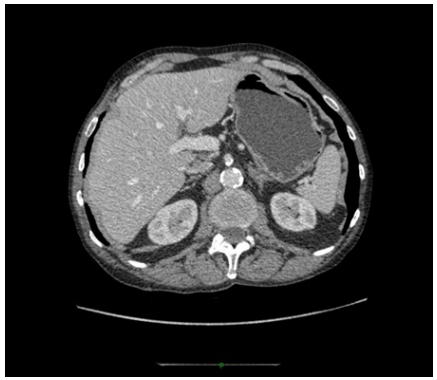
Figure 3-1 - Examples of proximal aortic calcification and corresponding semi-quantitative scores.



(a) No calcification (score 0)

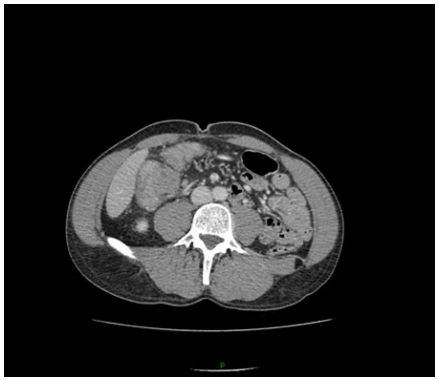


(b) Minor calcification (score 2)

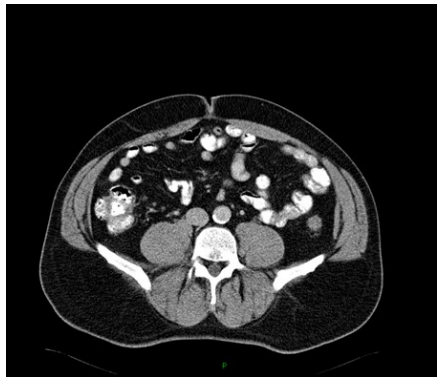


(c) Major calcification (score 4)

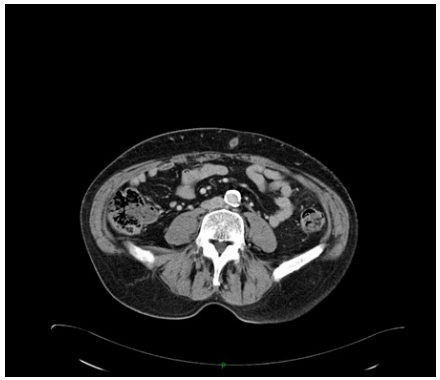
Figure 3-2 - Examples of distal aortic calcification and corresponding semi-quantitative scores.



(a) No calcification (score 0)

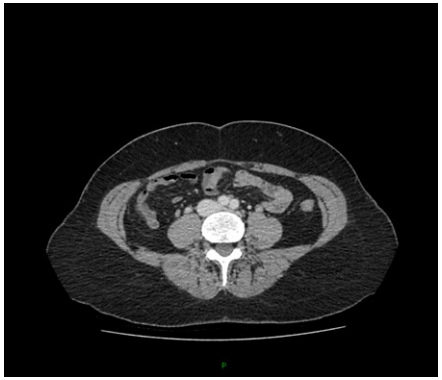


(b) Minor calcification (score 2)

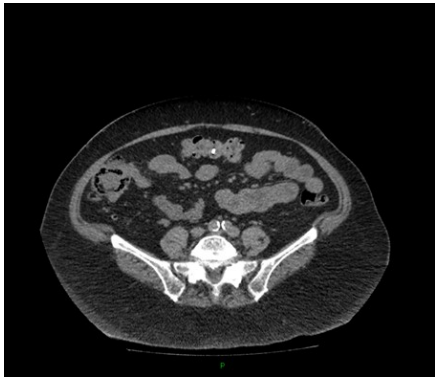


(c) Major calcification (score 4)

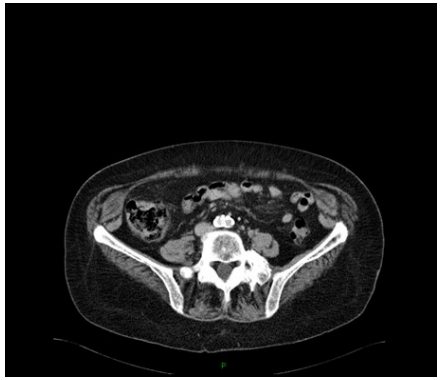
Figure 3-3 - Examples of common iliac arterial calcification and corresponding semi-quantitative scores.



(a) No calcification (score 0 for each common iliac)



(b) Minor calcification (score 2 for each common iliac)



(c) Major calcification (score 4 for each common iliac)

4 The relationship between aortic calcification and postoperative complications following colorectal cancer resection.

4.1 Introduction

Surgical resection of colorectal cancer offers the best chance of cure in stage I to III disease. Despite improvements in surgical technique and perioperative care, postoperative complications occur in up to 50% [309,380,495], predisposing to delays in adjuvant therapy, higher recurrence rates and impaired survival [311,312,496]. Early identification of patients most at risk is therefore key in reducing postoperative complications.

Host factors predictive of postoperative complications include increasing age, the presence of comorbidity, most often represented by the American Society of Anaesthesiologists (ASA) classification[497], and a history of smoking[397]. However, age is non-modifiable, ASA is subjective and lacks inter-rater reliability while smoking cessation requires a minimum period of 8 weeks to produce a beneficial effect on surgical outcome[400].

The degree of aortic calcification (AC) on the preoperative CT of patients undergoing abdominal surgery has been reported to be independently associated with higher rates of complications[489]. However, studies in patients undergoing colorectal resection have focused on anastomotic leak and included patients undergoing emergency surgery with varied underlying pathology, operative indication and technique, all of which influence complication rates [472,474-476,481,498]. Critically, previous studies have failed to assess the full spectrum of complications and do not account for the fact that AC is influenced by shear stress and varies throughout the length of the aorta[370]. Sites of anatomical branching such as the origin of mesenteric arteries and the bifurcation are disproportionately affected[486].

As previously stated, AC may help to identify those at highest risk of postoperative complications, rationalise the use of limited resources such as cardiopulmonary exercise testing and prehabilitation programmes and facilitate consideration of alternative surgical strategies such as end stoma

formation in patients at high risk of anastomotic leak. However, its relationship with clinical outcome following colorectal cancer resection first requires to be established in a cohort of sufficient size and homogeneity.

It was hypothesised that patients with AC would experience more complications following elective colorectal cancer resection, particularly infective complications, potentially as a consequence of limited ability to meet the increased tissue demand for oxygen in the perioperative period. This study therefore aimed to assess the relationship between the site and burden of abdominal AC and postoperative complications following colorectal cancer resection.

4.2 Methods

Consecutive patients undergoing potentially curative resection of colorectal cancer at Glasgow Royal Infirmary were retrospectively identified from a prospectively maintained database. This cohort was expanded from that reported in Chapter 3 to include patients with colon as well as rectal cancer operated on with curative intent between 2008 and 2016. The main inclusion criterion was the availability of pre-operative staging CT images within 6 months of surgery. Axial images of the chest, abdomen and pelvis acquired following intravenous contrast for colorectal cancer staging purposes were used. Exclusion criteria included a history of concurrent cancer, pathology other than adenocarcinoma or known metastatic disease at the time of surgery. Patients who underwent palliative or emergency procedures and those undergoing endoscopic management of polyp cancers or transanal resection of rectal cancers were also excluded.

Clinical and pathological data including perioperative complications were recorded prospectively. Pathological tumour stage was reported using the TNM staging system[213]. Complications within 30 days were graded using the Clavien-Dindo scale (I to V) according to the treatment required; complications requiring surgical intervention are regarded as major and classified as grade III and above[499]. Additionally, complications were classified as infective and non-infective[500]. Non-infective complications included persistent ileus, pulmonary embolus and cardiac events encompassing acute coronary syndrome and acute myocardial infarction. Infective complications included surgical site infections (wound or intra-abdominal (abscess/anastomotic leak)) and remote site infections (e.g. pneumonia). Anastomotic leak was defined as a communication between the intra- and extraluminal compartments arising from a defect in the intestinal wall at the anastomotic site[501]. Cases of suspected leak were confirmed on CT and verified by a radiologist. Definitions and incidence of postoperative complications are shown in Tables 4.1-4.2.

To assess the degree of AC, the novel semi-quantitative visual assessment method described in Chapter 3 was used. Calcification in the proximal and distal aorta was evaluated on three transverse images extracted from the preoperative staging CT. The proximal aorta at the level of the superior

mesenteric artery origin was located, its circumference divided into quadrants and a point assigned for each calcified quadrant. A slice showing the distal aortic circumference in its entirety immediately proximal to the bifurcation was then selected and the same scoring methodology applied. A slice immediately distal to the bifurcation was used to score the circumference of both common iliac arteries. This approach was taken to assess the influence of site and burden of calcification on clinical endpoints, taking into account the anatomical differences in arterial supply between the colon and rectum and the haemodynamic effects of shear stress at the aortic bifurcation.

All scans from the study cohort were assessed separately by two individuals (KK, CHF). All assessors were blinded to clinicopathological characteristics at the time of scan assessment.

This study was approved by the regional research ethics committee (reference number 17/WS/0200). The need for informed consent from patients was waived due to the retrospective nature of the study.

4.2.1 Statistical analysis

Descriptive statistics were used to summarise baseline characteristics. Proximal and distal AC scores were stratified with reference to the median into no calcification (total score = 0), minor calcification (greater than 0 but less than median value) and major calcification (greater than median). The Mantel-Haenszel test was used to assess associations between the degree of AC and clinicopathological characteristics. Binary logistic regression analysis was used to determine relationships between clinicopathological characteristics and postoperative complications. Variables with a p-value of less than 0.05 on univariate analysis were included in the multivariate model using a backward conditional method.

The intraclass correlation coefficient (ICC) was used to compare inter- and intra-rater reliability. ICC estimates and their 95% confident intervals were calculated based on a mean-rating, absolute-agreement, 2-way mixed-effects model. ICC was categorised as outlined in Chapter 3. A p value < 0.05 was considered

significant. Statistical analysis was performed using SPSS software (version 25.0; SPSS Inc.).

4.3 Results

Between 2008 and 2016, 820 patients underwent colorectal resection. Following exclusion of patients who had surgery for recurrent disease, local resection, emergency or palliative procedures and distant metastatic disease at time of surgery, 672 patients were included. Pre-operative CT imaging was not available in 7 patients and unsuitable for analysis for technical reasons including significant aortic contrast concentration (6 patients) and aberrant vascular anatomy (2 patients). The baseline demographic and clinicopathological characteristics of the remaining 657 patients are presented in Table 4.3.

Most patients were male (56%), aged over 65 years (63%) and ASA grade one or two (68%). The median age was 68 years (range 27-93). Current or ex-smokers comprised 55% of the study cohort. The majority of patients had pathological T-stage 3 (55%) and node-negative (65%) disease. Open surgery was undertaken in 405 patients (62%).

For intra-rater reliability, the ICC was 1.0 for proximal AC and 0.97 (95% CI 0.96 – 0.98) for distal AC. For inter-rater reliability, the ICC for proximal AC was 0.89 (95% CI 0.82 – 0.94) and for distal AC was 0.92 (95% CI 0.87 – 0.96).

When assessing proximal AC, 315 (48%) patients had no calcification and 342 (52%) had visible calcification; the median proximal AC score in those with calcification was 1 (range 1 to 4). For distal AC, 163 (25%) patients had no calcification and 494 patients (75%) had visible calcification; the median score was 4 (range 1 to 12). Proximal AC was categorised as minor in 208 patients (32%) and major in 134 patients (20%). Distal AC was minor in 234 patients (35%) and major in 260 patients (40%). Both proximal and distal AC were present in 322 patients (49%), proximal AC alone present in 20 patients (3%) and distal AC alone in 172 patients (26%).

Postoperative complications of any grade developed in 282 patients (43%), of which 60 (9%) were major complications (Clavien-Dindo grade III or higher). Infective complications developed in 185 patients (28%) (Table 4.1) while non-infective complications occurred in 131 (20%) patients (Table 4.2).

The associations between the degree of aortic calcification and clinico-pathological characteristics are shown in Table 4.5. For proximal AC, increasing age ($p<0.001$), higher ASA grade ($p<0.001$), positive smoking history ($p<0.001$), colonic tumour site ($p=0.025$) and higher T stage ($p=0.018$) were associated with an increasing burden of calcification. For distal calcification, similar associations with increasing age ($p<0.001$), ASA grade ($p<0.001$) and positive smoking history ($p<0.001$) as well as male gender ($p=0.002$) were associated with an increasing burden of calcification.

The associations between AC and postoperative complications are displayed in Table 4.6. Proximal AC was associated with the development of non-infective complications (28% vs 16%, $p=0.004$) but not major or infective complications. Distal AC was associated with the development of all complications (47% vs 34%, $p=0.015$), major complications (12% vs 5%, $p=0.015$) and non-infective complications (26% vs 14%, $p<0.001$).

Anastomotic leak occurred in 30 of 562 patients who underwent anastomosis formation (5.3%), with 22 patients (73%) requiring relaparotomy. The rate of AL was similar at 4.6% when patients who had defunctioning ileostomies (n=110) during the index procedure were excluded. The majority of patients (n=24, 80%) who developed AL had undergone anterior resection. There was no association between the presence of proximal AC and AL. A non-significant trend was noted between distal AC and AL (7% vs 3%, $p=0.077$).

Logistic regression was performed to determine the relationship between clinicopathological characteristics and all postoperative complications (Table 4.7). On univariate analysis, increasing age (OR 1.24, 95%CI 1.01 – 1.51, $p=0.040$), male gender (OR 1.54, 95% CI 1.13 – 2.11, $p=0.007$), higher ASA grade (OR 1.53, 95% CI 1.10 – 2.12, $p= 0.012$), positive smoking history (OR 1.76, 95% CI 1.29 – 2.42, $p = 0.001$), rectal tumour site (OR 1.49, 95%CI 1.08 – 2.07, $p=0.014$), open surgery (OR 2.54 (95%CI 1.54 – 2.97), $p=0.001$) and the presence of distal AC (OR 1.28, 95% CI 1.05 – 1.56, $p=0.015$) were associated with the development of postoperative complications. On multivariate analysis, factors which were independently related to postoperative complications included male gender (1.50, 95%CI 1.08 - 2.07, $p=0.015$), rectal tumour site (OR 1.51, 95%CI 1.07 – 2.12, $p=0.018$) and open surgery (OR 1.99, 95%CI 1.43 – 2.79, $p=0.001$).

Logistic regression was also performed to determine the relationship between clinicopathological characteristics and major complications (CDIII-V) (Table 4.7). On univariate analysis, positive smoking history (OR 2.56, 95%CI 1.42 – 4.64, p=0.002) and the presence of distal AC (OR 1.56, 95%CI 1.08 – 2.24, p=0.016) were related to major complications. However, on multivariate analysis, a positive smoking history was the only independent predictive of major complications (OR 2.56, 95%CI 1.42 – 4.64, p=0.002).

Finally, logistic regression was performed to determine the relationship between clinicopathological characteristics and non-infective complications (Table 4.7). On univariate analysis, increasing age (OR 1.64, 95%CI 1.27 – 2.12, p=0.001), open surgery (OR 1.54, 95%CI 1.02 – 2.32, p=0.041), an increased burden of proximal AC (OR 1.42, 95%CI 1.12 – 1.81, p=0.004) and distal AC (OR 1.53, 95%CI 1.19 – 1.98, p=0.001) were associated with the development of non-infective complications. On multivariate analysis, age was the sole independent predictor of non-infective complications (OR 1.48, 95%CI 1.12 – 1.96, p<0.001).

4.4 Discussion

Further to the derivation in Chapter 3 of a novel semi-quantitative AC score, this study confirms that it is possible to visually assess AC on routinely-obtained CT imaging and that it is reproducible across a large number of patients. Moreover, AC was related to complications following colorectal cancer resection. The presence of distal AC was more closely associated with complications than proximal AC. In contrast to the hypothesis, AC was associated with non-infective rather than infective complications. However, neither the site nor burden of AC displaced other independent markers of perioperative risk in multivariate analysis. Interestingly, no statistically significant association was observed between the degree of AC and anastomotic leak, in contrast to previous studies.

Associations between increasing levels of AC and cardiovascular risk factors including increasing age, ASA grade and smoking suggest that the method of assessment captured clinically-relevant atherosclerotic disease without the need for dedicated imaging software. The consistent association between an increasing degree of AC and non-infective complications suggests that AC may have a role to play in identifying patients at risk of non-infective complications, including cardiac-related peri-operative events.

The recent expansion of perioperative medicine has facilitated detailed assessment of high-risk patients in dedicated clinics[502]. Such review has been shown to reduce risk of complications through preoperative optimisation using multimodal prehabilitation, improved critical care use and enhanced shared decision-making between surgeons, anaesthetists and patients[503]. However, defining the high-risk patient and balancing demand with resource capacity remains challenging. To this end, characterisation of the frailty phenotype with static and dynamic markers that are readily available and are applicable by staff from differing specialties is highly desirable. At individual patient level, a high burden of AC may form part of the criteria used to trigger consideration of high-risk clinic review and further investigation of cardiac functional status including cardiopulmonary exercise testing. At organisational level, the presence of significant AC in combination with existing indicators of adverse outcome e.g. advanced age may aid decision-making regarding appropriate postoperative destination (i.e. critical care or ward level). As the population aged over 60

continues to expand[504] and the incidence of colorectal cancer continues to rise[3], radiographic biomarkers such as AC may assist with pragmatic resource utilisation in an increasingly burdened healthcare system.

Characterising the high-risk patient calls for subsequent intervention to mitigate risk. Improvements in cardiorespiratory fitness gained through preoperative exercise programmes correlate with reduced postoperative complications following CRC resection[505–507]. However, prehabilitation trial participants are often younger and less comorbid than non-trial participants[508]. Future trials and service development may be better served by including a radiographic biomarker such as AC as an inclusion criterion. Such markers are attractive as they represent personalised and cost-effective methods of identifying higher risk patients who are not detected by current subjective measures such as ASA grading.

A history of smoking was the only independent predictor of major complications in this study. Smoking accelerates atherosclerosis through vascular inflammation and endothelial dysfunction and is independently related to subclinical atherosclerosis[509]. However, smoking is strongly correlated with infective rather than non-infective complications[397]. Distal AC was also associated with increased odds of major complications but was not independent of smoking or open surgery. While smoking cessation has been associated with reduced complication rates, it requires intensive interventions over a period of several weeks, limiting its utility in patients managed within time-targeted cancer pathways[400]. Non-modifiable factors such as male gender and rectal cancer were associated with higher odds of complications while open surgery was an independent predictor of all complications. Use of minimally invasive approaches including robotic surgery for rectal cancer with its ergonomic advantages in restricted areas such as the male pelvis represents a feasible strategy to reduce postoperative complications and the attendant consequences[510].

The disparity between our results and previous studies investigating AC and anastomotic leak warrants discussion. Thoracic AC was independently associated with leak following oesophagectomy[470,478]. Unlike the mesenteric arteries, thoracic aortic branches are end arteries and AC here is more likely to impact on anastomotic healing. In colorectal surgery, the accepted leak rate is below

10%[398]. Leak rates in colorectal studies examining AC have ranged from 8 to 22% [474–476,481,498]. The leak rate was low at 5% among the 562 patients who underwent a primary anastomosis in this cohort. Moreover, all patients in this study underwent elective resection guided by the principles of oncological surgery. By comparison, four of six studies examining AC and leak included benign disease (e.g. diverticular disease) and emergency operations, both of which can influence complication rates[472,474,476,481]. One study of patients undergoing CRC resection in Iran reported a leak rate of 20% but included only patients with ASA grades 1 or 2 in whom CVD risk is likely to be low[475]. Shen and colleagues reported a leak rate of 7.8% among 423 Chinese patients undergoing rectal cancer resection and found an independent relationship between AC and leak[498]. In addition to the lower incidence of radiographically-detectable vascular calcification in Asian populations[497], the authors highlighted the low predictive value of the AC threshold derived in this cohort, suggesting the reported relationship with leak is not directly comparable to patients from Europe and North America.

It was observed that proximal AC was more prevalent among patients with colon cancer and higher T stage tumours. This was not the case for distal AC, prompting the hypothesis that the pathogenesis of colonic tumours may relate to the atherosclerotic burden of the main arterial supply. It is possible that in patients with significant proximal AC, tumours may evolve in a more hypoxic environment and in turn have more aggressive phenotype. Examination of the biology of colonic tumours in the context of the degree of AC and oncologic endpoints such as cancer-specific survival is warranted.

This study has several limitations. Its single-centre nature limits generalisability to the wider population undergoing colorectal resection. The use of visual AC quantification is subject to bias, although investigators were blinded to clinico-pathological data at the time of scan assessment. Mesenteric vascular abnormalities including stenosis or occlusion were not assessed due to previous work suggesting asymptomatic stenosis (<50% or >50%) or occlusion is not associated with adverse outcome[175].

In conclusion, radiographically-detectable aortic calcification is associated with post-operative complications following colorectal cancer resection and may aid

in identification of patients who could benefit from additional pre-operative investigation and optimisation.

Table 4-1 - Definition and incidence of infective complications

Complication	Definition	Incidence†
Infective		
Anastomotic leak	Failure of integrity of restored gastrointestinal continuity following resection and primary anastomosis including an abscess in vicinity of the anastomotic site; diagnosis confirmed on imaging or at re-operation	30 (5.3%)
Wound infection	Infection of the skin, subcutaneous and/or deeper tissues at an incision site with one or more of purulent discharge, isolated organism, at least one symptom of infection	81 (12.3%)
Respiratory tract infection	Any infectious disease of the upper or lower respiratory tract requiring treatment	45 (6.8)
Urinary tract infection	Bacterial or fungal infection on urine culture requiring treatment	12 (1.8)
Intra-abdominal collection	Collection distant to site of anastomosis requiring treatment including percutaneous drainage	17 (2.6%)

Table 4-2 - Definition and incidence of non-infective complications

Complication	Definition	Incidence†
Non-infective		
Atrial fibrillation	ECG-documented atrial fibrillation with HR >100 requiring treatment	25 (3.8%)
Acute coronary syndrome/ myocardial infarction	ECG changes consistent with myocardial ischaemia associated with raised serum troponin I levels	9 (1.4%)
Stroke	CT-confirmed features of acute ischaemic or haemorrhagic infarction	5 (0.8%)
DVT/PE	Imaging-confirmed thromboembolus requiring anti-coagulation	9 (1.4%)
Ileus	Absence of GI function associated with vomiting requiring nasogastric drainage	34 (5.2%)
Acute kidney injury	A rise in serum creatinine of 26 micromol/L or more within 48 hours or a fall in urine output to 0.5mL/kg/hr for more than 6 hours	7 (1.1%)
Surgical non-infective complication	Wound dehiscence without infection; acute herniation; high output stoma refractory to medical treatment	49 (7.5%)

† Cumulative values for incidence differ from those in results section due to more than one category of complication arising in the same patient

Table 4-3 - Baseline characteristics of patients undergoing elective resection of non-metastatic colorectal cancer between 2008 - 2016 (n = 657).

Characteristic		n (%)
Age (years)	< 65	241 (37)
	65 – 75	259 (39)
	> 75	157 (24)
Gender	Male	368 (56)
	Female	289 (44)
ASA grade	1	144 (22)
	2	303 (46)
	3	188 (29)
	4	22 (3)
BMI	< 30	428 (65)
	> 30	218 (33)
	Not recorded	11 (2)
Smoking status	Non-smoker	297 (45)
	Ex-smoker	266 (41)
	Current	94 (14)

Characteristic		n (%)
Tumour site	Colon	426 (65)
	Rectum	231 (35)
Surgical approach	Open	405 (62)
	Laparoscopic	252 (38)
T stage	0[a]	10 (1)
	1	89 (13)
	2	98 (15)
	3	358 (55)
	4	102 (16)
N stage	0	430 (65)
	1	167 (25)
	2	60 (9)

Abbreviations: ASA – American Society of Anaesthesiologists, BMI – body mass index.

[a] T Stage 0 – 10 patients with complete pathologic response following neoadjuvant chemoradiation for locally advanced rectal cancer.

Table 4-4 - Frequency of complications by type.

Complication type	Frequency n (%)
Any	280 (43)
Major (Clavien-Dindo grade III or greater)	60 (9)
Infective (total)	185 (28)
Surgical site infection	122 (19)
Remote site infection	63 (9)
Non-infective	131 (20)

Table 4-5 - Association between the degree of proximal and distal aortic calcification and clinico-pathological characteristics of patients undergoing colorectal cancer resection (Mantel-Haenszel test, significance level p<0.05).

		Proximal AC				Distal AC			
		None n=315 (48%)	Minor n=208 (32%)	Major n=134 (20%)	p-value	None n=163 (25%)	Minor n=234 (35%)	Major n=260 (40%)	p-value
Age	< 65	189 (78)	39 (16)	13 (6)	0.001	112 (47)	90 (37)	39 (16)	0.001
	65 – 75	96 (37)	103 (40)	60 (23)		42 (16)	98 (38)	119 (46)	
	> 75	30 (19)	66 (42)	61 (39)		9 (6)	46 (29)	102 (65)	
Gender	Male	168 (46)	132 (36)	68 (18)	0.889	79 (21)	125 (34)	164 (45)	0.002
	Female	147 (51)	76 (26)	66 (23)		84 (29)	109 (38)	96 (33)	
ASA grade	1 – 2	245 (55)	136 (30)	66 (15)	0.001	133 (30)	174 (39)	140 (31)	0.001
	3 - 4	70 (33)	72 (34)	68 (32)		30 (14)	60 (29)	120 (57)	
BMI[a]	< 30	200 (47)	131 (30)	97 (23)	0.112	112 (26)	124 (29)	192 (45)	0.073
	> 30	111 (51)	71 (33)	36 (16)		49 (23)	105 (48)	64 (29)	

		Proximal AC				Distal AC			
		None n=315 (48%)	Minor n=208 (32%)	Major n=134 (20%)	p-value	None n=163 (25%)	Minor n=234 (35%)	Major n=260 (40%)	p-value
Smoking status	Non-smoker	167 (56)	82 (28)	48 (16)	0.001	122 (41)	105 (35)	70 (23)	0.001
	Ex-smoker	112 (36)	92 (35)	62 (23)		28 (10)	103 (39)	135 (51)	
	Current	36 (38)	34 (36)	24 (26)		13 (14)	26 (28)	55 (58)	
Tumour site	Colon	190 (45)	142 (33)	94 (22)	0.025	100 (24)	149 (35)	177 (41)	0.144
	Rectum	125 (54)	66 (29)	40 (17)		63 (27)	85 (37)	83 (36)	
T stage[b]	1 – 2	109 (55)	55 (28)	33 (17)	0.018	52 (26)	73 (37)	72 (37)	0.327
	3 – 4	206 (45)	153 (33)	101 (22)		111 (24)	161 (35)	188 (41)	
N-stage	N0	198 (46)	139 (32)	93 (22)	0.157	108 (25)	144 (34)	178 (41)	0.498
	N≥1	117 (52)	69 (30)	41 (18)		55 (24)	90 (40)	82 (36)	

Abbreviations: AC – aortic calcification, ASA – American Society of Anaesthesiologists, BMI – body mass index.
[a] Missing cases: BMI - 11. [b] T stage 0 incorporated into T stage groups 1 – 2.

Table 4-6 - Associations between the degree of proximal and distal aortic calcification and postoperative complications in patients undergoing colorectal cancer resection (Mantel-Haenszel test, significance level p<0.05).

		Proximal AC				Distal AC			
		None n = 315 (48%)	Minor n = 208 (31%)	Major n = 134 (20%)	p-value	None n = 163 (25%)	Minor n = 234 (35%)	Major n = 260 (40%)	p-value
All complications	No	188 (60)	117 (56)	70 (52)	0.138	107 (66)	130 (56)	138 (53)	0.015
	Yes	127 (40)	91 (44)	64 (48)		56 (34)	104 (44)	122 (47)	
Major complications	No	286 (91)	192 (92)	119 (89)	0.661	155 (95)	213 (91)	229 (88)	0.015
	Yes	29 (9)	16 (8)	15 (11)		8 (5)	21 (9)	31 (12)	
Infective complications	No	225 (71)	150 (72)	97 (72)	0.821	126 (77)	161 (69)	185 (71)	0.240
	Yes	90 (29)	58 (28)	37 (28)		37 (23)	73 (31)	75 (29)	
Anastomotic leak[a]	No	264 (96)	167 (94)	101 (94)	0.334	143 (97)	191 (96)	198 (93)	0.077
	Yes	12 (4)	11 (6)	7 (6)		5 (3)	9 (4)	16 (7)	
Non-infective complications	No	264 (84)	166 (80)	96 (72)	0.004	140 (86)	195 (83)	191 (74)	0.001
	Yes	51 (16)	42 (20)	38 (28)		23 (14)	39 (17)	69 (26)	

Abbreviations: AC – aortic calcification.

Table 4-7 - Analysis of the relationship between clinico-pathological characteristics and postoperative complications in patients undergoing colorectal cancer resection (Binary logistic regression, significance level p<0.05).

	All complications				Major complications				Non-infective complications			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (<65 / 65-74/>75)	1.24 (1.01 – 1.51)	0.040	1.23 (0.99 – 1.53)	0.057	1.35 (0.96 – 1.90)	0.090	-	-	1.64 (1.27 – 2.12)	0.001	1.48 (1.12 – 1.96)	0.001
Sex (Female/Male)	1.54 (1.13 – 2.11)	0.007	1.50 (1.08 – 2.07)	0.015	1.03 (0.60 – 1.76)	0.915	-	-	1.25 (0.84 – 1.84)	0.269	-	-
ASA grade (I-II/III-IV)	1.53 (1.10 – 2.12)	0.012	1.37 (0.97 – 1.94)	0.077	1.47 (0.85 – 2.54)	0.163	-	-	1.30 (0.87 – 1.94)	0.200	-	-
Smoking history (No/Yes)	1.76 (1.29 – 2.42)	0.001	1.40 (0.89 – 2.19)	0.147	2.56 (1.42 – 4.64)	0.002	2.56 (1.42 – 4.64)	0.002	1.34 (0.91 – 1.98)	0.137	-	-

	All complications				Major complications				Non-infective complications			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
BMI (<30/ >30 kg/m2)	1.36 (0.98 – 1.89)	0.068	-	-	0.71 (0.39 – 1.29)	0.261	-	-	0.83 (0.55 – 1.26)	0.382	-	-
Tumour site (Colon/rectum)	1.49 (1.08 – 2.07)	0.014	1.51 (1.07 – 2.12)	0.018	1.16 (0.67 – 2.01)	0.589	-	-	1.08 (0.73 – 1.61)	0.692	-	-
Surgical approach (Laparoscopic/ Open)	2.54 (1.54 – 2.97)	0.001	1.99 (1.43 – 2.79)	0.001	1.17 (0.67 – 2.04)	0.575	-	-	1.54 (1.02 – 2.32)	0.041	1.47 (0.97 – 2.23)	0.071
TNM stage (I – II/III)	1.15 (0.83 – 1.59)	0.395	-	-	1.01 (0.58 – 1.77)	0.960	-	-	1.07 (0.82 – 1.59)	0.752	-	-
Proximal AC (None / Minor/ Major)	1.16 (0.95 – 1.42)	0.138	-	-	1.08 (0.77 – 1.51)	0.660	-	-	1.42 (1.12 – 1.81)	0.004	1.08 (0.80 – 1.46)	0.624
Distal AC (None / Minor / Major)	1.28 (1.05 – 1.56)	0.015	1.05 (0.83 – 1.33)	0.703	1.56 (1.08 – 2.24)	0.016	1.32 (0.90 – 1.94)	0.158	1.53 (1.19 – 1.98)	0.001	1.27 (0.96 – 1.69)	0.099

Abbreviations: AC aortic calcification, ASA American Society of Anaesthesiology, BMI body mass index, TNM Tumour, Node, Metastasis, OR odds ratio, CI confidence interval.

5 The relationship between aortic calcification and survival following colorectal cancer resection

5.1 Introduction

While major resection with curative intent is undertaken in the majority of patients with non-metastatic colorectal cancer, rates of resection vary with age from over 90% in those aged less than 75 years old to 76% of those over 75[511]. However, a proportion of patients undergoing curative surgery subsequently develop metastatic disease and die. Such treatment failures represent a complex interaction between tumour factors and adverse host characteristics.

Traditionally, tumour stage has been regarded as the main determinant of outcome. However, the influence of host characteristics is increasingly apparent. Comorbidity[323,512] and lifestyle factors such as smoking[513] and obesity[389] can affect both the short and long-term outcomes in patients with CRC. Pre-treatment evidence of systemic inflammation is also recognised as a key prognostic host factor in CRC, independent of tumour stage and comorbidity[353,354]. As such, staging systems evaluating both tumour and host characteristics may provide additional information that could be used to optimise patient selection for treatment.

Long-term survival following curative surgery is influenced by cancer-specific factors such as recurrence as well as non-cancer factors including the presence of significant comorbidity. Since calcification is a manifestation of atheromatous disease, it is likely that the clinical value of AC lies in its status as a surrogate marker of underlying cardiovascular disease. However, the observation in Chapter 4 that proximal AC was more prevalent among patients with colon cancer and associated with higher T stage tumours suggests a host-tumour interaction. It is possible that in patients with significant proximal AC, tumours may evolve in a more hypoxic environment and in turn have a more aggressive phenotype.

Previous studies have reported a higher prevalence of cardiovascular disease in patients with right-sided colorectal cancer but did not examine survival[176]. It is therefore unclear whether a significant burden of aortic calcification influences long-term outcome and if so, whether this is mediated through impaired overall survival or relates to adverse tumour characteristics and thereby influences oncological outcome.

It was hypothesised that patients with a significant degree of AC would experience inferior overall and cancer-specific survival. This study sought to characterise the relationship between the degree of AC present on CT and survival in patients undergoing curative colorectal cancer resection and aimed to explore the relationship between site and burden of AC and tumour location.

5.2 Methods

Consecutive patients who had potentially curative surgical resection of colorectal cancer between 2008 and 2016 at Glasgow Royal Infirmary were identified from a prospectively maintained database. Exclusion criteria were similar to Chapter 3, with additional exclusion of patients who died within 30 days of surgery (n=6).

Clinical and pathological data including long-term disease outcomes were extracted from patient records. Cancers of the right colon were deemed as those requiring right or extended right hemicolectomy while left including those cancers arising in the descending and sigmoid resected by left hemicolectomy or sigmoid colectomy. Splenic flexure tumours were excluded. Following colorectal cancer resection, patients were reviewed at 3 monthly intervals during year one and biannually for years two and three. Surveillance CTs of chest, abdomen and pelvis were performed annually and colonoscopy once during the three-year follow-up period. Recurrence was defined as new or recurrent disease identified during surveillance following apparently curative surgery (i.e. absence of metastatic or macroscopic disease at completion of surgery), verified histologically or radiologically after discussion in the colorectal cancer multidisciplinary team meeting. Recurrent disease during follow-up was classified as local if the first site of disease recurrence was pelvic or peritoneal and systemic if the first site of disease recurrence was distant to the primary, with the exception of the peritoneum. Cancer-specific survival was measured from the date of surgical resection until date of death from recurrent or metastatic colorectal cancer. Overall survival was measured from the date of surgical resection until date of death due to other causes. Survival data were censored in September 2019.

To assess the degree of AC, a novel semi-quantitative visual assessment method was used and scores categorised as described in the preceding chapters[514].

This study was approved by the regional research ethics committee (reference number 17/WS/0200). The need for informed consent from patients was waived due to the retrospective nature of the study.

5.2.1 Statistical analysis

Descriptive statistics were used to summarise baseline characteristics. The median and interquartile range (IQR) were used to describe the calcification scores. Associations between host and tumour factors were investigated using the Chi-squared or Mantel-Haenszel test. The relationships between clinico-pathological variables and survival were assessed using Kaplan Meier log rank and Cox regression analysis. Variables with a p-value of <0.05 on univariate analysis were entered into the multivariate model. Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA).

5.3 Results

Following exclusion of 6 patients who died within 30 days of surgery, 651 patients were included. The baseline clinical and pathological characteristics of are outlined in Table 5.1. Most patients were male (56%), 65 years or older (63%), ASA class 1 or 2 (68%) and current or ex-smokers (54%). The majority had TNM stage I or II disease (64%) based on final pathology. Tumours were moderately or well differentiated in 92% with clear surgical resection margins in 95%. Venous invasion was present in 373 cases (57%).

Median follow-up for survivors was 72 months (minimum 34 months). During follow up, 187 patients died; 101 deaths due to cancer and 86 deaths due to other causes. Deaths in relation to tumour site were assessable in 408 patients due to synchronous tumours in the remaining 16. In patients with right colon cancer, 23 cancer deaths (10%) and 47 non-cancer deaths (20%) occurred, while in patients with left colon cancer, 18 cancer-deaths (10%) and 25 non-cancer deaths (14%) were observed. For patients with rectal cancer, 39 cancer deaths (17%) and 28 non-cancer deaths (12%) occurred. Recurrence developed in 124 patients: 43 patients (7%) had local recurrence while 81 patients (12%) developed distant metastases.

The median score for proximal AC was 1 (IQR 1 – 2). The median score for distal AC was 4 (IQR 2 – 6). Proximal AC was absent in 313 patients (48%), minor in 207 patients (32%) and major in 131 patients (20%). Distal AC was absent in 163 patients (25%), minor in 232 patients (36%) and major in 256 patients (39%).

Associations between clinicopathological characteristics and AC are shown in Tables 5.2 and 5.3. Proximal AC was associated with increasing age, ASA grade, higher T-stage and smoking (all $p<0.05$). A higher proportion of patients with right colon cancer had a significant burden of proximal AC when compared with left colonic and rectal tumours ($p=0.002$). Distal AC was similarly associated with increasing age, ASA grade, smoking, right colon tumour site and female gender (all $p<0.05$).

The relationship between clinicopathological characteristics and overall survival is displayed in Table 5.4 and Figure 5.1. On univariate analysis, the following

factors were associated with overall survival in the whole cohort: a history of smoking, increasing age, ASA grade and TNM stage and an increasing burden of proximal and distal AC (all $p<0.05$). BMI over 30 kg/m^2 was associated with improved survival. On multivariate analysis, increasing age (HR 1.43, 95%CI 1.15 – 1.77, p=0.002), ASA (1.46, 95%CI 1.09 – 1.97, p=0.012), BMI (HR 0.59, 95%CI 0.43 – 0.81, p<0.001), TNM stage (HR 1.79, 95%CI 1.34 – 2.39, p=0.001) and degree of proximal AC (HR 1.37, 95%CI 1.12 – 1.68, p=0.002) were independently associated with overall survival. In patients undergoing colon cancer resection, similar factors to those derived on uni- and multivariate analysis for the whole cohort were associated with overall survival. In patients undergoing rectal cancer resection, overall survival was associated with increasing age (HR 1.64, 95%CI 1.17 – 2.30, p=0.004) and higher TNM stage (HR 1.73, 95%CI 1.07 – 2.79, p=0.026) in addition to proximal AC (HR 1.53, 95%CI 1.14 – 2.06, p=0.005) and distal AC (HR 1.57, 95%CI 1.14 – 2.16, p=0.006) on univariate analysis. These factors were not independently related to overall survival on multivariate analysis.

The relationship between clinicopathological characteristics and cancer-specific survival is displayed in Table 5.5 and Figure 5.2. In the whole cohort, higher TNM stage was associated with inferior cancer-specific survival (HR 3.33, 95% CI 2.16 – 5.14, p=0.001) while increasing BMI was associated with improved survival (HR 0.58, 95% CI 0.36 – 0.93, p=0.024). These factors remained independently associated with inferior cancer-specific survival on multivariate analysis. Following colon cancer resection, male gender, increasing TNM stage and degree of proximal AC were associated with inferior cancer-specific survival (all $p<0.05$). A higher burden of proximal AC (HR 1.63, 95%CI 1.13 – 2.36, p=0.009) was related to inferior cancer-specific survival on multivariate analysis, independent of gender (HR 2.05, 95%CI 1.07 – 3.92, p=0.030) and increasing TNM stage (HR 2.98, 95%CI 1.64 – 5.43, p<0.001). Higher TNM stage alone was associated with poorer cancer-specific survival in patients who had rectal cancer resection (HR 3.62, 95%CI 1.88 – 6.97, p<0.001).

In patients undergoing colon cancer resection, the relationship between proximal AC and cancer-specific survival was further investigated with respect to tumour site (right versus left colon) (Tables 5.6 and 5.7). In right colon cancer,

increasing TNM stage (HR 3.59, 95% CI 1.52 - 8.47, p=0.004) and degree of proximal AC (HR 1.74, 95% CI 1.03 - 2.95, p=0.039) were associated with poorer cancer-specific survival on univariate analysis and remained independently associated on multivariate analysis. This was not the case in patients with left colon cancer, in whom only TNM stage was associated with cancer-specific survival (HR 2.32, 95%CI 1.00 – 5.35, p=0.049).

5.4 Discussion

To our knowledge, this is the first study to demonstrate that the degree of aortic calcification evident on staging CT imaging is associated with inferior survival following colon cancer resection. Moreover, a greater burden of proximal AC was related to inferior cancer-specific survival in a stage-independent manner in patients with right colon cancer. These data suggest that the degree of proximal AC may be a simple radiologic marker that could identify patients at higher risk of poor long-term outcome following colon cancer resection.

Previous studies assessing the degree of AC in patients undergoing abdominal surgery have employed various methods of calcification assessment and evaluated varying aortic trajectories[471,474,475,477,479-481]. In this study, the proximal aorta at SMA level and distal aorta at the bifurcation were considered anatomically relevant regions in which to assess calcification. The SMA and its branches assume the major arterial supply following left colonic and rectal resection and remain integral to the supply of the retained colon following ligation of the ileocolic pedicle in right hemicolectomy. This may explain the fact that distal aortic calcification was not independently related to overall or cancer-specific survival in this study, regardless of tumour site. Calcification is common in areas of increased shear stress such as sites of arterial bifurcation[515]. Distal AC may therefore be more reflective of haemodynamic turbulence than the burden of atherosclerotic disease. Furthermore, as calcification progresses, arterial compliance diminishes[516,517], in turn increasing pressure in the proximal aorta. In patients with significant proximal AC, flow may be mechanically restricted, resulting in lower perfusion pressures to the corresponding colonic territory.

The perfusion characteristics of the colon and rectum have clear relevance when considering short-term outcomes such as anastomotic healing. However, the association with survival implies an impact beyond the perioperative period. Vascular calcification is an age-related phenomenon and a central pathophysiological component of atherosclerosis, a process common to diabetes, cardiovascular disease and renal impairment. The deposition of hydroxyapatite crystals in the intimal and medial components of the arterial wall results from an alteration in the phenotype of vascular smooth muscle cells stimulated by

osteogenic proteins and inflammatory cytokines such as TNF-alpha and interleukin-6[370]. The predictive value of arterial wall calcification in the setting of cardiovascular disease is striking: calcification in any coronary vascular wall can increase cardiovascular risk four-fold[363]. The relationship between a significant burden of AC and inferior overall survival demonstrated in this study is therefore not unexpected. It was, however, independent of age and comorbidity measures such as ASA grade, suggesting the effect is not simply reflective of recognised causes of vascular calcification.

The relationship between significant proximal AC and cancer-specific survival in patients undergoing right colonic resection appears to be a novel observation. In this cohort, only TNM stage, an established indicator of oncologic outcome, and the presence of proximal AC were related to cancer-specific survival on multivariate analysis. These results should be interpreted with caution as events were limited in sub-groups of colonic site. However, an association between tumour site and arterial calcification has previously been reported by Wang and co-workers, who found higher rates of cardiovascular disease and radiologically-determined calcification of the coronary arteries, thoracic and abdominal aorta in patients with right colon cancer when compared with left[176]. One potential explanation for the association between proximal AC and inferior cancer-specific survival may lie in its effect on perfusion. Lower perfusion pressures in patients with heavily calcified aortas may give rise to tumours with a greater tolerance of reduced oxygen tension. A key stimulator of epithelial to mesenchymal transition, tumour hypoxia is recognised as a characteristic which acts to increase metastatic potential[518]. In the adjuvant setting, such effects may also be compounded by reduced efficacy of oxygen-dependent cytotoxic therapies, with chemo-resistance well-recognised in hypoxic tumours[519]. Evaluation of hypoxic markers in the local tumour microenvironment would enable elucidation of the relationship between systemic calcification and the perfusion context of the tumour.

Factors such as systemic inflammation may also be implicated. The presence of a systemic inflammatory response prior to colorectal cancer resection has been associated with higher T stage tumours and is more prevalent in right colon cancer. As a key component of atherosclerotic plaque formation, the presence

of systemic inflammation is regarded as a non-traditional risk factor for future cardiovascular events. Assessment of the preoperative SIR profiles of patients in conjunction with evaluation of AC is warranted to clarify the mechanism by which AC and colon cancer interact to impact survival.

It is clear that further work is required to validate these findings and clarify the potential underlying mechanisms. However, the clinical implications also require consideration. Surgical resection of colorectal cancer is aimed at long-term cure but comorbidity, particularly cardiovascular disease, is prevalent and likely to impact both quality of life and survival in those who achieve cure[520]. The opportunity to utilise a diagnosis of cancer as a salient incentive to institute lifestyle changes that have been shown to modify such risk is therefore attractive. Indeed, increased visceral adiposity and reduced skeletal muscle density are associated with the development of major cardiac events in colorectal cancer survivors[521]. The paradoxical association between high BMI and improved survival evident in this study has previously been observed and attributed to the non-linear relationship between BMI and mortality[522], highlighting the need to use more detailed body composition metrics in survival analysis. Adherence to national guidance on nutrition and physical activity has translated to improved survival in a large prospective study of colon cancer survivors[523]. Recent work has also demonstrated reversibility of aortic stiffening in response to exercise training[524], suggesting exercise programmes instituted prior to and continued following surgery may produce durable improvements in cardiovascular risk. It is incumbent on the multidisciplinary team treating such patients to highlight the importance of instituting and maintaining lifestyle change in order to optimise long-term outcome.

This study requires interpretation in light of its limitations. While clinical and pathological data were prospectively collected, the retrospective and single-centre nature of the study warrant external validation. Future work should also aim to include measures of cardiovascular comorbidity to enable a more meaningful assessment of the relevance of AC to overall survival.

To conclude, this study demonstrates that the degree of proximal AC represents a potential indicator of inferior overall and cancer-specific survival in patients undergoing colon cancer resection. The underlying drivers are unclear, but

systemic inflammation, tumour hypoxia and comorbidity may all represent relevant contributors. These factors warrant further study, as does the possibility that patients with significant cardiovascular comorbidity may be less likely to receive or complete adjuvant chemotherapy, resulting in the potential for higher rates of recurrence and impaired survival in this group. Finally, it is possible that AC may have further clinical value in highlighting patients who could benefit from lifestyle interventions to improve survival.

Table 5-1 - Baseline characteristics of patients undergoing resection of non-metastatic colorectal cancer between 2008 - 2016 (n=651).

Variable		N (%)
Age	<65	240 (37)
	65 - 75	257 (39)
	>75	154 (24)
Gender	Male	363 (56)
	Female	288 (44)
ASA grade	1	144 (22)
	2	301 (46)
	3	187 (29)
	4	19 (3)
BMI[a]	<30	424 (65)
	>30	217 (33)
Smoking history	No	302 (46)
	Yes	349 (54)
Tumour site	Colon	424 (65)
	Rectum	227 (35)

Variable		N (%)
TNM stage	0[b]	10 (1)
	I	156 (24)
	II	260 (40)
	III	225 (35)
Differentiation[a]	Well/moderate	596 (92)
	Poor	43 (6)
Margin involvement	Absent	616 (95)
	Present	35 (5)
Venous invasion	Absent	278 (43)
	Present	373 (57)

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI).

[a] Missing cases – BMI (n=10), differentiation (n=12)

[b] TNM stage 0 – 10 cases of rectal cancer with complete pathological response following neoadjuvant chemoradiation.

Table 5-2 - Associations between the degree of proximal AC and clinico-pathological characteristics (n=651).

Characteristic		No calcification n = 313 (%)	Minor calcification n = 207 (%)	Major calcification n = 131 (%)	p-value
Age	< 65	189 (79)	39 (16)	12 (5)	0.001
	65 – 75	95 (37)	103 (40)	59 (23)	
	> 75	29 (19)	65 (42)	60 (39)	
Gender	Male	166 (46)	131 (36)	66 (18)	0.880
	Female	147 (51)	76 (26)	65 (23)	
ASA grade	1 – 2	243 (54)	136 (31)	66 (15)	0.001
	3 - 4	70 (34)	71 (35)	65 (31)	
BMI[a]	< 30	199 (47)	131 (31)	94 (22)	0.151
	> 30	110 (51)	71 (23)	36 (17)	
Smoking status	Non-smoker	170 (56)	85 (28)	47 (16)	0.001
	Current or ex-smoker	143 (41)	122 (35)	84 (24)	
Tumour site [b]	Right	92 (40)	81 (35)	58 (25)	0.002
	Left	98 (52)	59 (31)	33 (17)	
	Rectal	123 (54)	66 (29)	38 (17)	

Characteristic		No calcification n = 313 (%)	Minor calcification n = 207 (%)	Major calcification n = 131 (%)	p-value
T-stage	1 – 2	107 (55)	55 (28)	33 (17)	0.032
	3 – 4	206 (45)	152 (33)	98 (22)	
N-stage	Node-negative	197 (46)	139 (33)	91 (21)	0.156
	Node-positive	116 (52)	68 (30)	40 (18)	
Venous invasion	Present	134 (48)	94 (34)	50 (18)	0.522
	Absent	179 (48)	113 (30)	81 (22)	
Vital status	Alive	244 (53)	148 (31)	72 (16)	0.001
	Cancer-death	39 (45)	24 (28)	23 (27)	
	Non-cancer death	26 (26)	39 (39)	36 (35)	
Recurrence	Nil	254 (48)	171 (32)	102 (20)	0.539
	Local	22 (51)	10 (23)	11 (26)	
	Distant	37 (46)	26 (32)	18 (22)	

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI). [a] Missing cases = 10 [b] n = 648 (3 resections for synchronous tumours involving right and left colon)

Table 5-3 - Association between the degree of distal AC and clinico-pathological characteristics (n=651).

Characteristic		No calcification n = 163 (%)	Minor calcification n = 232 (%)	Major calcification n = 256 (%)	p-value
Age	< 65	112 (47)	90 (38)	38 (15)	0.001
	65 – 75	42 (16)	97 (38)	118 (46)	
	> 75	9 (6)	45 (29)	100 (65)	
Gender	Male	84 (29)	108 (38)	96 (33)	0.004
	Female	79 (22)	124 (34)	160 (44)	
ASA grade	1 – 2	133 (30)	174 (39)	138 (31)	0.001
	3 - 4	30 (15)	58 (28)	118 (57)	
BMI[a]	< 30	112 (26)	123 (29)	189 (45)	0.076
	> 30	49 (23)	105 (48)	63 (29)	
Smoking status	Non-smoker	122 (41)	104 (35)	69 (24)	0.001
	Current or Ex-smoker	41 (11)	125 (36)	183 (52)	
Tumour site [b]	Right	48 (21)	80 (35)	103 (45)	0.027
	Left	52 (27)	68 (36)	70 (37)	
	Rectal	63 (28)	84 (37)	80 (35)	

Characteristic		No calcification n = 163 (%)	Minor calcification n = 232 (%)	Major calcification n = 256 (%)	p-value
T-stage	1 – 2	52 (27)	73 (37)	70 (36)	0.286
	3 – 4	111 (24)	159 (35)	186 (41)	
N-stage	Node-negative	108 (25)	143 (34)	176 (41)	0.465
	Node-positive	55 (24)	89 (40)	80 (36)	
Venous invasion	Present	68 (25)	106 (38)	104 (37)	0.710
	Absent	95 (25)	126 (34)	152 (41)	
Vital status	Alive	133 (29)	167 (36)	164 (35)	0.001
	Cancer-death	18 (20)	34 (40)	34 (40)	
	Non-cancer death	12 (12)	31 (31)	58 (57)	
Recurrence	Nil	138 (26)	179 (34)	210 (40)	0.574
	Local	11 (26)	16 (37)	16 (37)	
	Distant	14 (17)	37 (46)	30 (37)	

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI). [a] Missing cases: BMI 10. [b] n = 648 (3 resections for synchronous tumours involving right and left colon)

Table 5-4 - Cox regression analysis of factors associated with overall survival.

	All resections (n=651)				Colonic resections (n=424)				Rectal resections (n=227)			
	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Age (<65/65-75/>75)	1.71 (1.41 - 2.08)	0.001	1.43 (1.15 - 1.77)	0.002	1.80 (1.41 - 2.30)	0.001	1.52 (1.15 - 2.02)	0.004	1.64 (1.17 - 2.30)	0.004	1.42 (0.98 - 2.06)	0.061
Sex (Female/male)	1.34 (1.00 - 1.80)	0.053	-	-	1.53 (1.06 - 2.22)	0.025	1.42 (0.97 - 2.08)	0.075	1.05 (0.64 - 1.71)	0.858	-	-
ASA grade (I-II/III-IV)	1.82 (1.36 - 2.42)	0.001	1.46 (1.09 - 1.97)	0.012	1.92 (1.34 - 2.75)	0.001	1.56 (1.07 - 2.29)	0.021	1.61 (0.97 - 2.66)	0.066	-	-
BMI (<30/>30)	0.56 (0.41 - 0.78)	0.001	0.59 (0.43 - 0.81)	0.001	0.46 (0.30 - 0.68)	0.001	0.52 (0.35 - 0.77)	0.001	0.82 (0.48 - 1.40)	0.471	-	-
Smoking history (No/yes)	1.43 (1.07 - 1.92)	0.017	1.31 (0.96 - 1.79)	0.091	1.51 (1.05 -2.18)	0.027	1.42 (0.95 - 2.10)	0.086	1.29 (0.79 - 2.11)	0.310	-	-

	All resections (n=651)				Colonic resections (n=424)				Rectal resections (n=227)			
	Univariate HR (95% CI)	**p-value**	**Multivariate HR (95% CI)**	**p-value**	**Univariate HR (95% CI)**	**p-value**	**Multivariate HR (95% CI)**	**p-value**	**Univariate HR (95% CI)**	**p-value**	**Multivariate HR (95% CI)**	**p-value**
TNM stage (I-II/III)	1.72 (1.29 - 2.29)	0.001	1.79 (1.34 - 2.39)	0.001	1.71 (1.19 - 2.45)	0.003	1.89 (1.32 - 2.73)	0.001	1.73 (1.07 - 2.79)	0.026	1.57 (0.97 - 2.56)	0.066
Proximal AC (None/minor/major)	1.66 (1.39 - 1.98)	0.001	1.37 (1.12 - 1.68)	0.002	1.72 (1.18 - 2.16)	0.001	1.57 (1.18 - 2.10)	0.002	1.53 (1.14 - 2.06)	0.005	1.20 (0.84 - 1.72)	0.319
Distal AC (None/minor/major)	1.47 (1.21 - 1.77)	0.001	0.93 (0.73 - 1.19)	0.575	1.41 (1.11 - 1.79)	0.005	0.75 (0.54 - 1.03)	0.076	1.57 (1.14 - 2.16)	0.006	1.34 (0.95 - 1.91)	0.095

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI).

Table 5-5 - Cox regression analysis of factors associated with cancer-specific survival.

	All resections (n=651)				Colonic resections (n=424)				Rectal resections (n=227)			
	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Age (<65/65-75/>75)	1.23 (0.93 -1.63)	0.145	-	-	1.26 (0.87 - 1.83)	0.230	-	-	1.37 (0.87 - 2.14)	0.171	-	-
Sex (Female/male)	1.41 (0.91 - 2.20)	0.120	-	-	2.31 (1.22 - 4.38)	0.010	2.05 (1.07 – 3.92)	0.030	0.78 (0.42 - 1.46)	0.435	-	-
ASA (I-II/III-IV)	1.03 (0.66 - 1.63)	0.887	-	-	1.08 (0.60 - 1.96)	0.801	-	-	1.08 (0.53 - 2.21)	0.836	-	-
BMI (<30/>30)	0.58 (0.36 - 0.93)	0.024	0.59 (0.37 - 0.94)	0.026	0.47 (0.25 - 0.89)	0.020	0.69 (0.36 – 1.33)	0.267	0.85 (0.42 - 1.70)	0.640	-	-
Smoking history (No/yes)	0.95 (0.63 - 1.46)	0.827	-	-	1.02 (0.57 - 1.80)	0.952	-	-	0.86 (0.46 - 1.61)	0.627	-	-

	All resections (n=651)				Colonic resections (n=424)				Rectal resections (n=227)			
	Univariate HR (95% CI)	**p-value**	**Multivariate HR (95% CI)**	**p-value**	**Univariate HR (95% CI)**	**p-value**	**Multivariate HR (95% CI)**	**p-value**	**Univariate HR (95% CI)**	**p-value**	**Multivariate HR (95% CI)**	**p-value**
TNM stage (I-II/III)	3.33 (2.16 - 5.14)	0.001	3.32 (2.15 - 5.12)	0.001	3.11 (1.73 - 5.56)	0.001	2.98 (1.64 - 5.43)	0.001	3.62 (1.88 - 6.97)	0.001	-	-
Degree of proximal AC (None/minor/major)	1.28 (0.98 - 1.67)	0.073	-	-	1.44 (1.00 - 2.06)	0.049	1.63 (1.13 - 2.36)	0.009	1.13 (0.75 - 1.70)	0.577	-	-
Degree of distal AC (None/minor/major)	1.13 (0.86 - 1.48)	0.371	-	-	1.09 (0.76 - 1.56)	0.657	-	-	1.24 (0.83 - 1.87)	0.285	-	-

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI).

Table 5-6 - Cox regression analysis of factors associated with cancer-specific survival in patients with right colon cancer (n=231).

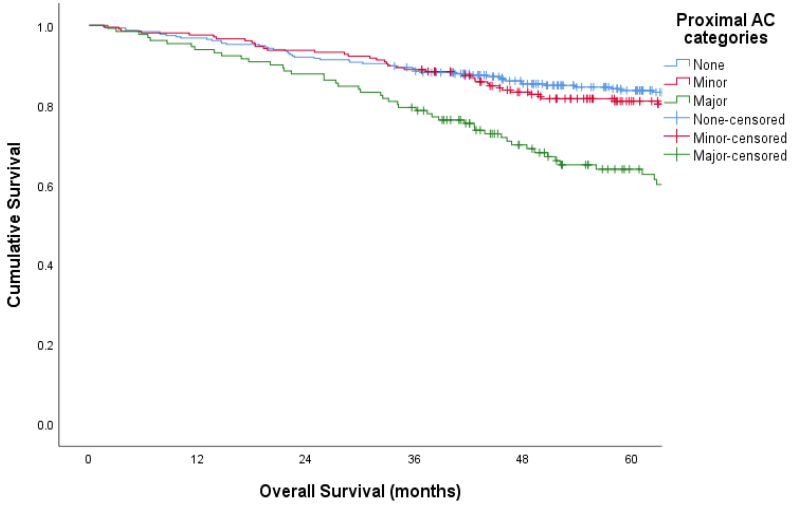
	Univariate HR (95% CI)	**p-value**	**Multivariate HR (95% CI)**	**p-value**
Age (<65/65-75/>75)	1.03 (0.60 - 1.77)	0.917	-	-
Sex (Female/male)	2.10 (0.86 - 5.11)	0.101	-	-
ASA (I-II/III-IV)	1.70 (0.75 - 3.86)	0.204	-	-
Smoking history (No/yes)	0.83 (0.37 - 1.88)	0.657	-	-
TNM stage (I-II/III)	3.59 (1.52 - 8.47)	0.004	4.09 (1.72 - 9.71)	0.001
Degree of proximal AC (None/minor/major)	1.74 (1.03 - 2.95)	0.039	1.92 (1.14 – 3.24)	0.014
Degree of distal AC (None/minor/major)	1.16 (0.68 – 1.99)	0.596	-	-

Table 5-7 - Cox regression analysis of factors associated with cancer-specific survival in in patients with left colon cancer (n=190).

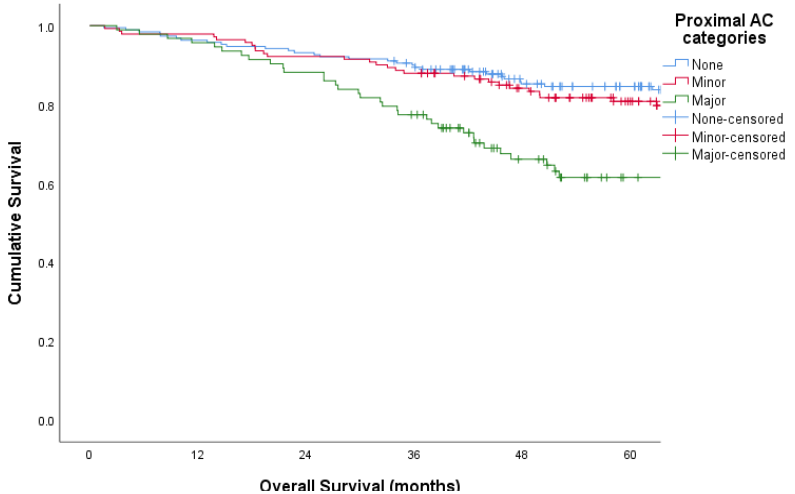
	Univariate HR (95% CI)	**p-value**	**Multivariate HR (95% CI)**	**p-value**
Age (<65/65-75/>75)	1.35 (0.79 - 2.31)	0.278	-	-
Sex (Female/male)	2.29 (0.89 - 5.84)	0.084	-	-
ASA (I-II/III-IV)	0.46 (0.16 - 1.37)	0.164	-	-
Smoking history (No/yes)	1.05 (0.45 - 2.42)	0.914	-	-
TNM stage(I-II/III)	2.32 (1.00 - 5.35)	0.049	-	-
Degree of proximal AC (None/minor/major)	1.07 (0.62 - 1.86)	0.798	-	-
Degree of distal AC (None/minor/major)	0.90 (0.54 – 1.51)	0.696	-	-

Figure 5-1 - Kaplan-Meier curves demonstrating the relationship between proximal AC and overall survival.

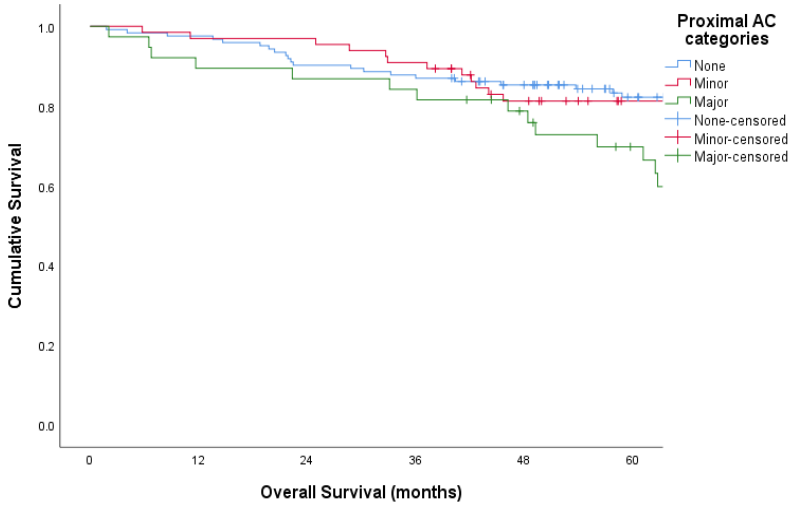
a) Whole cohort – proximal AC and OS (p<0.001)



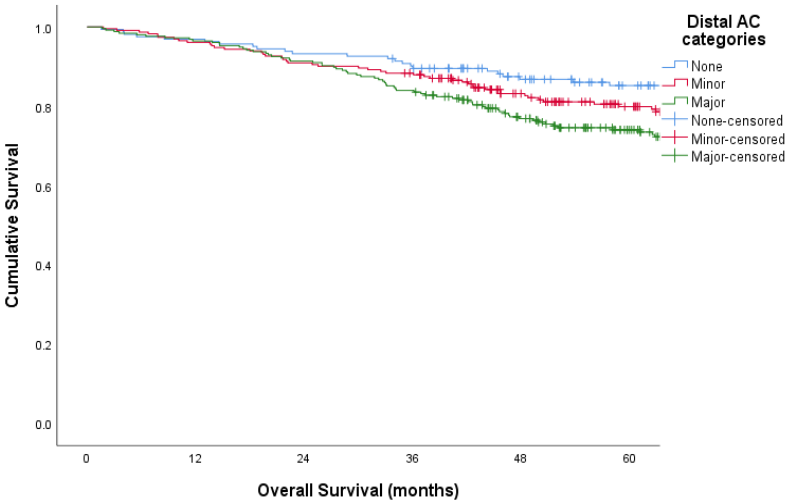
b) Colon cancer – proximal AC and OS (p<0.001)



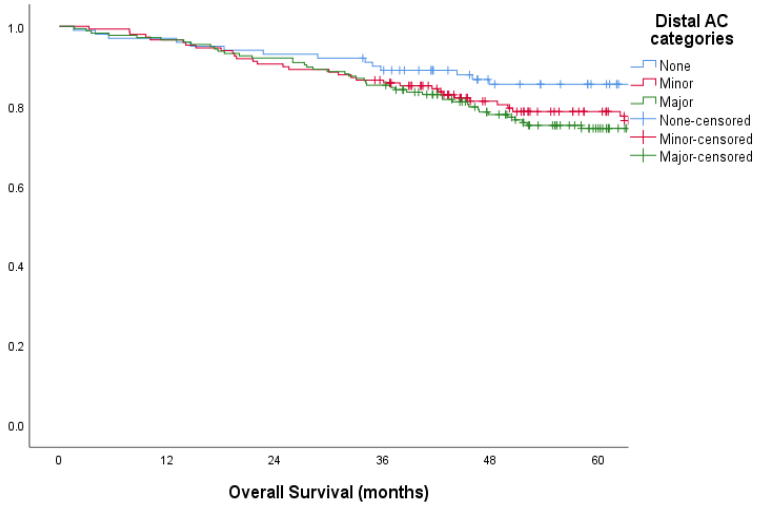
c) Rectal cancer – proximal AC and OS (p=0.017)



d) Whole cohort – distal AC and OS (p<0.001)



e) Colon cancer – distal AC and OS (p=0.013)



f) Rectal cancer – distal AC and OS (p=0.014)

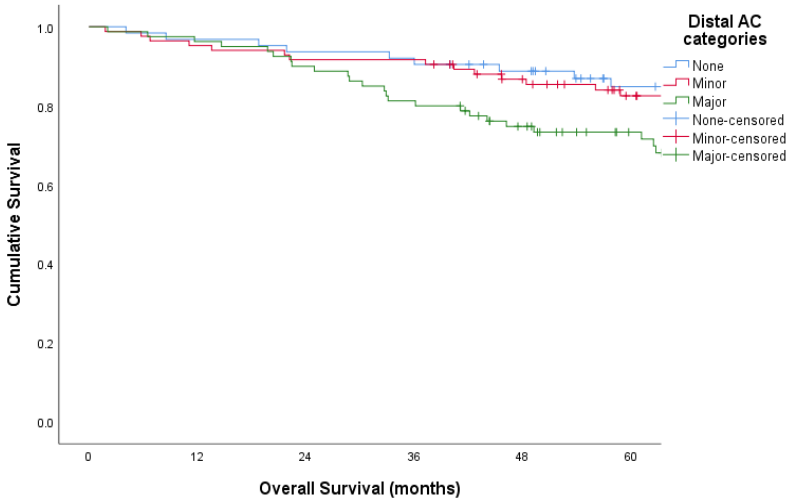
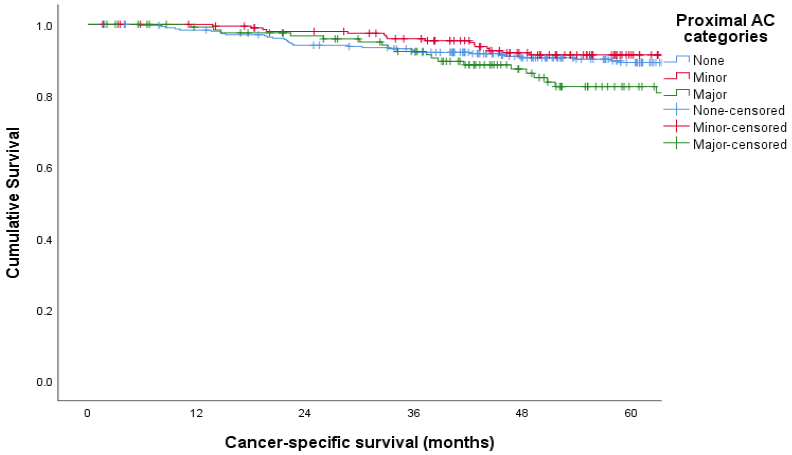
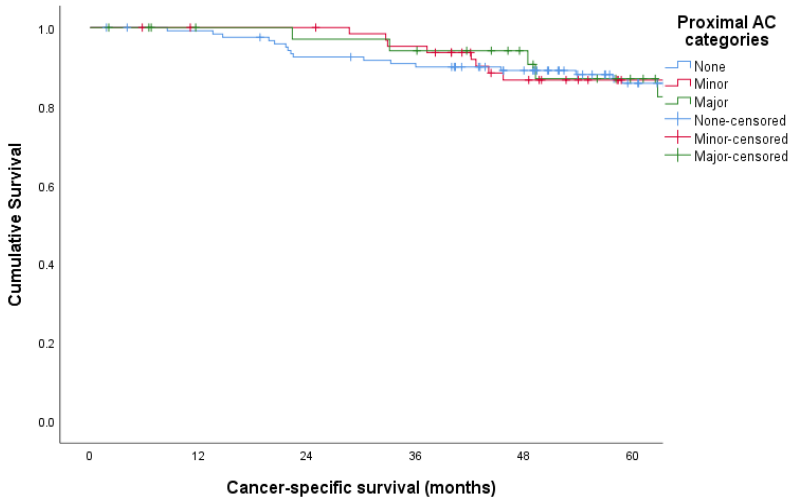


Figure 5-3 - Kaplan-Meier curves demonstrating the relationship between proximal AC and cancer-specific survival.

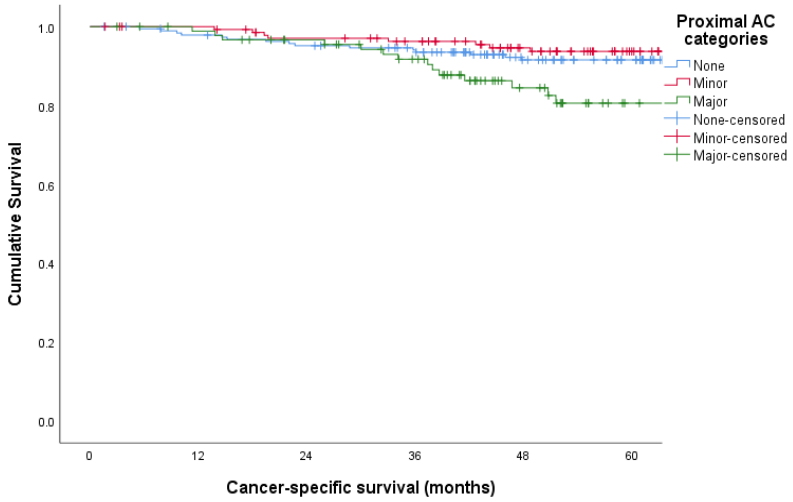
a) Whole cohort – proximal AC and CSS (p=0.067)



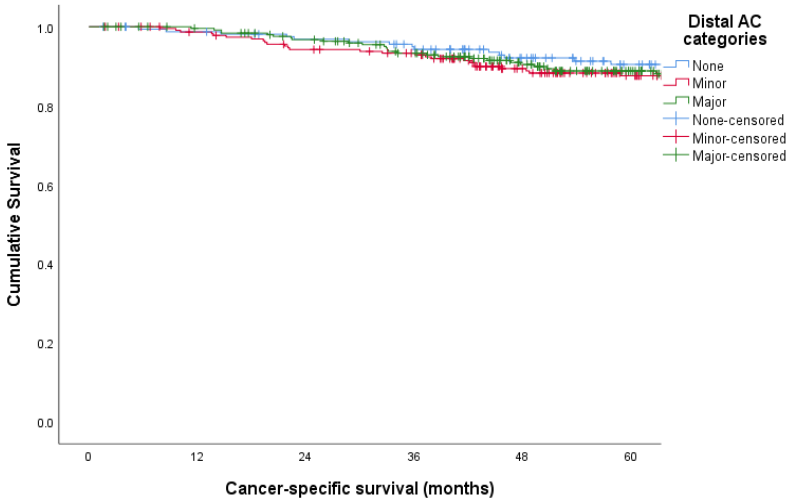
b) Colon cancer – proximal AC and CSS (p=0.026)



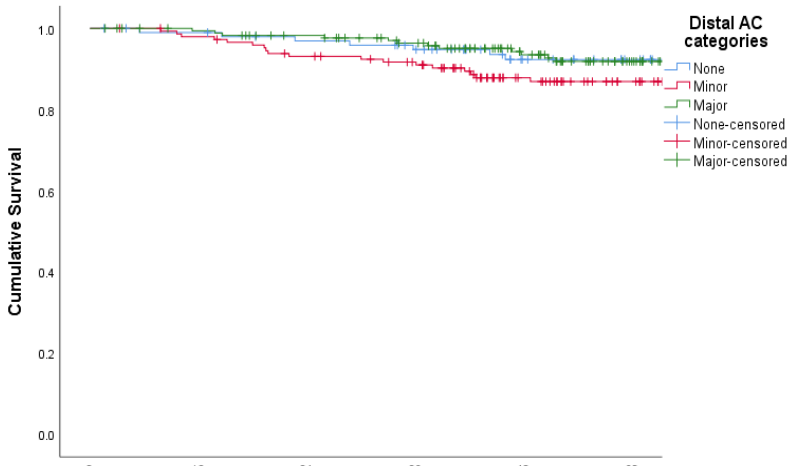
c) Rectal cancer – proximal AC and CSS (p=0.837)



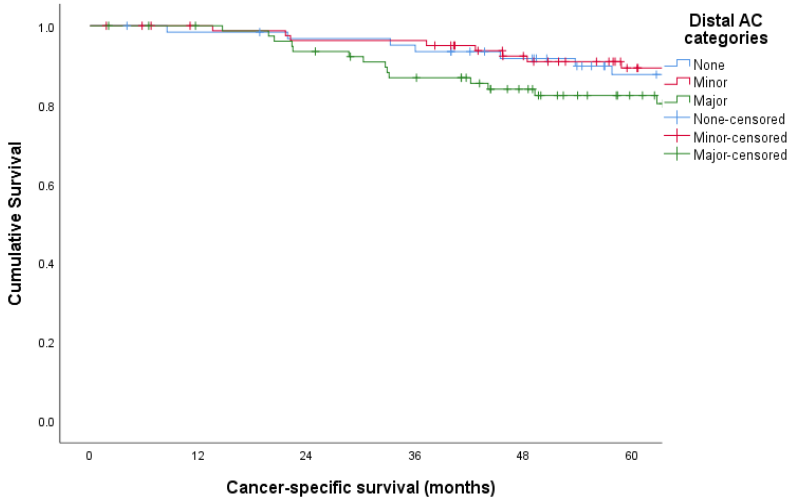
d) Whole cohort – distal AC and CSS (p=0.453)



e) Colon cancer – distal AC and CSS (p=0.065)



f) Rectal cancer – distal AC and CSS (p=0.242)



6 Aortic calcification and response to neo-adjuvant therapy in locally advanced rectal cancer

6.1 Introduction

In the preceding chapter, a correlation between the tumour site and location and degree of AC was observed. This association appeared most relevant in colon cancer. However, it is becoming increasingly clear that rectal cancer differs significantly from colon cancer in its biology and treatment. While the management of colon cancer has remained largely unchanged, rectal cancer management has evolved significantly. The introduction of total mesorectal excision (TME) in the 1980s contributed to a substantial decline in local recurrence rates[172]. Further improvements in local control have been gained through the use of radiotherapy in combination with a radio-sensitising agent prior to surgery in those with locally advanced (T stage 3 or 4 and/or node-positive) disease[249,525]. In the UK, involvement of the circumferential resection margin (CRM) by tumour, lymph node, lymphovascular and/or perineural disease on staging MRI is used to identify patients who may benefit from neoadjuvant chemoradiotherapy (NACRT)[458]. The aim of such treatment is to downstage the involved CRM and enable curative resection.

In 15-20% of patients, a pathological complete response (pCR) occurs where no viable tumour is found on histological examination of the resection specimen[236]. On this basis, the concept of non-operative management was described[526]. Close observation of patients with evidence of a clinical complete response (cCR) on imaging and endoscopy following NACRT is now used in selected cases, avoiding the morbidity of surgery while providing comparable oncological outcomes[527–529]. However, TME following NACRT remains the standard of care for patients with locally advanced rectal cancer (LARC) who have an incomplete response or opt for operative management[458].

The differential response among patients treated with NACRT for LARC highlights heterogeneity in tumour characteristics. A proportion of patients have radiosensitive tumours with significant or complete regression in response to radiotherapy, while others demonstrate little change. Hypoxia within the tumour

microenvironment has long been recognised as a factor associated with poor response to radiotherapy[454]. This hypoxia results from an imbalance between oxygen supply and demand during carcinogenesis and leads to the formation of abnormal tumour vasculature[530]. Systemic factors such as anaemia are also implicated in impaired oxygen delivery and reduced radiotherapy efficacy. Several studies have confirmed anaemia to be a negative prognostic indicator in response to NACRT for rectal cancer with greater clinical downstaging[531] and higher rates of pathological regression[450,451,532] reported in non-anaemic patients.

It is likely that NACRT response is not solely determined by intrinsic tumour characteristics. The interplay between host and tumour characteristics is an important mediator of treatment response. As a marker of cardiovascular disease, aortic calcification may influence the dynamics of mesenteric flow by decreasing vessel pliability and reducing arterial diameter. Such macrovascular flow disturbance may compound the effects of systemic factors including anaemia and local factors including tumour hypoxia.

The work presented in previous chapters highlighted that AC can be measured reliably using the semi-quantitative method and is associated with relevant short- and long-term outcomes. Reliable biomarkers of radiotherapy response, however, remain elusive. Grading of the response to NACRT has become a critical component of the management of LARC. The exponential rise in the use of magnetic resonance imaging (MRI) for locoregional staging pre- and post-NACRT has led to the development of tumour regression grades (TRGs) based on MRI findings[533]. The reference standard remains the degree of tumour regression present within the histological resection specimen which can be graded by a variety of pathological TRGs[235,241,534]. Observational studies attempting to correlate pathological and MRI-derived TRGs have, however, shown low levels of agreement[239,535]. Attempts to define the genotype associated with cCR in patients undergoing NACRT have been made but factors such as intra-tumoural heterogeneity as well as the resource implications of gene sequencing techniques have prevented clinical translation[536]. A simple, clinically relevant method of stratifying patients according to likely response to NACRT therefore represents a valuable tool in the management of patients with LARC.

It was hypothesised that in patients with rectal cancer, significant AC may result in limited response to NACRT. The present study aims to explore the relationship between host factors including the degree of AC and response to NACRT in patients with LARC and assess these in an external cohort.

6.2 Methods

Consecutive patients from Glasgow Royal Infirmary (GRI) with histologically proven rectal cancer who underwent neoadjuvant chemoradiation for LARC between 2008 and 2016 were identified from a prospectively maintained database. Exclusion criteria included patients who received short course radiotherapy or systemic chemotherapy only. LARC was defined as an involved CRM (tumour, lymph node, lymphovascular and/or perineural disease $\leq$1mm from the mesorectal fascia)[537]. Referral for consideration of NACRT was made following formal discussion in the colorectal cancer multidisciplinary team (MDT) meeting.

All patients underwent staging following histopathological confirmation of rectal adenocarcinoma using contrast-enhanced CT imaging of the thorax, abdomen and pelvis to rule out distant metastatic disease. In patients with no contraindication, locoregional staging with pelvic MRI was performed. Tumour height was recorded as the distance in centimetres between the tumour and the anal verge on radiological staging and classified as low (<5cm), mid (5-10cm) and upper (>10cm). Clinical stage was evaluated using digital rectal examination, endoscopic and radiological findings prior to treatment. Patients were re-staged and their imaging reviewed by the MDT on completion of NACRT. Rectal resection incorporating TME was performed using an open or laparoscopic approach approximately 8 weeks following completion of NACRT.

Clinico-pathological characteristics including details of the chemoradiation regimen, duration and dose were extracted from electronic patient records. Patients received long-course radiation at a dose of 45Gy in 25 fractions over 5 weeks as standard. This was combined with a radio-sensitising agent, most commonly oral capecitabine. Patients with a history of significant cardiovascular disease were administered bolus 5-fluorouracil in weeks 1 and 5 of treatment in place of capecitabine.

The semi-quantitative visual method described in Chapter 4 was used to assess the degree of AC.

Pathological data were derived from reports issued at the time of resection. Tumours were staged using the Tumour, Nodes, Metastases (TNM) classification[213] and according to the Royal College of Pathologists Dataset[537]. Response to NACRT was determined using T- and N-downstaging, the degree of histopathological tumour regression and the Neoadjuvant Rectal (NAR) score[538].

The reporting pathologist's impression of response to preoperative therapy was recorded using the tumour regression score advocated by the AJCC[241]. In addition, the degree of tumour regression was assessed using the Mandard[534] and Rödel[235] grading systems. The Mandard TRG was originally derived in specimens from oesophageal cancer resection. It uses a semi-quantitative approach to classify the proportion of residual cancer to scar tissue in the resection specimen. Similarly, the Rödel TRG assesses the amount of viable tumour in relation to the amount of fibrosis. The features of each TRG are outlined in Table 6.1.

The Neoadjuvant Rectal (NAR) score, a surrogate endpoint developed for use in clinical trials to predict long-term outcome following NACRT for rectal cancer[538], was calculated for each patient using pre-treatment data. The formula (Figure 6.1) incorporates clinical T stage and pathological T and N stage to produce a score between 0 and 100; lower scores are suggested to indicate short-term benefit which may relate to improved survival. The difference between the pre-treatment clinical and post-treatment pathological T- and N-stage were used to assess T- and N-downstaging.

A cohort of 333 LARC patients at St Marks Hospital and Academic Institute (SMH) was identified from a prospectively maintained database between May 2007 and November 2016. Of them, 49 patients underwent NARCT following discussion of their cases at the local colorectal cancer MDT. Upon completion of the NACRT, TME was performed. Data on clinical and radiological staging were not available; therefore, pCR rates were used to assess NACRT response. AC was assessed by one rater (ID). A sample of 30 scans was scored separately by two raters (KK, ID) to assess inter-rater reliability.

6.2.1 Statistical analysis

Baseline characteristics were grouped according to standard thresholds and summarised using descriptive statistics. The intraclass correlation coefficient (ICC) was used to compare inter-rater reliability as reported in previous chapters. Associations between clinico-pathological characteristics and response to NACRT were assessed using Chi-squared test for association, Mantel-Haenszel or Fisher's exact test where appropriate. Statistical analysis was performed using SPSS software (version 26, IBM, Armonk, NY).

Ethical approval for the study was provided by the London North West University Healthcare NHS Trust (20/LO/0370). The need for patient consent was waived due to the retrospective nature of the study.

6.3 Results

Between 2008 and 2016, 231 patients underwent rectal cancer resection with curative intent at Glasgow Royal Infirmary. Of these, 86 patients were considered to have LARC on baseline clinical, imaging and endoscopic evaluation and were referred for consideration of NACRT following MDT discussion. In total, 79 patients proceeded to NACRT. Exclusions included two patients who had previously undergone pelvic radiotherapy for testicular and prostate cancer respectively, two patients with missing clinical records, two patients who received neoadjuvant systemic chemotherapy alone and one patient who underwent short-course radiotherapy.

The baseline characteristics of patients in the GRI cohort are displayed in Table 6.2. The majority of patients were male (n=46, 58%), aged over 65 years (n=40, 51%) and had a history of smoking (n=44, 56%). Most patients had cT stage 3 or greater tumours (n=75, 95%) in the mid and upper rectum (n=44, 56%) and node positive disease (n=60, 76%).

The NACRT regimen consisted of long-course radiotherapy (25 fractions of 45Gy delivered over 5 weeks) in combination with oral capecitabine in 66 patients (84%). In 13 patients (16%), 5-fluoruracil (5-FU) was administered during weeks 1 and 5 of radiotherapy; 3 patients received concurrent folinic acid. NACRT was associated with toxicities in 23 patients (29%), with dose reductions or treatment interruptions occurring in 8 patients (10%).

MRI following NACRT was carried out in 21 patients (27%). All patients proceeded to surgery following NACRT. Surgical resection was performed by abdominoperineal resection in 40 patients (51%), anterior resection in 35 patients (44%) and Hartmann's procedure in 4 patients (5%). All but one patient who underwent anterior resection had a primary anastomosis. Margin involvement was confirmed on histopathology in 12 patients (15%), of whom 5 (42%) later developed local recurrence. A further 7 patients (10%) who had R0 resections developed local recurrence during surveillance.

A pCR was reported in 10 patients (13%). The majority of patients who had a pCR had low rectal tumours (60%). In those with an incomplete response, T-

downstaging occurred in 26 patients (38%) and N-downstaging in 34 patients (49%). Response to NACRT graded by the RCP TRG was complete in 10 patients (13%), near complete in 16 patients (20%), partial in 31 patients (39%) and poor in 22 (28%). Response to NACRT graded by the Mandard TRG was reported as complete in 11 patients (14%), rare residual cancer in 14 patients (18%), predominantly fibrosis in 13 patients (17%), residual cancer outgrowing fibrosis in 21 patients (26%) and absence of regression in 20 patients (25%). Response to NACRT graded by the Rödel TRG was complete in 10 patients (13%), intermediate in 40 (51%) and poor in 29 (37%). The NAR score was less than 8 in 12 patients (15%), 8 to 16 in 43 patients (54%) and greater than 16 in 24 patients (30%).

Associations between baseline characteristics including age, gender, pre-treatment haemoglobin level, cT stage, cN stage, tumour height and response to NACRT are displayed in Tables 6.3-6.6. No associations between pre-treatment host or tumour characteristics and pCR were evident. A statistically significant association was noted between lower cT stage tumours and complete or intermediate response to NACRT using the RCP TRG (p=0.021). A non-significant trend between tumour height <5cm and complete response as graded by the RCP, Mandard and Rödel TRGs was noted. Expected associations between higher cT stage and degree of T-downstaging and nodal positivity and degree of N-downstaging were noted. No further statistically significant associations between baseline characteristics and response to NACRT using the Mandard TRG, Rödel TRG or T-downstaging were evident. A higher NAR score was associated with higher cN stage (p=0.002) and tumour height <5cm (p=0.002).

The associations between the degree of AC and response to NACRT are shown in Table 6.7. Proximal AC was absent in 45 patients (57%), minor in 19 patients (24%) and major in 15 patients (19%) while distal AC was absent in 25 patients (32%), minor in 23 patients (29%) and major in 31 patients (39%). There were no statistically significant associations between the degree of proximal or distal AC and response to NACRT as measured by pCR rates, RCP, Mandard and Rödel TRGs, T-downstaging, N-downstaging, or NAR score.

Between 2007 and 2016, 333 patients with available CT imaging underwent rectal cancer resection with curative intent at St Mark's Hospital. Of these, 49 patients proceeded to NACRT. The baseline characteristics of patients in the

study cohort are displayed in Table 6.8. The majority of patients were male (n=37, 75%), aged less than 65 years (n=29, 59 %) and were ASA grade 1 or 2 (n=43, 87%). A pCR occurred in 8 patients (16%).

Proximal AC was absent in 36 patients (74%), minor in 6 patients (12%) and major in 7 patients (14%) while distal AC was absent in 20 patients (41%), minor in 15 patients (31%) and major in 14 patients (28%). For inter-rater reliability, the ICC for proximal AC was 0.92 (95% CI 0.84 – 0.96) and for distal AC was 0.88 (95% CI 0.75 – 0.94).

There were no statistically significant associations between the development of a pCR and age, gender or the degree of proximal or distal AC in the St Mark's cohort (Table 6.9). This remained the case when patients who developed a pCR in either cohort were pooled (Table 6.10).

6.4 Discussion

In this study, neither the degree of proximal nor distal aortic calcification was associated with response to NACRT in patients with margin-threatening rectal cancer. Distal AC was more common than proximal AC in both the SMH and GRI cohorts. When compared to the patients with rectal cancer who did not undergo NACRT (data not shown), the proximal and distal calcification rates were similar, suggesting that the degree of calcification in this study cohort was representative of that in patients with rectal cancer who proceeded directly to surgery. Importantly, AC assessment was performed in an external cohort and its use demonstrated to be feasible and reproducible.

The absence of an association between the degree of calcification and NACRT response may be related to several factors. The small number of patients with a complete response (n=10, 13% GRI, n=8, 16% SMH) is likely to limit our ability to detect a relationship. This may also underlie the lack of association between treatment response and anaemia, present in only 10 patients from the GRI cohort. Using the TRGs, most patients were categorised as having an intermediate response, making differentiation of factors predisposing to a complete or poor response difficult. However, binary response measures such as T- and N-downstaging were not associated with the degree of aortic calcification. It is also plausible that the atherosclerotic burden of the abdominal aorta is unrelated to radiotherapy response. Supplementing the findings reported here with tissue-based assessment of hypoxic markers would better define whether AC is associated with tumour hypoxia. Prospective collection of tissue at varying timepoints in patients with LARC undergoing NACRT coupled with dynamic assessment of arterial flow using MR imaging techniques would enable a clearer appreciation of the relationship between AC and tumour hypoxia.

The optimal endpoint for assessment of response to neoadjuvant therapy is a source of ongoing debate. Tumour regression grades are commonly associated with high rates of interobserver variability[239]. The variable diagnostic performance of MRI[539] and the need for multiple integrated sequences to improve predictive capacity for pCR[540,541] limit their use. Moreover, wide variation in pCR rates across institutions has been attributed to differences in

the thoroughness of pathological examination[542]. The NAR score was developed as a surrogate endpoint for use in clinical trials which involve assessment of response to NACRT but its predictive value has been disputed in subsequent studies[543]. The use of multiple metrics of tumour response was therefore undertaken in this study. However, no association between these measures and the degree of aortic calcification was evident in either cohort. It was notable that variables such as tumour height which are associated with NACRT response in other series did not consistently show significant associations with measures of NACRT response, suggesting an expanded sample size is required to validate the study findings.

Relatively few studies have examined the relationship between aspects of comorbidity and treatment response. Anderson and colleagues found hypertension to be the sole component of the metabolic syndrome (hypertension, obesity, hypertriglyceridaemia, elevated fasting glucose and reduced HDL cholesterol) associated with reduced odds of complete response in a cohort of 102 patients with a pCR rate of 17%[544]. However, a limited number of patients had metabolic syndrome in this study (6%) while 50% had hypertension which was poorly defined in the study methods. Blood pressure was not measured before or during NACRT and it is possible that patients with a history of hypertension were normotensive on minimal medication, limiting the reliability of the findings. A further study examining the impact of diabetes mellitus on response to NACRT in 102 patients with rectal cancer reported similar rates of tumour down-staging between diabetic and non-diabetic patients but a difference in pCR rates[545]. None of the patients with diabetes were found to have a complete response compared with 24% of their non-diabetic counterparts. Although the small cohort size limits how generalisable the results are, the possibility that microvascular rather than macrovascular calcification, as is common in diabetes, could influence radiotherapy response warrants further exploration.

Comparison of the tumour microenvironment characteristics and the degree of calcification in the patients in this study would have provided clarity on the relationship between aortic calcification and markers of tumour hypoxia. However, availability of tissue for analysis from patients within the cohort was

limited. Future studies examining NACRT response in relation to patient and tumour characteristics are required. Moreover, the paucity of data in relation to the effect of comorbidity on NACRT response suggests integration of comorbidity indices would provide more context to assess the clinical relevance of aortic calcification in patients with rectal cancer.

Several practical aspects must also be considered: the use of NACRT for LARC within the UK is variable[546] as demonstrated by the differences in the proportion of patients undergoing NACRT in the two cohorts described here (34% GRI, 15% St Mark's). In addition, use of NACRT in the UK is generally reserved for poor prognosis tumours i.e. low margin-threatening node-positive tumours whereas NACRT in North America is not restricted to such so-called "ugly" tumours. Debate continues regarding the optimal format of NACRT (short-course radiotherapy versus long-course chemoradiation) while trials are underway examining the addition of systemic chemotherapy to the neoadjuvant treatment schedule (total neoadjuvant therapy)[547]. Such differences in treatment indication and format necessitate examination of the influence of host factors on NACRT response in a large contemporary cohort. Techniques such as propensity-score matching may be required to enable reliable comparison between cohorts from UK, Europe and North America.

As described, this study has several limitations. In addition, data on tumour volume pre- and post-NACRT as a response metric were limited. Similarly, only a small number of patients had both pre- and post-NACRT MR imaging available to enable MRI-based assessment of treatment response. The St Mark's dataset contained a small proportion of patients undergoing NACRT and was limited by the absence of clinical staging and MRI data, restricting response to treatment analysis to pCR only. However, the reference standard for NACRT response remains histopathological examination, suggesting such additional proxy measures may have little impact on the study findings.

In conclusion, in the absence of an available larger cohort in which to examine NACRT response in relation to host characteristics, these data suggest that aortic calcification does not appear to significantly influence treatment response. Further work to assess the degree of hypoxia within the tumour

microenvironment may provide additional information on the relationship between vascular calcification, tumour hypoxia and NACRT response.

Table 6-1 - Tumour regression grades and the corresponding histopathological criteria.

Tumour Regression Grade	Score	Description
Royal College of Pathologists	0	No viable cancer cells (complete response)
	1	Single cells or rare small groups of cancer cells (near-complete response)
	2	Residual cancer with evident tumour regression, but more than single cells or rare small groups of cancer cells (partial response)
	3	Extensive residual cancer with no evident tumour regression (poor or no response)
Mandard	1	Complete regression - absence of residual cancer and fibrosis
	2	Presence of rare residual cancer
	3	An increase in the number of residual cancer cells, but predominantly fibrosis
	4	Residual cancer outgrowing fibrosis
	5	Absence of regressive changes
Rödel	Poor (0-1)	No regression or dominant tumour mass with obvious fibrosis and/or vasculopathy
	Intermediate (2-3)	Dominant fibrotic change with few tumour cells or groups (easy to find) or very few tumour cells in fibrotic tissue with or without mucous substance
	Complete (4)	No tumour cells, only fibrotic mass (total regression or response)

Table 6-2 - Baseline demographics of patients undergoing neoadjuvant chemoradiation (n=79).

Variable		n (%)
Age	<65	39 (49)
	65 - 75	33 (42)
	>75	7 (9)
Gender	Female	33 (42)
	Male	46 (58)
ASA grade	1	20 (25)
	2	38 (48)
	3	20 (25)
	4	1 (1)
BMI*	<30	65 (82)
	>30	13 (17)
Smoking history	No	35 (44)
	Yes	44 (56)
Tumour height (distance from anal verge, cm)	<5	35 (44)
	5 - 10	26 (33)
	>10	18 (23)

Variable		n (%)
cT stage	2	4 (5)
	3	61 (77)
	4	14 (18)
cN stage*	0	18 (23)
	1	23 (29)
	2	37 (47)

Abbreviations: ASA American Society of Anaesthesiology, BMI body mass index, cT/N clinical tumour/node stage, NACRT neoadjuvant chemoradiotherapy.

*Missing cases 1

Table 6-3 - Associations between baseline clinico-pathological characteristics and response to NACRT by histopathological response.

		Incomplete response n = 69	Complete response n = 10	p-value
Age (years)	< 65	36 (92)	3 (8)	0.608
	65 – 75	26 (79)	7 (21)	
	> 75	7 (100)	0 (0)	
Gender	Female	29 (88)	4 (12)	0.592
	Male	40 (87)	6 (13)	
Pre-NACRT haemoglobin (g/L)[a]	Normal	59 (86)	10 (14)	0.236
	Low	10 (100)	0 (0)	
cT stage	2 - 3	55 (84)	10 (16)	0.124
	4	14 (100)	0 (0)	
cN stage[b]	0	14 (78)	4 (22)	0.167
	1 - 2	54 (90)	6 (10)	
Tumour height (cm)	<5	29 (83)	6 (17)	0.226
	5 – 10	23 (88)	3 (12)	
	>10	17 (94)	1 (6)	

Abbreviations: cT/N clinical tumour/node stage, NACRT neoadjuvant chemoradiotherapy. [a] Normal range 130-180 g/L for males, 110-165 g/L for females [b] Nodal stage data missing for 1 patient

Table 6-4 - Associations between baseline clinico-pathological characteristics and response to NACRT graded by the Royal College of Pathologists, Mandard and Rödel tumour regression grades (TRG).

		Royal College of Pathologists TRG			p-value	Mandard TRG			p-value	Rödel TRG			p-value
		0 n = 10	1-2 n = 47	3 n = 22		1 n = 11	2-4 n = 48	5 n = 20		4 n = 10	2-3 n = 40	0-1 n = 29	
Age (years)	< 65	3 (8)	26 (67)	10 (25)	0.969	4 (10)	25 (64)	10 (26)	0.704	3 (8)	22 (56)	14 (36)	0.733
	65 – 75	7 (21)	16 (49)	10 (30)		7 (21)	17 (51)	9 (27)		7 (21)	13 (39)	13 (39)	
	> 75	0 (0)	5 (71)	2 (29)		0 (0)	6 (86)	1 (14)		0 (0)	5 (71)	2 (28)	
Gender	Female	4 (12)	17 (52)	12 (36)	0.273	4 (12)	18 (55)	11 (33)	0.233	4 (12)	14 (42)	15 (46)	0.293
	Male	6 (13)	30 (65)	10 (22)		7 (15)	30 (65)	9 (20)		6 (13)	26 (57)	14 (30)	
Pre-NACRT haemoglobin[a]	Normal	10 14)	39 (56)	20 (29)	0.794	11 (16)	40 (58)	18 (26)	0.638	10 (15)	33 (48)	26 (38)	0.762
	Low	0 (0)	8 (80)	2 (20)		0 (0)	8 (80)	2 (20)		0 (0)	7 (70)	3 (30)	

		Royal College of Pathologists TRG			p-value	Mandard TRG			p-value	Rödel TRG			p-value
		0 n = 10	1-2 n = 47	3 n = 22		1 n = 11	2-4 n = 48	5 n = 20		4 n = 10	2-3 n = 40	0-1 n = 29	
cT stage	2 - 3	10 (15)	40 (62)	15 (23)	0.021	10 (15)	40 (62)	15 (23)	0.253	10 (15)	33 (51)	22 (34)	0.107
	4	0 (0)	7 (50)	7 (50)		1 (7)	8 (57)	5 (36)		0 (0)	7 (50)	7 (50)	
cN stage[b]	0	4 (22)	10 (56)	4 (22)	0.270	4 (22)	12 (67)	2 (11)	0.079	4 (22)	9 (50)	5 (28)	0.201
	1 - 2	6 (10)	37 (62)	17 (28)		7 (12)	35 (58)	18 (30)		6 (10)	31 (52)	23 (38)	
Tumour height (cm)	<5	6 (17)	20 (57)	9 (26)	0.294	7 (20)	22 (63)	6 (17)	0.068	6 (17)	18 (52)	11 (31)	0.276
	5 – 10	3 (11)	16 (62)	7 (27)		3 (11)	15 (58)	8 (31)		3 (12)	12 (46)	11 (42)	
	>10	1 (6)	11 (61)	6 (33)		1 (6)	11 (62)	6 (33)		1 (6)	10 (56)	7 (39)	

Abbreviations: cT/N clinical tumour/node stage, NACRT neoadjuvant chemoradiotherapy. [a] Normal range 130-180 g/L for males, 110-165 g/L for females [b] Nodal stage data missing for 1 patient

Table 6-5 - Associations between baseline clinico-pathological characteristics and response to NACRT by T- and N-downstaging.

		T-downstaging		p-value	N-downstaging		p-value
		No n = 43	Yes n = 36		No n = 39	Yes n = 39	
Age (years)	< 65	22 (56)	17 (44)	0.623	21 (54)	18 (46)	0.569
	65 – 75	15 (46)	18 (54)		13 (41)	19 (59)	
	> 75	6 (86)	1 (14)		5 (71)	2 (28)	
Gender	Female	20 (61)	13 (39)	0.351	20 (61)	13 (39)	0.109
	Male	23 (50)	23 (50)		19 (42)	26 (58)	
Pre-NACRT haemoglobin[a]	Normal	39 (56)	30 (44)	0.327	33 (49)	35 (51)	0.598
	Low	4 (40)	6 (60)		5 (50)	5 (50)	
cT stage	2 - 3	39 (60)	26 (40)	0.032	29 (45)	36 (55)	0.134
	4	4 (29)	10 (71)		9 (69)	4 (31)	
cN stage[b]	0	8 (44)	10 (56)	0.299	18 (100)	0 (0)	<0.001
	1 - 2	35 (58)	25 (42)		21 (35)	39 (65)	
Tumour height (cm)	<5	15 (43)	20 (57)	0.136	20 (59)	14 (41)	0.395
	5 – 10	17 (65)	9 (35)		9 (35)	17 (65)	
	>10	11 (61)	7 (39)		9 (50)	9 (50)	

Abbreviations: cT/N clinical tumour/node stage, NACRT neoadjuvant chemoradiotherapy.[a] Normal range 130-180 g/L for males, 110-165 g/L for females [b] Nodal stage data missing for 1 patient

Table 6-6 - Associations between baseline clinico-pathological characteristics and response to NACRT by Neoadjuvant Rectal (NAR) score.

		NAR Score			p-value
		<8 n = 12	8-16 n = 43	>16 n = 24	
Age (years)	< 65	3 (8)	25 (25)	11 (28)	0.765
	65 – 75	8 (24)	15 (46)	10 (30)	
	> 75	1 (14)	3 (43)	3 (43)	
Gender	Female	5 (15)	15 (46)	13 (39)	0.303
	Male	7 (15)	28 (61)	11 (24)	
Pre-NACRT haemoglobin (g/L)[a]	Normal	12 (17)	35 (51)	22 (32)	0.806
	Low	0 (0)	8 (80)	2 (20)	
cT stage	2 - 3	11 (17)	35 (54)	19 (29)	0.404
	4	1 (7)	8 (57)	5 (36)	
cN stage[b]	0	6 (33)	11 (61)	1 (6)	0.002
	1 - 2	2 (10)	31 (52)	23 (38)	
Tumour height (cm)	<5	8 (23)	23 (66)	4 (11)	0.002
	5 – 10	3 (12)	12 (46)	11 (42)	
	>10	1 (6)	8 (44)	9 (50)	

Abbreviations: cT/N clinical tumour/node stage, NACRT neoadjuvant chemoradiotherapy. [a] Normal range 130-180 g/L for males, 110-165 g/L for females. [b] Nodal stage data missing for 1 patient.

Table 6-7 - Comparison of response to NACRT by the degree of calcification.

		Proximal AC				Distal AC			
		None n = 45	Minor n = 19	Major n = 15	p-value	None n = 25	Minor n = 23	Major n = 31	p-value
pCR	No	39 (57)	17 (25)	13 (18)	0.931	23 (33)	18 (26)	28 (41)	0.923
	Yes	6 (60)	2 (20)	2 (20)		2 (20)	5 (50)	3 (30)	
TRG – Royal College of Pathologists	Complete (0)	6 (60)	2 (20)	2 (20)	0.720	2 (20)	5 (50)	3 (30)	0.652
	Intermediate (1-2)	28 (60)	10 (21)	9 (19)		17 (36)	12 (26)	18 (38)	
	Poor (3)	11 (50)	7 (32)	4 (18)		6 (27)	6 (27)	10 (46)	
TRG – Mandard	Complete (1)	7 (64)	2 (18)	2 (18)	0.923	3 (27)	5 (46)	3 (27)	0.473
	Intermediate (2-4)	27 (56)	11 (23)	10 (21)		16 (33)	14 (29)	18 (38)	
	Poor (5)	11 (55)	6 (30)	3 (15)		6 (30)	4 (20)	10 (50)	

		Proximal AC				Distal AC			
		None n = 45	Minor n = 19	Major n = 15	p-value	None n = 25	Minor n = 23	Major n = 31	p-value
TRG – Rödel	Complete (4)	6 (60)	2 (20)	2 (20)	0.793	2 (20)	5 (50)	3 (30)	0.606
	Intermediate (2-3)	24 (60)	8 (20)	8 (20)		15 (38)	10 (25)	15 (38)	
	Poor (0-1)	15 (52)	9 (31)	5 (17)		8 (28)	8 (28)	13 (45)	
T-downstaging	No	22 (51)	14 (33)	7 (16)	0.704	13 (30)	12 (28)	18 (42)	0.642
	Yes	23 (64)	5 (14)	8 (22)		12 (33)	11 (31)	13 (36)	
N-downstaging	No	22 (58)	10 (26)	6 (16)	0.592	10 (26)	13 (34)	15 (40)	0.580
	Yes	22 (55)	9 (23)	9 (23)		15 (38)	9 (22)	16 (40)	
NAR score	Low (<8)	6 (50)	2 (17)	4 (33)	0.924	2 (17)	5 (42)	5 (42)	0.555
	Intermediate (8-16)	27 (63)	10 (23)	6 (14)		15 (35)	11 (26)	17 (40)	
	High (>16)	12 (50)	7 (29)	5 (21)		8 (33)	7 (29)	9 (38)	

Abbreviations: AC aortic calcification, NAR neoadjuvant rectal score, pCR pathologic complete response, TRG tumour regression grade.

Table 6-8 - Baseline demographics of patients undergoing neoadjuvant chemoradiation (St Mark's cohort, n=49).

Variable		n (%)
Age (years)[a]	<65	29 (59)
	65 - 75	10 (20)
	>75	9 (18)
Gender	Female	12 (25)
	Male	37 (75)
ASA grade[a]	1	10 (20)
	2	33 (67)
	3	4 (8)
	4	1 (2)
BMI[a]	<30	38 (78)
	>30	6 (12)

Abbreviations: ASA American Society of Anaesthesiologists, BMI body mass index.

[a] Missing cases: Age (n=1), ASA (n=1), BMI (n=5).

Table 6-9 - Associations between baseline clinico-pathological characteristics and response to NACRT by histopathological response in St Mark's cohort.

		Incomplete response n = 41	Complete response n = 8	p-value
Age (years)[a]	< 65	22 (76)	7 (24)	0.235
	65 – 75	10 (100)	0 (0)	
	> 75	8 (89)	1 (11)	
Gender	Female	9 (75)	3 (25)	0.386
	Male	32 (86)	5 (14)	
Proximal AC	None	29 (81)	7 (19)	0.621
	Minor	6 (100)	0 (0)	
	Major	6 (86)	1 (14)	
Distal AC	None	15 (75)	5 (25)	0.175
	Minor	13 (87)	2 (13)	
	Major	13 (93)	1 (7)	

Abbreviations: AC – aortic calcification.

[a] Missing cases: Age (n= 1)

Table 6-10 - Associations between baseline clinico-pathological characteristics and response to NACRT by histopathological response in the combined Glasgow Royal and St Mark's cohort (n=128).

		Incomplete response n = 110	Complete response n = 18	p-value
Age (years)[a]	< 65	58 (85)	10 (24)	0.557
	65 – 75	36 (84)	7 (16)	
	> 75	15 (94)	1 (6)	
Gender	Female	38 (84)	7 (16)	0.721
	Male	72 (87)	11 (13)	
Proximal AC	None	68 (84)	13 (16)	0.575
	Minor	23 (92)	2 (8)	
	Major	19 (86)	3 (14)	
Distal AC	None	38 (84)	7 (16)	0.365
	Minor	31 (82)	7 (18)	
	Major	41 (91)	4 (9)	

Abbreviations: AC – aortic calcification.

[a] Missing cases: Age (n= 1)

7 Aortic calcification and tolerance of adjuvant therapy following colorectal cancer resection

7.1 Introduction

Following curative resection of colorectal cancer, a proportion of patients will proceed to adjuvant chemotherapy (ACT). The aim of such treatment in the short term is to eliminate residual circulating tumour cells or micrometastatic disease to reduce recurrence and improve long-term survival. The presence of high-risk pathological features including nodal involvement[257], poor differentiation[221], tumour perforation[225], perineural[232] or lymphovascular invasion [226,231] is associated with an increased risk of recurrence following curative resection. Recommendation of patients for ACT is made by the colorectal MDT following histological examination of the resected specimen, with consideration given to the patient's wishes, comorbidity and performance status[458].

Several landmark trials in the 1990s established the fluoropyrimidine 5-fluorouracil (5-FU) in combination with leucovorin, a folinic acid derivative that enhances 5-FU activity, as the standard of care for node-positive colon cancer[261-264]. The addition of oxaliplatin, a platinum derivative, to 5FU and leucovorin was subsequently demonstrated to improve disease-free survival[207,273]. Capecitabine, the oral pro-drug of 5-fluorouracil, was later confirmed to confer similar benefit in conjunction with oxaliplatin[208,275]. Double agent, 5-FU-based chemotherapy remains the accepted standard of care for adjuvant treatment in patients with colorectal cancer.

Tolerance of ACT is variable. Oxaliplatin is associated with long-term peripheral neurotoxicity while capecitabine can lead to acute cardiotoxicity including coronary vasospasm and related acute coronary syndromes. Quality of life in both the short- and long-term is impacted by ACT toxicity. Oncologists consider the risk of such adverse events with the potential benefit of reduced recurrence and improved survival. This, together with individual risk of cardiovascular toxicity, is assessed predominantly by subjective means.

It is well recognized that older, more comorbid patients are often excluded from trials and in the non-trial setting are less likely to be referred for or proceed to adjuvant therapy despite the presence of high-risk pathological features[548–553]. The result is a relative lack of real-world data on the characteristics, in particular, the cardiovascular status of patients undergoing adjuvant chemotherapy and their corresponding short-term outcomes. In this setting, assessment of aortic calcification may represent an indicator of CV risk and ability to tolerate chemotherapy that can be readily applied. Moreover, chemotherapy relies on adequate drug delivery to target tissues. In patients with significant atheromatous disease, poorer perfusion could impact on treatment efficacy, exposing patients to toxicity with limited survival benefit. It is plausible that if substantiated, AC may represent a stratification tool for patients in future clinical trials of ACT.

The hypothesis of this study was that patients with a greater burden of AC would have poorer tolerance and completion of ACT. This study therefore aimed to assess the characteristics of patients receiving ACT following curative colorectal cancer resection and the associations between clinicopathological factors including the degree of AC and proxy measures of chemotherapy tolerance.

7.2 Methods

Consecutive patients who had undergone elective curative resection of stage II – III colorectal cancer at Glasgow Royal Infirmary between 2008 and 2016 were identified. Patients were eligible for inclusion if, based on final pathology, there was nodal involvement by tumour or the presence of other high-risk pathological features including venous invasion, T4 disease, tumour obstruction, tumour perforation, poor differentiation and perineural or lymphatic vascular invasion. Referral for adjuvant therapy followed discussion in the colorectal cancer multidisciplinary meeting where consideration of the risk of recurrence was balanced with patient suitability for further treatment.

All patients with nodal involvement or high-risk pathological features were retrospectively identified from a prospectively-maintained colorectal cancer database. Demographics and baseline clinical and pathological characteristics were extracted from the patient's medical e-record. Pathological tumour stage was reported using the TNM staging system[213]. The database of the oncology treatment centre was queried to extract the corresponding chemotherapy treatment records of patients who had proceeded to adjuvant therapy. The intended regimen and number of cycles planned were abstracted from oncology clinic letters prior to treatment. The regimen, total dose and final number of cycles received were extracted from the chemotherapy treatment records. The planned total dose was calculated by multiplying the intended dose by the intended number of cycles for each agent. The percentage of actual dose received for each agent was calculated by dividing the total dose received by the planned total dose. Patients who switched regimens were not included in the analysis of patients who required dose reductions due to difficulties defining the planned and received doses. Dose reductions and number of cycles completed were recorded. Toxicities are not currently captured by the oncology database and, where available, were extracted from oncology clinic letters.

Regimens included single agent capecitabine or 5-FU (in combination with leucovorin as modified de Gramont) or double agent chemotherapy (capecitabine and oxaliplatin (CAPOX)/5-fluorouracil and oxaliplatin with leucovorin (FOLFOX)). Duration was 4 or 8 cycles for capecitabine and CAPOX, 6 or 12 cycles for FOLFOX and 12 cycles for modified de Gramont. A proportion of

patients between March 2008 and November 2013 were recruited to the SCOT study[554], an international multicentre randomised trial assessing 3 months versus 6 months treatment with oxaliplatin-containing chemotherapy.

Aortic calcification was scored in the proximal and distal aorta as described in Chapter 3 using preoperative CT images.

Ethical approval was obtained from the West of Scotland Research Ethics Committee (reference number 17/WS/0200) and the Caldicott guardian. The need for informed consent from patients was waived due to the retrospective nature of the study.

7.2.1 Statistical analysis

Descriptive statistics were used to summarise baseline characteristics. The median and interquartile range (IQR) were used to describe the calcification scores. The degree of calcification was grouped into categories using the median score: no calcification (score 0), minor (less than median) or major (greater than median). Completion of intended cycles and requirement for dose reduction and change of regime were recorded as binary variables. The percentage of planned dose received was expressed as a continuous variable. Associations between chemotherapy treatment characteristics and the degree of AC were investigated using the Mantel-Haenszel test or Fisher's exact test. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA).

7.3 Results

Of 651 patients who survived curative resection, 177 had stage II disease with high-risk pathological features and 227 had stage III disease. The most common high-risk feature in those with stage II disease was venous invasion, present in 153 patients (86%). Among 404 potentially eligible patients, 194 commenced adjuvant chemotherapy. The median time to commencement of adjuvant therapy was 53 days (IQR 46 – 62).

The baseline characteristics of the 194 patients who started adjuvant therapy compared with those who were eligible but did not receive adjuvant chemotherapy (n=210) are displayed in Table 7.1. Patients who received adjuvant chemotherapy were more likely to be under 75 years of age (94% vs 60%, p<0.001), ASA grade 1 or 2 (76% vs 61%, p<0.001) and non-smokers (54% vs 40%, p=0.004). Patients who had adjuvant chemotherapy were more likely to have no proximal (63% vs 34%, p<0.001) or distal AC (34% vs 15%, p<0.001). In total, 162 patients (83%) received chemotherapy with capecitabine and 30 patients (15%) received 5-FU; oxaliplatin was included in 141 (73%). Single agent chemotherapy was undertaken in 53 patients (27%).

The intended number of cycles was completed in 139 patients (72%). Of those who did not complete the intended number of cycles, the median percentage of cycles received was 50% (IQR 25% - 75%). The regime was changed during treatment in 17 patients (9%): 4 patients who were originally intended for FOLFOX chemotherapy, 12 patients due to receive XELOX chemotherapy and 1 patient scheduled to received capecitabine alone.

Excluding those who switched regime, 48 patients (27%) required a dose reduction. Of 159 patients, 21 (13%) required to have the dose of capecitabine reduced; 5 patients (24%) received over 90% and 15 patients (71%) received over 75% of the intended capecitabine dose. Of 33 patients, 7 (21%) required to have the dose of 5FU reduced; 5 patients (71%) received over 90% and all patients received over 75% of the intended 5FU dose. Of 141 patients receiving oxaliplatin, 45 patients (32%) required to have the dose of oxaliplatin reduced; 5

patients (11%) received over 90% and 15 patients (33%) received over 75% of the intended dose of oxaliplatin.

Table 7.2 shows the association between clinicopathological characteristics and completion of chemotherapy, requirement for dose reduction and change of regime. As age increased, the proportion of patients who failed to complete the planned number of cycles increased (22% aged<65 vs 33% aged>75, p=0.082). An association was evident between a history of smoking and failure to complete the intended number of cycles (36% smokers versus 22% non-smokers, p=0.030). An association was also noted between an increasing burden of proximal AC and failure to complete the intended cycles (24% no proximal AC versus 33% minor proximal AC versus 44% major proximal AC, p=0.041). The proportion of patients not completing chemotherapy was higher among those on single-agent regimens than on double agent regimens (49% vs. 21%).

A trend towards increasing BMI and need for chemotherapy dose reduction (35% BMI >30 vs 22% BMI<30, p=0.058) was noted. Tumours with a more advanced TNM stage were also associated with the need for a dose reduction (31% TNM stage III versus 16% of TNM stage II, p=0.046). A trend was noted in the requirement for dose reductions in patients receiving chemotherapy containing 5-FU compared with capecitabine-based regimens (41% versus 24%, p=0.059). Patients on double agent chemotherapy were significantly more likely to require a dose reduction than those on single agent regimens (33% versus 13%, p=0.008).

An inverse association was noted between regime change and increasing age: as age increased, the proportion of patients switching regimens decreased (12% versus 0%). Female gender was associated with a change of chemotherapy from the intended regime (14% versus 5%, p=0.040). Patients on single agent regimens were less likely to require a change of regime compared with those on double agent regimens (2% versus 11%).

Table 7.3 examines chemotherapy type and dose reduction for capecitabine, 5FU and oxaliplatin in relation to the degree of AC. Patients who had double agent chemotherapy were more likely to have no evidence of proximal AC (69% vs 47%, p=0.040) or distal AC (37% vs 25%, p=0.068). There were no statistically

significant associations between dose reduction for any single chemotherapy component and the degree of proximal and distal AC.

Figures 7.1 and 7.2 display the survival curves for patients with stage 3 disease undergoing ACT by degree of proximal and distal AC respectively. There was no statistically significant differences evident in cancer-specific survival when assessed as a function of the degree of AC present among those undergoing ACT.

7.4 Discussion

The present study confirms that among eligible patients with resected stage II and III colorectal cancer, those who did not receive ACT were more likely to have AC. Furthermore, patients with AC who proceeded to ACT more frequently received single-agent therapy. The observation that the degree of AC was not significantly associated with dose reduction also highlights potential undertreatment in this group. However, the increased proportion of patients with proximal AC who failed to complete chemotherapy suggests that this group may be unable to tolerate full-dose, double-agent chemotherapy and, more generally, may derive limited benefit.

Several trends noted among the cohort undergoing adjuvant chemotherapy here point towards judicious use of chemotherapy. Overall adherence to chemotherapy was high, with over 70% of patients completing the planned number of cycles and over 70% of patients who were maintained on their original regime doing so at full dose. This suggests both appropriate patient selection for adjuvant therapy and use of appropriate regimens that may be less likely to result in toxicity. Patients with a more advanced TNM stage required dose reduction more frequently than those with earlier stage disease (31% vs 16%, p=0.046). This could stem from a reluctance to expose patients to potential toxicity in the absence of a solid evidence base for adjuvant therapy in node-negative disease. However, it may reflect underlying compromise related to more aggressive disease and cancer-associated phenomena such as sarcopenia that can dysregulate metabolism of cytotoxic drugs. While double agent chemotherapy was associated with a greater number of dose reductions and regime changes than single agent therapy, the proportion of patients completing chemotherapy was higher compared to those on single agent chemotherapy. This may be indicative of clinician assessment of baseline fitness, where patients with a degree of comorbidity or frailty are likely to have been preferentially assigned to single agent therapy in an attempt to modify the risk-benefit ratio.

Unlike the trend observed with proximal AC, patients with a BMI over 30 were equally represented among the cohort undergoing adjuvant chemotherapy and those who did not. However, such patients were significantly more likely to

require dose reductions during treatment. Recent studies have called for sex-specific muscle mass rather than body surface area to be used for chemotherapy dose calculation[555,556]. This may better account for the altered pharmacodynamics of chemotherapeutics in patients with sarcopenic obesity where reduced muscle mass may be masked by excess adiposity. It appears that based on history, clinicians are adept at rationalising chemotherapy to those without a significant cardiac history, but the development of a personalised approach to dosing based on more nuanced measures of body composition may be required to optimise chemotherapy adherence.

While randomised trial evidence of safety, tolerance and survival benefit from adjuvant chemotherapy in elderly and comorbid patients is available in both the pre and post-double agent ACT era[466,550,557,558], increasing age and comorbidity remain significant concerns for both surgeons and oncologists treating patients with colorectal cancer[548,549]. This may reflect the fact that elderly patients included in trials are generally regarded as a subgroup of fit patients who are not directly comparable to those seen in regular clinical practice. System-specific measures such as AC and more generalised indicators of fitness such as body composition derived from routinely obtained cross-sectional imaging may enable a greater appreciation of physiological age and the end-organ effects of comorbidity. Combining such data with that on quality of life from patients during and after treatment in both trial and non-trial settings is essential to enable a more personalised discussion of risks and benefits. In the face of an expanding elderly population and increasing multi-morbidity, the need for such data to guide shared decision-making and assist in rationalising of cost-intensive resources such as chemotherapy is clear.

This study has several limitations. The cohort undergoing chemotherapy was small, limiting analysis to univariate with no adjustment for confounders. Moreover, data was not available on the reasons for not referring for adjuvant chemotherapy. Toxicities were inconsistently recorded in a non-standard fashion and were therefore not included in the assessment of chemotherapy adherence. The single centre nature of the study also limits applicability, as previous work has suggested that local factors such as socioeconomic status may influence treatment[559]. Both patients with colon and rectal cancer were included in this

study. The differing treatment pathway of patients with margin-threatening rectal cancer is acknowledged. However, the aim was to describe the characteristics and adherence of the patients receiving adjuvant therapy. It was therefore an active decision to retain patients with rectal cancer in the cohort. Strengths include the fact that all patients were discussed in a colorectal cancer multidisciplinary meeting following resection and the decision to refer to oncology was therefore made in a forum rather than by the treating surgeon alone. The cohort included both trial and non-trial participants, reflecting real world practice. The inclusion of body composition metrics as variables of interest is planned in future multi-centre work assessing the role of patient factors in determinants of outcome from surgical and cytotoxic therapy for colorectal cancer.

In conclusion, this study has highlighted that AC may be associated with poorer adherence to chemotherapy but requires validation in an expanded cohort. In combination with other host factors such as body composition, it may be useful in highlighting patients with sub-clinical comorbidity and/or frailty that may be unmasked during ACT and associated with adverse effects. Further studies to investigate its clinical utility are required.

Table 7-1 - Baseline characteristics of patients with resected colorectal cancer by adjuvant chemotherapy status (n-404).

Characteristic		No adjuvant chemotherapy n=210 (%)	Adjuvant chemotherapy n=194 (%)	p-value
Age (years)	< 65	50 (24)	99 (51)	0.001
	65 – 75	76 (36)	83 (43)	
	> 75	84 (40)	12 (6)	
Gender	Male	113 (54)	114 (59)	0.316
	Female	97 (46)	80 (41)	
ASA grade	1 - 2	127 (61)	147 (76)	0.001
	3 - 4	83 (39)	47 (24)	
BMI[a]	< 30	148 (73)	120 (62)	0.022
	> 30	55 (27)	73 (38)	
Smoking history	No	84 (40)	105 (54)	0.004
	Yes	126 (60)	89 (46)	
Tumour site	Colon	133 (63)	134 (69)	0.223
	Rectum	77 (37)	60 (31)	
Surgical approach	Laparoscopic	61 (29)	89 (46)	0.001
	Open	149 (71)	105 (54)	
Clavien-Dindo complications	Grade 0	123 (59)	124 (64)	0.125
	Grade I – II	63 (30)	57 (29)	
	Grade III+	24 (11)	13 (7)	
TNM stage	II	129 (61)	48 (25)	0.001
	III	81 (39)	146 (75)	

Characteristic		No adjuvant chemotherapy n=210 (%)	Adjuvant chemotherapy n=194 (%)	p-value
Venous invasion	Absent	38 (18)	49 (25)	0.080
	Present	172 (82)	145 (75)	
Proximal AC	None	71 (34)	122 (63)	0.001
	Minor	75 (36)	54 (28)	
	Major	64 (30)	18 (9)	
Distal AC	None	32 (15)	65 (34)	0.001
	Minor	64 (31)	84 (43)	
	Major	114 (54)	45 (23)	
Intended chemotherapy	Capecitabine	-	51 (26)	-
	CAPOX	-	111 (57)	
	FOLFOX	-	30 (15)	
	Mod de Gramont	-	2 (1)	

[a] Missing cases – BMI 8 (no chemotherapy = 7, chemotherapy = 1)

Table 7-2 - Associations between clinicopathologic characteristics and adjuvant chemotherapy completion among patients with resected colorectal cancer (n=194).

		Completed cycles			**Dose reduced[a]**			**Regime changed**		
		No (n= 55 28%)	Yes (n=139 72%)	p-value	No (n= 129 73%)	Yes (n=48 27%)	p-value	No (n= 177 91%)	Yes (n= 17, 9%)	p-value
Age	< 65	22 (22)	77 (78)	0.082	61 (70)	26 (30)	0.121	87 (88)	12 (12)	0.069
	65 – 75	29 (35)	54 (65)		56 (72)	22 (28)		78 (94)	5 (6)	
	> 75	4 (33)	8 (67)		12 (100)	0 (0)		12 (100)	0 (0)	
Gender	Male	29 (25)	85 (75)	0.284	80 (74)	28 (26)	0.655	108 (95)	6 (5)	0.040
	Female	26 (33)	54 (67)		49 (71)	20 (29)		69 (86)	11 (14)	
ASA grade	1 – 2	40 (27)	107 (73)	0.533	96 (72)	37 (28)	0.715	133 (91)	14 (9)	0.507
	3 - 4	15 (32)	32 (68)		33 (75)	11 (25)		44 (94)	3 (6)	
BMI[a]	< 30	33 (27)	87 (73)	0.849	84 (78)	24 (22)	0.058	108 (90)	12 (10)	0.454
	> 30	21 (29)	52 (71)		44 (65)	24 (35)		68 (93)	5 (7)	
Smoking history	No	23 (22)	82 (78)	0.030	66 (68)	31 (32)	0.111	97 (92)	8 (8)	0.541
	Yes	32 (36)	57 (64)		63 (79)	17 (21)		80 (90)	9 (10)	
Tumour site	Colon	36 (27)	98 (73)	0.493	82 (69)	37 (31)	0.088	119 (89)	15 (11)	0.099
	Rectum	19 (32)	41 (68)		47 (81)	11 (19)		58 (97)	2 (3)	
TNM stage	II	17 (34)	33 (66)	0.304	41 (84)	8 (16)	0.046	49 (98)	1 (2)	0.077
	III	38 (26)	106 (74)		88 (69)	40 (31)		128 (89)	16 (11)	

		Completed cycles			Dose reduced[a]			Regime changed		
		No n= 55 (28%)	Yes n=139 (72%)	p-value	No n= 129 (73%)	Yes n=48 (27%)	p-value	No n= 177 (91%)	Yes n= 17 (9%)	p-value
Proximal AC	None	29 (24)	93 (76)	0.041	77 (69)	35 (31)	0.137	112 (92)	10 (8)	0.417
	Minor	18 (33)	36 (67)		40 (80)	10 (20)		50 (93)	4 (7)	
	Major	8 (44)	10 (56)		12 (80)	3 (20)		15 (83)	3 (17)	
Distal AC	None	18 (28)	47 (72)	0.320	44 (73)	16 (27)	0.326	60 (92)	5 (8)	0.350
	Minor	20 (24)	64 (76)		52 (67)	26 (33)		78 (93)	6 (7)	
Distal AC	Major	17 (38)	28 (62)		33 (85)	6 (15)		39 (87)	6 (13)	
Chemotherapy base	Capecitabine	48 (29)	114 (71)	0.374	112 (76)	36 (24)	0.059	148 (91)	14 (9)	0.557
	5FU	7 (22)	25 (78)		17 (59)	12 (41)		29 (91)	3 (9)	
Chemotherapy type	Single agent	26 (49)	27 (51)	0.001	45 (87)	7 (13)	0.008	52 (98)	1 (2)	0.045
	Double agent	29 (21)	112 (79)		84 (67)	41 (33)		125 (89)	16 (11)	

a) n = 177

Table 7-3 - Associations between degree of AC and adjuvant chemotherapy type and dose reduction among patients with resected colorectal cancer.

		Proximal AC			p-value	Distal AC			p-value
		None	Minor	Major		None	Minor	Major	
Chemotherapy type	Single	25 (47)	23 (43)	5 (10)	0.040	13 (25)	24 (45)	16 (30)	0.068
	Double	97 (69)	31 (22)	13 (9)		52 (37)	60 (43)	29 (20)	
Capecitabine reduced	No	86 (61)	43 (31)	12 (8)	0.860	49 (35)	56 (40)	36 (26)	0.876
	Yes	13 (62)	5 (24)	3 (14)		5 (24)	14 (67)	2 (9)	
5FU reduced	No	18 (72)	4 (16)	3 (12)	0.767	8 (32)	11 (44)	6 (24)	0.583
	Yes	5 (71)	2 (29)	0 (0)		3 (43)	3 (43)	1 (14)	
Oxaliplatin reduced	No	66 (69)	19 (20)	11 (11)	0.585	37 (39)	37 (39)	22 (22)	0.873
	Yes	31 (69)	12 (27)	2 (4)		15 (33)	23 (51)	7 (16)	

Figure 7-1 Kaplan-Meier curve demonstrating cancer-specific survival in patients undergoing ACT by degree of proximal AC (p=0.975).

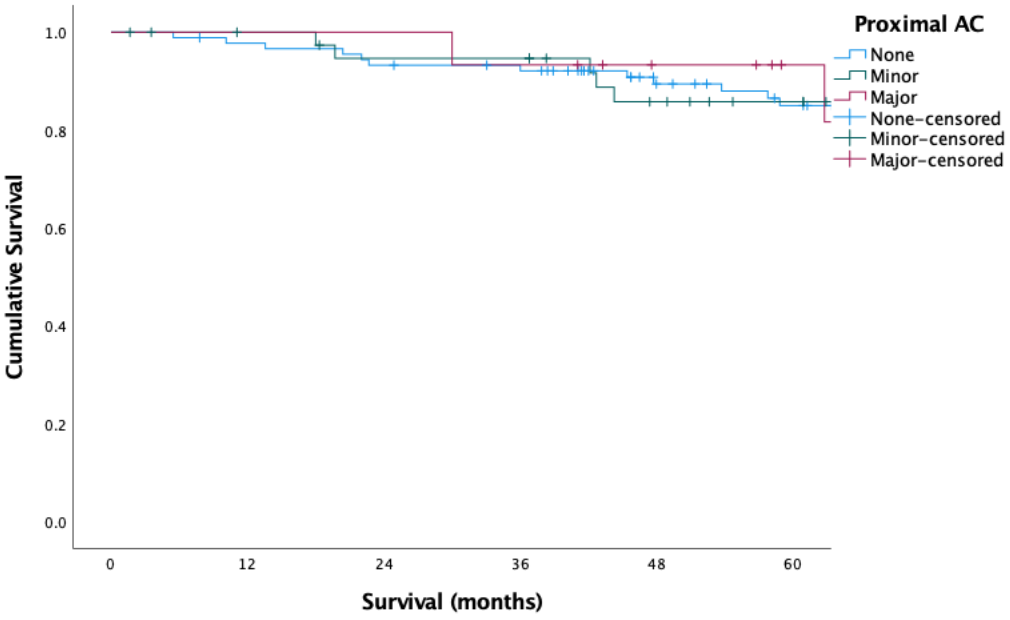
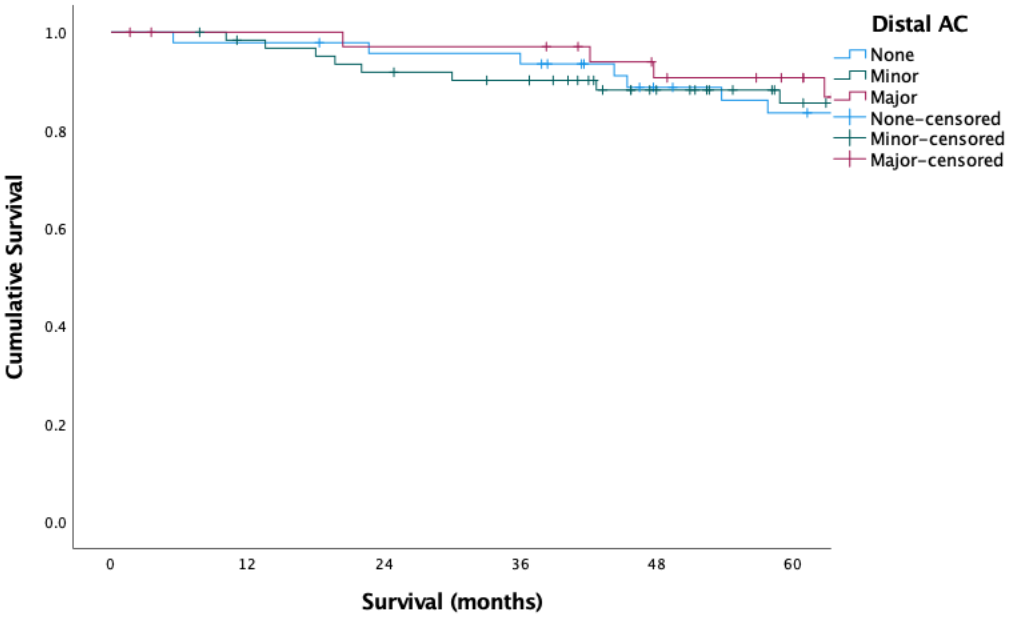


Figure 7-2 - Kaplan-Meier curve demonstrating cancer-specific survival in patients undergoing ACT by degree of distal AC (p=0.712).



8 Assessment of the relationship between aortic calcification and tumour hypoxia in colorectal cancer

8.1 Introduction

In previous chapters, the relationship between aortic calcification and clinical outcome has been examined. When specifically considering cancer outcomes, patients with right colon cancer who had more atherosclerotic disease within the aorta at the level of the SMA origin experienced inferior cancer-specific survival. No association between the degree of AC and higher rates of recurrence was evident in Chapter 5. While associations between a greater burden of AC and inability to tolerate or complete adjuvant chemotherapy were noted in Chapter 7, the comparatively small numbers of patients for whom adjuvant chemotherapy impacts survival make this unlikely to be a significant mediator. The hypothesis that a significant burden of proximal AC may promote a more ischaemic tumour environment with consequent upregulation of aggressive tumour features remains plausible.

The presence of tumour hypoxia on immunohistochemical analysis and multi-omics profiling has previously been reported to be a poor prognostic indicator in operable colorectal cancer [560,561]. The relationship between host atherosclerotic burden and tumour hypoxia has yet to be explored. Tumour hypoxia arises from an imbalance between oxygen supply and demand during carcinogenesis [562]. This is likely to be a dynamic process, with fluctuations in oxygen levels influenced by factors such as cellular metabolism as well as local blood flow. Tumour hypoxia is a key stimulator of epithelial to mesenchymal transition, a characteristic which acts to increase metastatic potential [518].

Carbonic anhydrase IX (CA IX) is a metabolic marker of tumour hypoxia and can be used to quantify areas of low oxygen tension [563]. A cell surface metallo-enzyme, it has a large extracellular domain, transmembrane region and short intracytoplasmic tail and is present in tumour tissue but not surrounding normal tissue. CA IX assists cell survival in the lower extracellular pH of hypoxic tumours by catalysing the reversible hydration of carbon dioxide to bicarbonate. It is upregulated by hypoxia-inducible factor HIF-1a, a marker influenced by hypoxic

and non-hypoxic stimuli. However, CA IX is comparatively stable and demonstrates lasting expression compared to HIF-1a, rendering it a reliable marker of tumour hypoxia [564].

It is unknown whether tumour hypoxia relates to markers of macrovascular health. Aortic calcification results in reduced vessel pliability [565] and may disrupt normal laminar flow within the aorta. Altered haemodynamics may result, influencing the flow of oxygenated blood to colorectal tumours.

It was hypothesised that increasing degrees of calcification may predispose to more hypoxic tumours in patients with operable colorectal cancer. The aim of this study was to assess the correlation between aortic calcification and tumour cell expression of carbonic anhydrase IX in patients with resected stage I-III colorectal cancer and evaluate the impact of these factors on survival.

8.2 Methods

Patients

Consecutive patients undergoing curative resection of TNM I-III colorectal cancer at Glasgow Royal Infirmary with available tissue microarray (TMA) cores and preoperative CT imaging were included. These patients formed a subset of the cohort reported in Chapter 4. Clinical and pathological characteristics were recorded as described in chapter 4. Ethical approval was provided by the NHS Research Scotland Greater Glasgow and Clyde Biorepository (reference number 544).

Assessment of CA IX

Immunohistochemistry for CA IX was performed at the Institute of Cancer Sciences, University of Glasgow. The protocol in Appendix 2 was followed. TMA slides were de-waxed using Histoclear (National Diagnostics, CA, USA) and rehydrated using graded ethanol and water. Heat-induced antigen retrieval was performed under pressure in a microwave using citrate buffer at pH 6. Endogenous peroxidase activity was blocked with 3% H_2O_2 and non-specific antibody binding blocked using 10% casein (v/v) (Vector Laboratories, Upper Heyford, UK) in antibody diluent (Agilent, Stockport, UK). TMAs were stained with CA IX (Bioscience, Slovakia) at 1:800 in diluent and incubated overnight at 4^{o}C. TMAs were then incubated for 30 minutes at room temperature in ImmPress (Vector Laboratories). Thereafter, DAB substrate (Vector Laboratories) was added and slides incubated for a further 5 minutes at room temperature. The slides were then counterstained in Harris Haematoxylin (ThermoFisher, Renfrew, UK), dehydrated in increasing gradients of ethanol and Histoclear and mounted using Pertex (Cellpath, Newton, UK). TMAs were scanned using Hamamatsu NanoZoomer Digital Slide Scanner and visualised using NanoZoomer NDP Digital Pathology viewer. Antibody specificity was confirmed using positive and negative cell pellets and a single band on a western blot (Figures 8.1 and 8.2). To rule out nonspecific staining, negative control slides (no antibody) were included (Figure 8.3).

Tumour cell CA IX expression in the membrane and cytoplasm was assessed using the weighted Histoscore method [566] and calculated as follows: (% of unstained tumour cells × 0) + (% of weakly stained tumour cells × 1) + (% of moderately stained tumour cells × 2) + (% of strongly stained tumour cells × 3) to give a score ranging from 0 to 300. Three 0.6 mm cores were scored per patient to take account of tumour heterogeneity. Each core was scored separately and an average score from the 3 cores obtained. Scoring was performed by a single blinded observer (KK) and 10% of cores co-scored by an independent observer blinded to clinicopathological characteristics and the primary observer's scores (JE).

Proximal and distal AC were scored on preoperative staging CT images as described previously. Overall and cancer-specific survival as defined in Chapter 5 were determined.

8.2.1 Statistical analysis

Descriptive statistics were used to summarise baseline characteristics. Proximal and distal AC scores were stratified with reference to the median as described in Chapter 3.0. The optimal cut-off for membranous and cytoplasmic CA IX expression and cancer-specific survival was determined using log-rank statistics from R studio (RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA). The Mantel-Haenszel test was used to assess associations between the degree of CA IX staining, AC and clinicopathological characteristics. The relationships between clinicopathological variables and survival were investigated using Kaplan Meier log rank analysis. A p-value of less than 0.05 was considered statistically significant.

The intra-class correlation coefficient (ICC) was used to assess intra- and inter-observer reliability. ICC estimates and their 95% confidence intervals (CI) were calculated based on a mean-rating (k = 2), one-way random effects model. ICC results were categorised as described in Chapter 3. Analysis was performed using SPSS Statistics for Mac (version 27.0; SPSS Inc., Chicago, IL, USA).

8.3 Results

In total, 651 patients undergoing curative colorectal cancer resection at Glasgow Royal Infirmary between 2008 to 2016 had complete follow up data and available CT imaging. Of these, 240 patients had corresponding TMA cores. Tumour cores were missing or inadequate for 17 patients, leaving 223 patients with complete data for evaluation.

The baseline clinical and pathological characteristics are outlined in Table 8.1. Most patients were male (53%), 65 years or older (65%), ASA class 1 or 2 (57%) and current or ex-smokers (58%). The majority had T stage 3 tumours (74%) and node-negative disease (65%) based on final pathology. Tumours were moderately or well differentiated in 91% with venous invasion present in 124 cases (56%).

Median follow-up for survivors was 93 months (minimum 75 months). During follow up, 82 patients died: 33 deaths due to cancer and 49 deaths due to other causes. Recurrence developed in 46 patients: 16 patients (7%) had local recurrence while 30 patients (14%) developed distant metastases.

Staining was positive for membranous CA IX in 146 patients (65%) and cytoplasmic CA IX in 141 patients (63%). Both membranous and cytoplasmic staining were present in 134 patients (60%). The median score for membranous CA IX was 7 (range 0-280) and for cytoplasmic CA IX was 7 (range 0 – 222). The cut-off point generated by log-rank analysis was 110 for membranous staining and 0 for cytoplasmic staining. On this basis, membranous CA IX staining was low in 211 patients (95%) and high in 12 patients (5%). Cytoplasmic CA IX expression was low in 81 (36%) and high in 141 (64%). Examples of low and high membranous and cytoplasmic CA IX expression are shown in Figures 8.4 and 8.5.

The associations between tumour cell CA IX expression and clinico-pathological characteristics are displayed in Table 8.2. There was no association between CA IX expression and patient characteristics including age, gender, BMI, ASA grade and smoking history. Similarly, there was no evidence of an association between membranous or cytoplasmic CA IX expression and the degree of proximal AC on CT. There was a non-significant trend between cytoplasmic CA IX expression and

distal AC. As the degree of AC increased, cytoplasmic CA IX expression decreased.

Pathological characteristics including T-stage, N-stage and presence of high-risk features such as venous invasion were not associated with tumour cell CA IX expression in this cohort. However, high membranous CA IX expression was positively associated with right-sided tumour location (11% right vs 2% left or rectal, p=0.006).

The Kaplan-Meier survival curves for tumour cell CA IX expression are shown in Figures 8.6-8.9. There was no evidence of a relationship on univariate analysis between membranous CA IX expression and cancer-specific or overall survival. For cytoplasmic CA IX expression, a non-significant association was evident: inferior survival was evident in patients with low CA IX expression compared with patients whose tumours had high levels of cytoplasmic CA IX (p=0.077). The small numbers in the high membranous group mandate cautious interpretation due to the risk of type II error.

Inter-rater reliability was excellent. The ICC for cytoplasmic CA IX expression was 0.92 (95% CI 0.88 - 0.95) and 0.94 (95% CI 0.91 - 0.96) for membranous CA IX expression. Visual inspection of scatter plots (Figures 8.10 and 8.12) and Bland-Altman plots (Figures 8.11 and 8.13) confirmed good correlation between raters for both sites of expression.

8.4 Discussion

This small study suggests that there may be an association between tumour cell cytoplasmic CA IX expression and the degree of AC visible on CT. This appears to be an inverse relationship, whereby increasing levels of calcification in the distal aorta are associated with low cytoplasmic CA IX expression that in turn may be associated with inferior cancer-specific survival. While these data are preliminary, the potential for macrovascular disease as represented by AC to influence tumour cell oxygenation and impact outcome remains possible.

The limited numbers mandate cautious interpretation. Only 5% of the cohort had high membranous CA IX expression. The association between right-sided tumour location and high membranous CA IX expression therefore needs validation in a larger cohort. The association between CA IX and cancer-specific survival was limited to cytoplasmic expression. However, the small number of survival events may have restricted our ability to detect a signal with membranous staining. This is particularly relevant as activation of CA IX stimulates translocation from cytoplasm to membrane. Therefore, low levels of cytoplasmic CA IX may represent activation rather than absence of hypoxia. However, all cases of high membranous CA IX expression were found to have cytoplasmic staining in this cohort, suggesting the mechanisms underpinning CA IX expression and survival are more complex.

In Chapter 5, inferior cancer-specific survival in patients with right-sided tumours and increasing degrees of proximal AC was noted on sub-group analysis. Right sided tumours constituted 37% of the cohort reported here. It is therefore not possible to further explore whether tumour hypoxia as represented by CA IX is implicated in poorer cancer-specific survival by virtue of aggressive features such as epithelial to mesenchymal transition.

Previous studies assessing CA IX expression in relation to clinical outcomes in patients with colorectal cancer are limited. In a study of 133 patients with resected colorectal cancer, no association between tumour cell expression and clinical outcome was evident, while tumour-associated stromal cell CA IX expression was associated with poorer overall survival [560]. Among 186 patients with resected TNM stage I-IV colorectal cancer and a median 23 months follow

up, 89% had positive CA IX staining; this was moderate to strong in only 7% [567]. No association with recurrence or survival was found. In contrast, poorer overall survival was associated with elevated CA IX expression in 85 patients with locally advanced rectal cancer undergoing neoadjuvant chemoradiation and resection [568]. However, it has been reported that the duration of ischaemia from arterial ligation to resection and fixation can alter levels of hypoxia-related protein expression in rectal cancer [569]. Of 166 patients with all-stage rectal cancer, 40% of whom had preoperative radiotherapy, CA IX was present in 44% of full resection specimens with moderate or strong staining evident in 29% [570]. The latter was associated with poor response to neoadjuvant therapy, inferior disease-free and cancer-specific survival. It was unclear if patients with metastatic disease were included in the cohort. In addition, the event rate was low with 16 patients developing recurrence and 28 cancer deaths. CA IX was not reported according to location, further restricting interpretation of its significance.

More recently, hypoxic gene signatures derived from multi-omics profiling of colorectal cancer have been associated with aggressive tumour features and adverse prognosis [561]. Minimal clinical data and unadjusted survival analysis were reported, necessitating further studies to expand our understanding of the mechanistic aspects of tumour hypoxia and robustly define its clinical impact in colorectal cancer.

It is clear that larger studies in more homogenous patient cohorts are required. Comparison with other markers of hypoxia including HIF-1a, glucose transporter-1 (GLUT-1) proteins, vascular endothelial growth factor (VEGF) and the chemokine receptor CXCR4 would enable a more nuanced appreciation of the presence of hypoxia within the tumour and surrounding microenvironment and its clinical relevance. Access to a contemporary cohort with both tissue and CT scan availability is necessary to further explore the potential association between distal AC and cytoplasmic CA IX expression. Work to expand the cohort in order to facilitate such a study is underway. The incorporation of scores generated by formal comorbidity assessment would provide much needed data to address the evidence gap around host characteristics, tumour and microenvironment characteristics and outcome. Therapeutic manipulation using CA IX inhibitors to combat the negative role of hypoxia in chemo- and

radiotherapy resistance has been proposed. However, further work to appreciate the underpinning mechanisms of tumour hypoxia and its interplay with oxygen-dependent therapies is first required.

In conclusion, this study suggests there may be an inverse correlation between an increasing degree of distal AC and cytoplasmic CA IX expression that appears to be associated with inferior CSS. Further studies to investigate this in a larger cohort are required.

Table 8-1 Baseline demographics and clinico-pathological features of patients undergoing curative colorectal cancer resection with available TMA cores (n=223).

Variable		N (%)
Age	<65	78 (35)
	65 - 75	81 (36)
	>75	64 (29)
Gender	Male	119 (53)
	Female	104 (47)
ASA grade	1 – 2	127 (57)
	3 - 4	96 (43)
BMI[a]	<30	136 (61)
	>30	83 (37)
Smoking history	No	93 (42)
	Yes	130 (58)
Tumour site	Colon	151 (68)
	Rectum	72 (32)

Variable		N (%)
T stage	I - II	59 (26)
	III	164 (74)
N stage	Negative	144 (65)
	Positive	79 (35)
Differentiation[b]	Well/moderate	203 (91)
	Poor	15 (7)
Venous invasion[c]	Absent	84 (38)
	Present	124 (56)

Abbreviations: Aortic calcification (AC), American Society of Anesthesiologists (ASA), body mass index (BMI).

Missing cases – a BMI (n=4) b Differentiation (n=5), c Venous invasion (n=15)

Table 8-2 Associations between tumour cell CA IX expression and clinico-pathological characteristics among patients undergoing curative colorectal cancer resection (n=223).

		Membrane CA IX		p-value	Cytoplasmic CA IX		p-value
		Low n = 211 (95%)	High n = 12 (5%)		Low n = 81 (36%)	High n = 141 (64%)	
Age	< 65	73 (94)	5 (6)	0.643	29 (37)	49 (63)	0.882
	65 – 75	77(95)	4 (5)		30 (37)	51 (63)	
	> 75	61 (95)	3 (5)		23 (36)	41 (64)	
Gender	Male	114 (96)	5 (4)	0.404	45 (38)	74 (62)	0.729
	Female	97(93)	7 (7)		37 (36)	67 (64)	
ASA grade	1 – 2	121 (95)	6 (5)	0.617	45 (24)	82 (65)	0.634
	3 - 4	90 (94)	6 (6)		37 (38)	59 (62)	

		Membrane CA IX			Cytoplasmic CA IX		
		Low n = 211 (95%)	High n = 12 (5%)		Low n = 81 (36%)	High n = 141 (64%)	
BMI[a]	< 30	130 (96)	6 (4)	0.596	50 (37)	86 (63)	0.926
	> 30	78 (94)	5 (6)		30 (36)	53 (64)	
Smoking history	No	90 (93)	7 (7)	0.287	30 (31)	67 (69)	0.111
	Yes	121 (96)	5(4)		52 (41)	74 (59)	
Proximal AC	None	90 (95)	5(5)	0.638	31 (33)	64 (67)	0.437
	Minor	72 (96)	3 (4)		31 (41)	44 (59)	
	Major	49 (93)	4 (7)		20 (38)	33 (62)	
Distal AC	None	49(96)	2 (4)	0.970	16 (31)	35 (69)	0.084
	Minor	76 (93)	6 (7)		26 (32)	56(68)	

	Major	86 (96)	4 (4)		40 (44)	50 (56)	
Tumour site[b]	Right	74 (89)	9 (11)	0.006	30 (36)	53 (64)	0.935
	Left/rectal	136 (98)	3 (2)		51 (37)	88 (63)	
T-stage	1 – 2	57 (97)	2 (3)	0.429	20 (33)	39 (66)	0.594
	3 – 4	154 (94)	10 (6)		62 (38)	102 (62)	
N-stage	Node-negative	137 (95)	7 (5)	0.642	55 (38)	89 (62)	0.532
	Node-positive	74 (74)	5 (6)		27 (34)	52 (66)	
Venous invasion	Present	84 (94)	5 (6)	0.898	31 (35)	58 (65)	0.624
	Absent	127 (95)	7 (5)		51 (38)	83(62)	

Figure 8-1 - Examples of positive (left) and negative (right) staining using cell pellets.

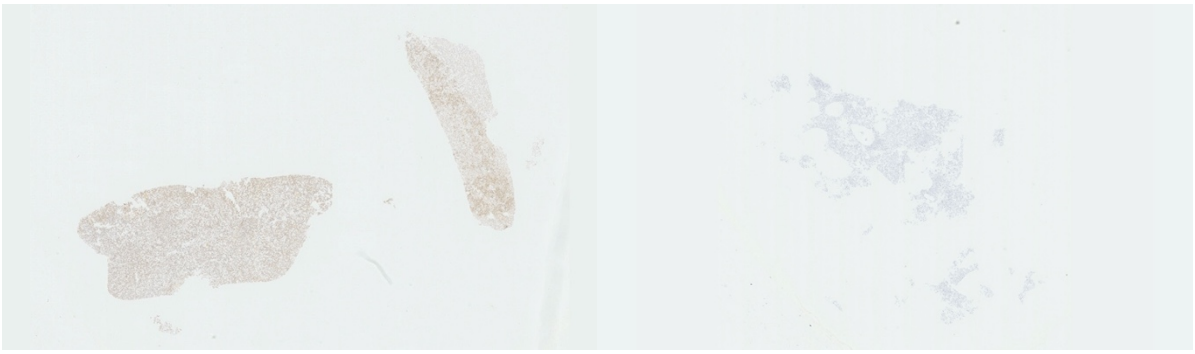


Figure 8-2 - Negative (no antibody) control.

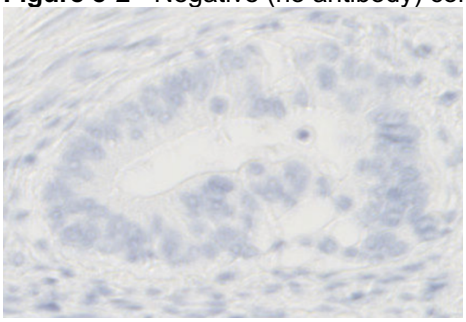


Figure 8-3 - Examples of low (left) and high (right) membranous CA IX expression.

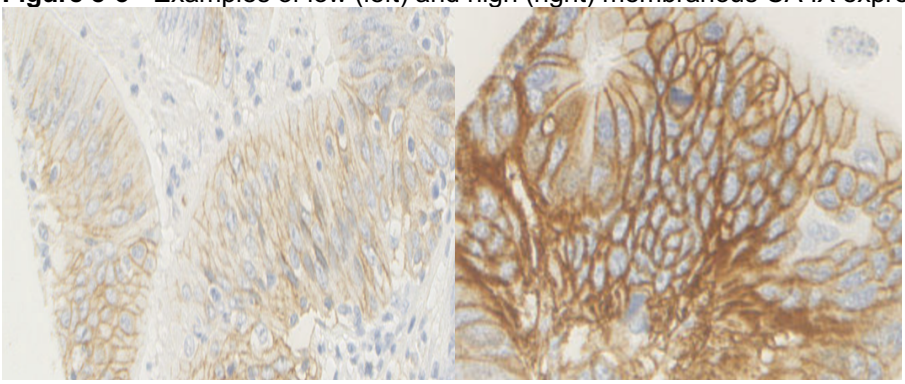


Figure 8-4 - Examples of low (left) and high (right) cytoplasmic CA IX staining.

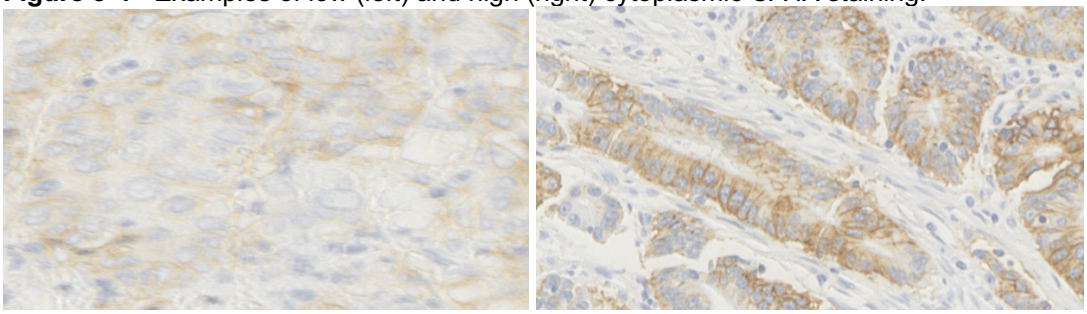


Figure 8-5 - Kaplan-Meier curves demonstrating the relationship between membranous CA IX expression and cancer-specific survival (P=0.511).

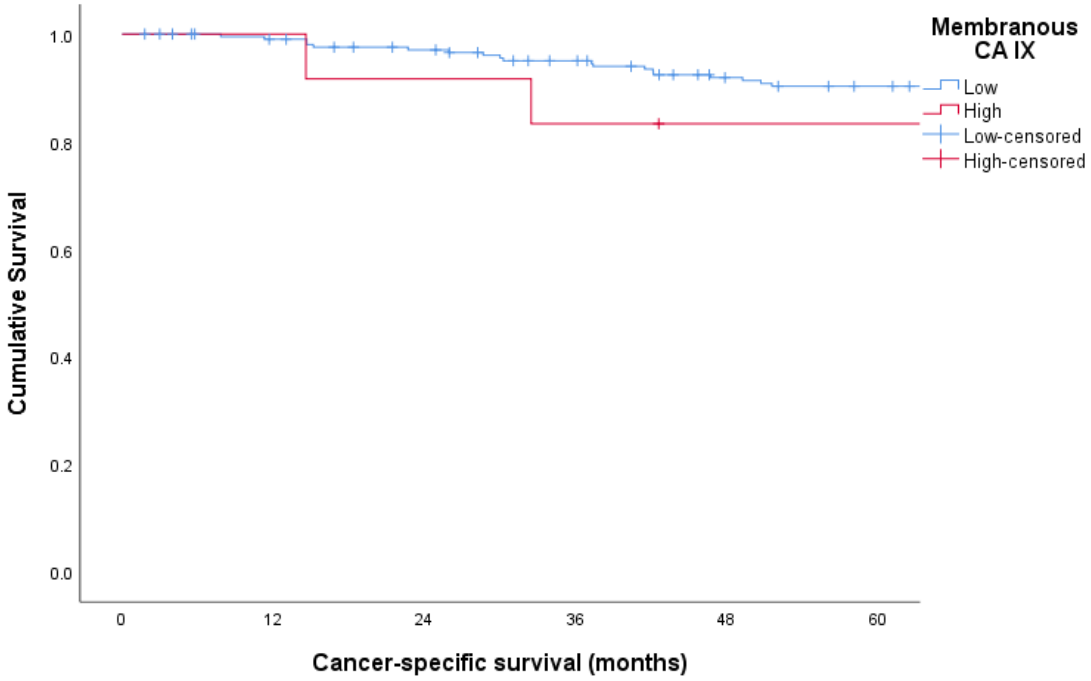


Figure 8-6 - Kaplan-Meier curves demonstrating the relationship between membranous CA IX expression and overall survival (P=0.427).

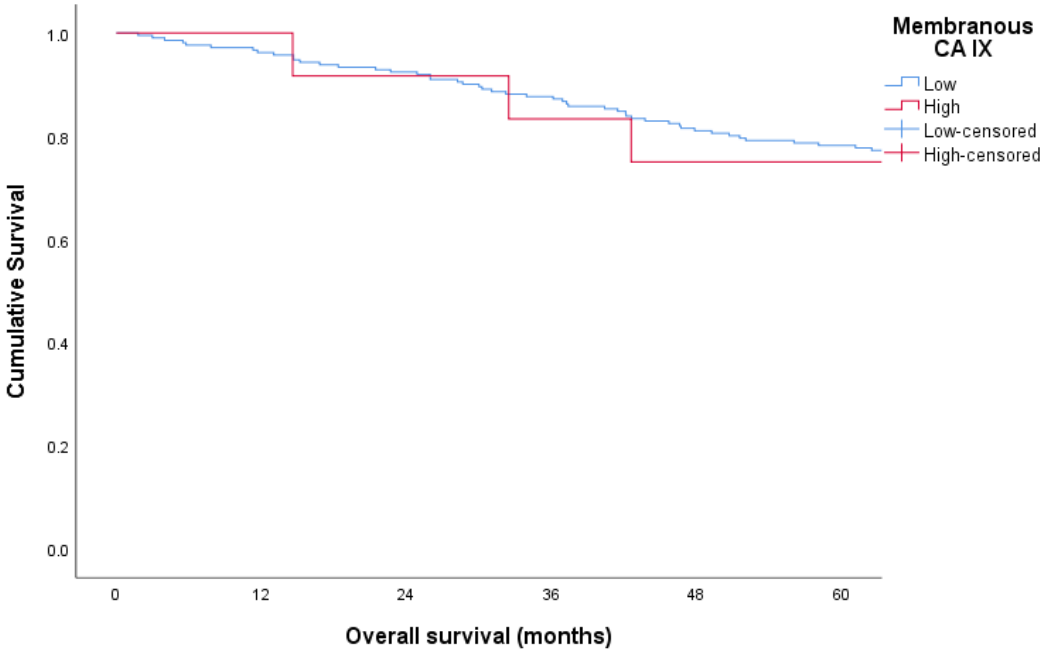


Figure 8-7 - Kaplan-Meier curves demonstrating the relationship between cytoplasmic CA IX expression and cancer-specific survival (P=0.077).

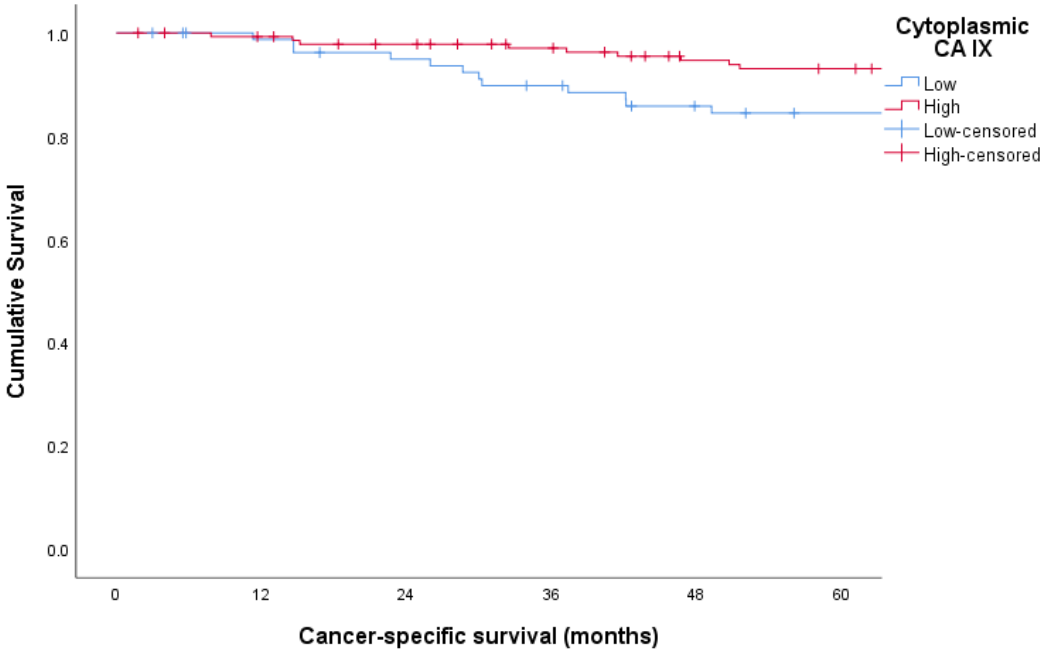


Figure 8-8 - Kaplan-Meier curves demonstrating the relationship between cytoplasmic CA IX expression and overall survival (P=0.189).

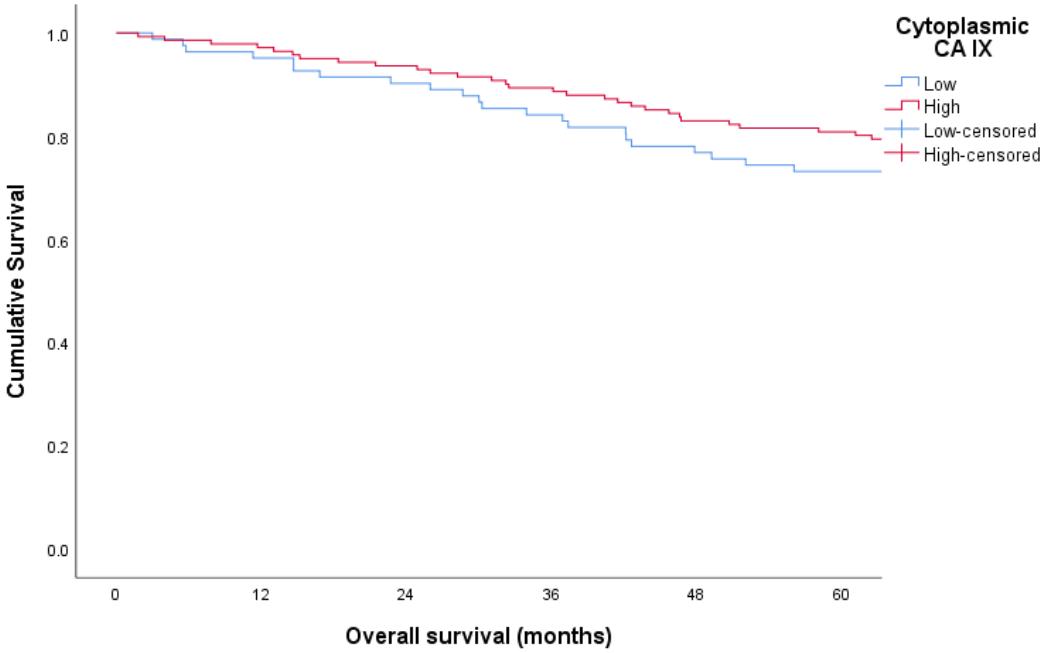


Figure 8-9 - Scatter plot of membranous CA IX scores between 2 raters.

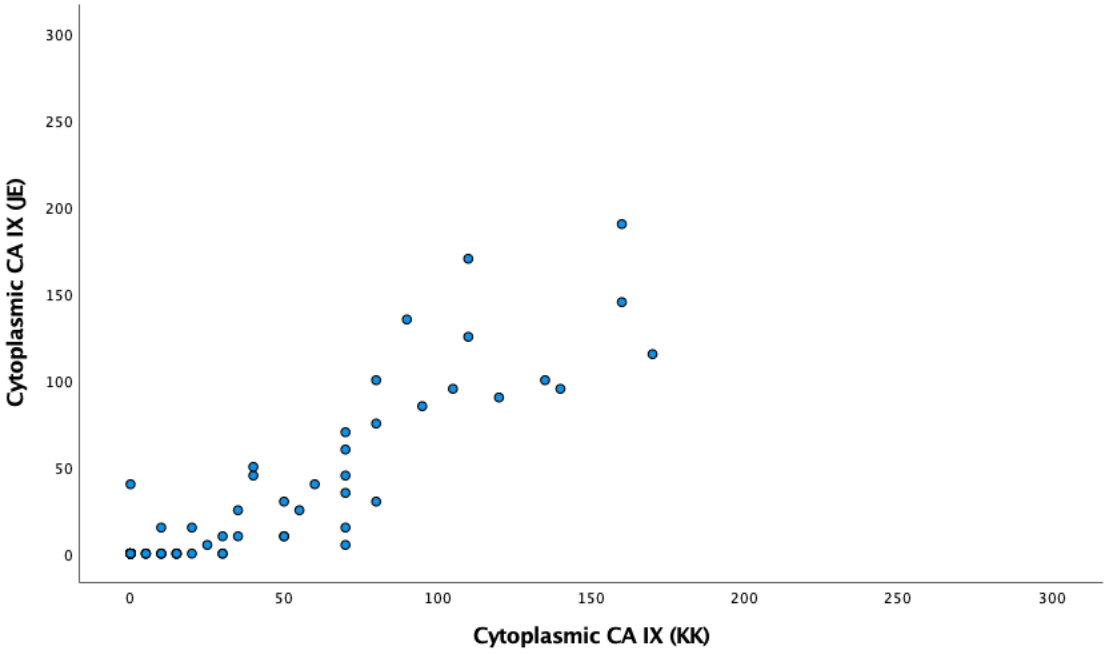


Figure 8-10 - Bland-Altman plot of membranous CA IX scores between 2 raters.

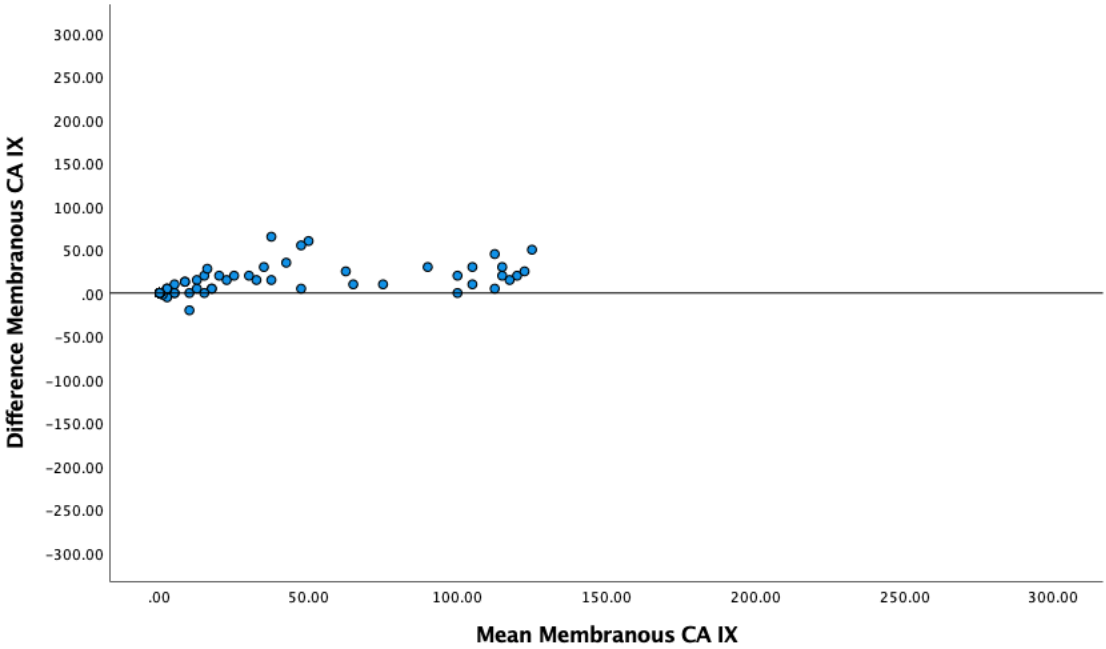


Figure 8-11 - Scatter plot of cytoplasmic CA IX scores between 2 raters.

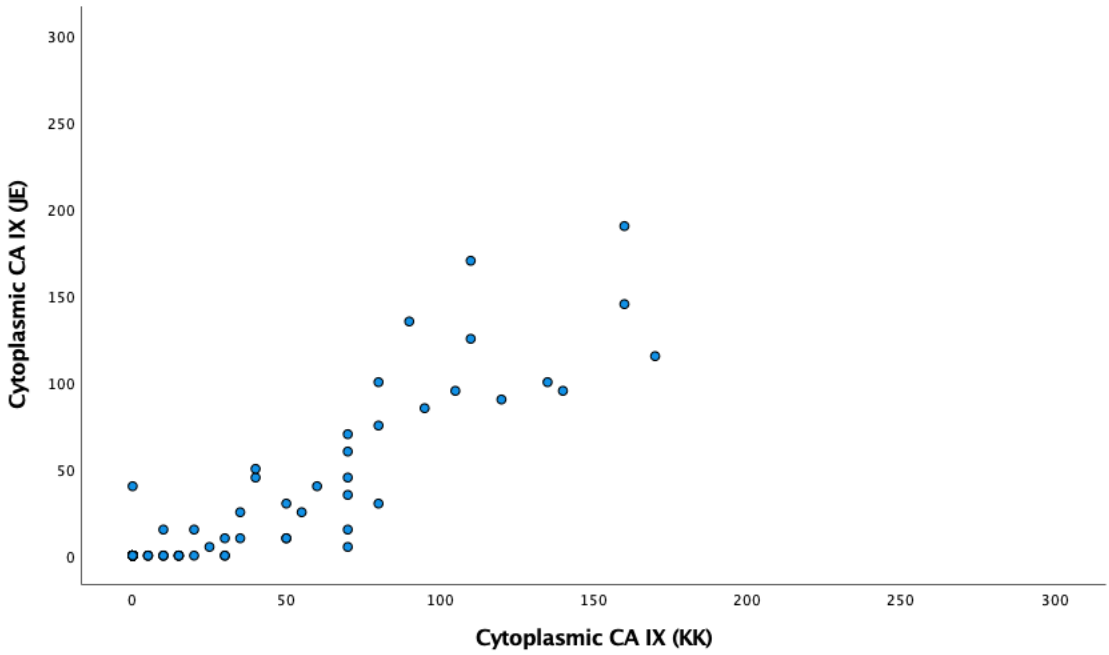
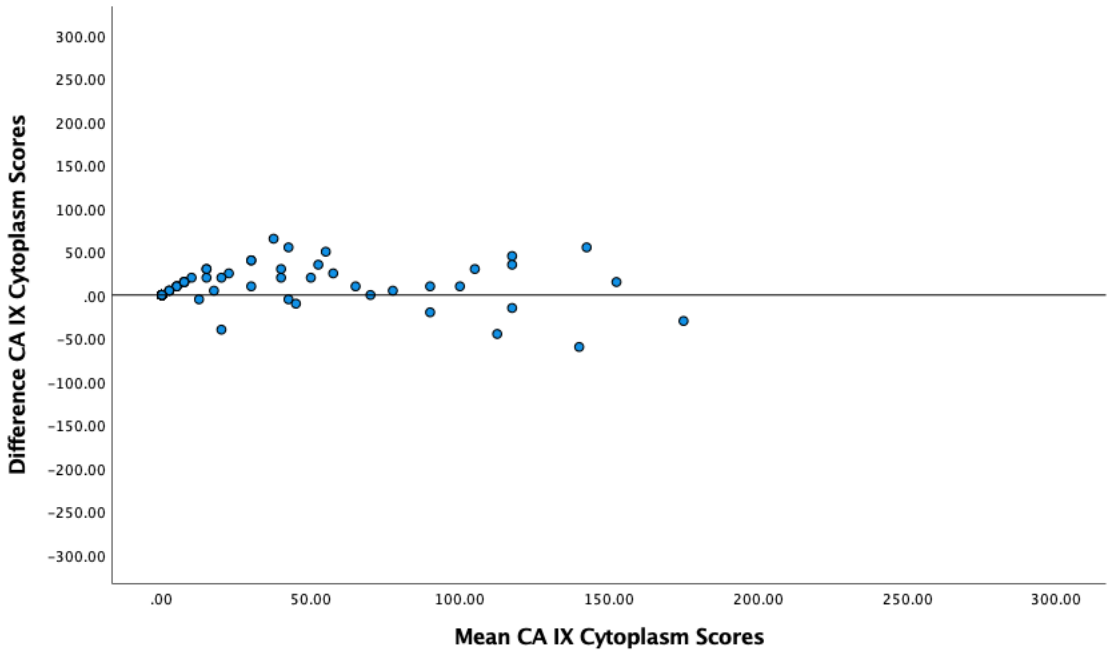


Figure 8-12 - Bland-Altman plot of cytoplasmic CA IX scores between 2 raters.



9 An investigation of the relationship between aortic calcification and systemic inflammation in patients undergoing curative colorectal cancer resection

9.1 Introduction

As a feature of both colorectal cancer and atherosclerotic cardiovascular disease, systemic inflammation is an important potential mediator of the relationships identified in previous chapters between AC and inferior outcome. Acute inflammation, a normal homeostatic response to infection, temporarily induces activation of immune and non-immune cells to protect the host from infection and promote tissue repair[138,571572]. The resulting local or systemic inflammatory response is usually terminated when clearance of the stimuli, whether infectious or sterile, is achieved.

In chronic inflammation, however, active termination of the inflammatory response fails to occur. Promulgation of the systemic inflammatory response (SIR) triggered by a non-infectious agent leads to a state of sustained chronic inflammation[573]. The SIR is of a lower order of magnitude than an acute inflammatory response[138], involves multiple organs and results in detectable alterations in circulating levels of white cells including neutrophils, lymphocytes, monocytes, platelets and acute-phase proteins, such as C-reactive protein (CRP) and albumin. The magnitude of the SIR can be assessed using these biomarkers.

In cardiovascular disease, pro-inflammatory cytokines such as interleukin-6 promote leucocyte infiltration to the arterial intima and alter smooth muscle cell function, contributing to atheromatous plaque formation[347,348]. Increased expression of endothelial adhesion molecules facilitating infiltration are common at arterial branching points[349]. While a varied cast of inflammatory mediators are implicated in atheroma formation, C-reactive protein (CRP), the downstream effector of the IL-6 pathway, has been used as an indicator of future risk of cardiovascular events. In patients with known atherosclerotic disease, a raised CRP is independently predictive of future cardiovascular events[574] and death[351].

In colorectal cancer, the SIR holds similar prognostic value[40,352]. Tumour-associated inflammation is a complex phenomenon characterised by both the local immune and systemic inflammatory response. The temporal influence of systemic inflammation in colorectal carcinogenesis remains unclear, but it is accepted that the SIR underlies cancer-associated cachexia in advanced disease. In patients with operable colorectal cancer, the presence of a preoperative SIR measured using the modified Glasgow Prognostic Score (mGPS) is prognostic of inferior survival, independent of established determinants including tumour stage[353] and host comorbidity[354].

While CRP as a biomarker offers limited mechanistic insight into the underlying pathogenesis of cardiovascular disease and colorectal cancer, it is a readily available and well validated prognostic marker in both populations[575]. The mGPS, based on CRP and albumin levels, can be used to assess for the presence of systemic inflammation. Given their shared inflammatory aetiology, it is possible that systemic inflammation is a manifestation of pre-existing aortic calcification in patients with colorectal cancer. In this case, patients with a significant burden of aortic calcification may be more likely to have a raised mGPS and by extension, poorer prognosis.

It has previously been demonstrated that patients with right sided tumours and those with more advanced T stage exhibit higher rates of systemic inflammation[576]. Given the relationships identified in previous chapters between a higher burden of AC, right-sided tumour location and inferior survival, it is possible that these factors are interrelated through the presence of a SIR. Furthermore, characterising the clinical utility of AC requires comparison of the strength of these relationships with an established measure such as the mGPS. Therefore, this study aimed to investigate the hypothesis that AC is associated with higher rates of systemic inflammation and determine whether AC is more closely related to survival than the mGPS following elective, curative intent colorectal cancer resection.

9.2 Methods

Patients who had undergone potentially curative resection of histologically proven colorectal cancer were identified from a prospectively maintained CRC database. Exclusion criteria were similar to Chapter 5. In addition, patients for whom the preoperative blood results were not available were excluded. Tumour were staged according to the fifth edition of the AJCC TNM manual[213] and the second edition of the Royal College of Pathologists dataset for colorectal cancer reporting[537].

The results of routine serum blood samples obtained at the pre-operative assessment visit prior to the date of surgery were extracted from the electronic medical record. Systemic inflammation was assessed using the modified Glasgow Prognostic score (mGPS). The mGPS was recorded as 0 for those patients with an albumin greater than 35 g/L and CRP less than 10mg/L, 1 for those with CRP greater than 10mg/L and 2 for those with an albumin value of less than 35 g/L and CRP greater than 10mg/L. A mGPS score of greater than 0 was regarded as evidence of systemic inflammation.

Aortic calcification was assessed on abdominopelvic pre-operative CT imaging and the scores categorised as described in Chapter 3.

The follow up schedule described in Chapter 5 applied to all patients in this cohort. Cancer-specific survival (CSS) was measured from the date of surgical resection until date of death from recurrent or metastatic colorectal cancer. Overall survival (OS) was measured from the date of surgical resection until date of death due to other causes. Data was censored on 06.09.2019.

9.2.1 Statistical analysis

Descriptive statistics were used to summarise the baseline characteristics of the cohort. The median and interquartile range (IQR) were used to describe the calcification scores. The associations between clinico-pathological characteristics, systemic inflammation and aortic calcification were assessed using the chi-squared or Mantel-Haenszel test for trend as appropriate. The relationship between clinico-pathologic variables and survival was assessed using

the log-rank Kaplan-Meier method and multivariate Cox regression analysis. Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA).

9.3 Results

Of 657 patients who underwent curative resection of colorectal cancer between 2008 and 2016, 640 patients met the inclusion criteria. Exclusions included patients who died within 30 days of resection (n=6) and those for whom preoperative blood results were not available (n=11).

Baseline characteristics are shown in Table 9.1. Most patients were over 65 years old (73%) and male (55%). The majority of tumours were located in the colon (65%). AC was less common in the proximal aorta (absent in 49% of patients) than in the distal aorta (absent in 25% of patients). Preoperative systemic inflammation was present in 150 patients (23%).

On examining the associations between calcification and systemic inflammation (Table 9.2), it was evident that a higher proportion of patients with calcification were inflamed when compared with those with no calcification (proximal AC and raised mGPS 53% vs 19% p=0.027, distal AC and raised mGPS 52% vs 16%, p=0.016). The trend did not follow a linear pattern and the difference was most evident when patients with no calcification were compared with patients with either minor or major calcification. On examining patients with colon cancer, an association between a raised mGPS and an increased burden of calcification was evident both in the proximal and distal aorta, but the difference was not statistically significant. No statistically significant association between the mGPS and the burden of proximal or distal calcification was evident in patients with rectal cancer.

Associations between SIR status and clinico-pathological characteristics are displayed in Table 9.3. Increasing age, higher T stage, right colonic tumour site and the presence of distal AC were associated with a raised mGPS.

The relationships between clinicopathological factors and overall survival are displayed in Table 9.4. On univariate analysis, increasing age, smoking, higher ASA grade, more advanced T stage and node-positive disease were associated with inferior survival, in addition to a raised mGPS and the presence of proximal and distal AC. On multivariate analysis, increasing age, higher ASA grade, node-

positive disease, a raised mGPS and the presence of proximal AC were independently related to inferior overall survival.

For cancer-specific survival (Table 9.5), advanced T stage and node-positive disease were associated on both univariate and multivariate analysis. No relationship was evident on univariate analysis between other clinico-pathological characteristics and cancer-specific survival, including the site and degree of AC and the presence of systemic inflammation.

Based on the observation in Chapter 5 that proximal AC was related to cancer-specific survival in patients with right colon cancer, a subset analysis was undertaken to assess whether AC remained independently related when assessed in conjunction with SIR status and TNM stage (Table 9.6). On univariate analysis, an increasing burden of proximal AC and more advanced TNM stage were associated with right colon cancer-specific survival; the mGPS and the degree of distal AC was not significantly related. On multivariate analysis, both proximal AC and TNM stage remained independently related, although the hazard ratio for TNM stage was much greater than that of proximal AC.

Kaplan-Meier curves demonstrating the impact of the presence, alone or in combination, of AC and SIR on overall and cancer-specific survival are shown in Figures 9.1 and 9.2. Poorer overall survival was evident in patients with a SIR. When combined with AC, survival was further impaired. AC and SIR status were not associated with cancer-specific survival.

9.4 Discussion

Building on the relationship identified in Chapter 5 between aortic calcification and overall survival, this study confirms systemic inflammation to be an independent factor influencing survival in addition to proximal AC. A stronger relationship between an elevated mGPS and inferior overall survival was indicated by the larger hazard ratio associated with the mGPS. However, the previously identified association between proximal AC and inferior cancer-specific survival in right colon cancer was not influenced by the presence of a SIR. The mechanisms underlying this independent relationship do not appear be strongly inflammatory in origin, suggesting alternative pathways mediate the interaction between local atherosclerosis and colon carcinogenesis.

It was hypothesised that aortic calcification may be a mediator of the influence of systemic inflammation on survival following colorectal cancer resection. Smoking, obesity and impaired glucose tolerance are independent risk factors for cardiovascular disease and are recognised inducers of chronic inflammation[577-579]. Population studies have demonstrated raised CRP levels in participants with such risk factors in the absence of a history of cardiovascular disease[580]. Correlation between higher baseline CRP values and the presence of coronary arterial calcification has been reported in a large cohort of asymptomatic patients[581]. However, a CRP threshold of 3 mg/L is recommended for the purposes of cardiovascular risk stratification of asymptomatic patients[350,582]. A threshold of 10 mg/L is used in the mGPS to differentiate between the presence and absence of systemic inflammation. It can be inferred that the magnitude of the SIR associated with cardiovascular disease is not directly comparable to that in established colorectal cancer.

The presence of systemic inflammation is intricately linked with local inflammation in the context of colorectal cancer. While peritumoral inflammation can confer prognostic benefit through increased infiltration of cytotoxic T cells[583-585], systemic inflammation is associated with impaired cell-mediated immunity that can aid the development and progression of colorectal cancer[586]. Not all patients with colorectal cancer are systemically inflamed and in those who are, a clear link between carcinogenesis and the induction of systemic inflammation remains elusive. However, the presence of high-risk

pathological features such as tumour necrosis have been implicated[587]. In colorectal cancer, tumour necrosis has been associated with both the presence of systemic inflammation and reduced local lymphocytic infiltration of the primary tumour, translating to inferior survival[355]. Necrosis is thought to result from an imbalance in tumour oxygen demand and supply and stimulates neo-angiogenesis, with implications for haematogenous dissemination[356,357]. It is conceivable that patients with pre-existing vascular calcification may be more predisposed to developing an ischaemic tumour microenvironment and resulting tumour necrosis. Characterising the tumour microenvironment in greater detail in a large sample as suggested in the preceding chapter is required to investigate and validate this hypothesis. However, in view of the lack of association between AC and cancer-specific survival in the cohort including all colon and rectal cancer, any possible correlation may be anticipated to be minor.

There appears, however, to be an association between the presence of calcification and inferior cancer-specific survival in patients with right colon cancer. Given the limited number of survival events in subgroups of tumour site, it is clear that the association between AC and right colonic tumour location requires assessment in a larger and ideally external cohort. Indeed, Wang and colleagues reported a higher prevalence of cardiovascular disease in patients with right-sided tumours[176], raising the possibility of an interaction between host comorbidity and tumour formation. It is possible that this finding reflects the segmental nature of colonic perfusion, whereby calcification of the proximal aortic region from which the right colon arterial supply arises influences outcome. However, right-sided tumours are characterised by different genomic features and host characteristics including female gender and advanced age[588]. Clearly, multiple factors may underlie an association between proximal AC and tumour factors. Further studies are required.

This study is limited by its retrospective nature and the use of the mGPS as the singular measure of systemic inflammation. Consideration was given to the use of other measures of SIR such as the neutrophil to lymphocyte ratio (NLR) that have been examined in patients with colorectal cancer. However, the optimal NLR threshold used to define systemic inflammation remains a source of debate with previous studies using differing values[589]. Furthermore, the aim of the study

was to assess the association between aortic calcification and systemic inflammation using the mGPS as it incorporates CRP, a prognostic indicator in patients with cardiovascular disease and colorectal cancer. The applicability of the results may be influenced by the fact that measurement of CRP in the preoperative period is not routinely performed as part of preoperative assessment of patients with colorectal cancer. Future work comparing alternative measures of the SIR in the context of the degree of calcification may therefore be worthwhile. Smoking, comorbidity and obesity are also relevant patient characteristics known to influence baseline CRP levels. Their contribution to the burden of AC and its impact on short- and long-term outcome in patients with colorectal cancer is examined elsewhere in this thesis.

In conclusion, this study confirmed that the presence of systemic inflammation as represented by the mGPS is more closely related to inferior survival than the degree of AC. In contrast to the study hypothesis, the relationship between AC and cancer-specific survival in right colon cancer does not appear to be clearly related to the presence of systemic inflammation. Future studies examining this in larger cohorts of colonic tumour site are required to validate these findings, while more detailed tissue-based assessment as outlined in Chapter 8 is required to investigate the hypothesis that atherosclerotic disease correlates with tumour hypoxia in patients with operable colorectal cancer.

Table 9-1 - Baseline characteristics of study population (n=640).

Variable		N (%)
Age	<65	237 (37)
	65 - 75	252 (39)
	>75	153 (24)
Gender	Female	286 (45)
	Male	354 (55)
Tumour site	Colon	418 (65)
	Rectum	222 (35)
mGPS	0	490 (77)
	1	64 (10)
	2	86 (13)
Proximal AC	None	311 (49)
	Minor	202 (31)
	Major	127 (20)
Distal AC	None	622 (25)
	Minor	227 (36)
	Major	251 (39)

Table 9-2 - Associations between the site and degree of calcification and systemic inflammation in patients undergoing elective curative colorectal cancer resection (n=640).

	mGPS	Proximal AC				Distal AC			
		None n (%)	Minor n (%)	Major n (%)	p-value	None n (%)	Minor n (%)	Major n (%)	p-value
All	0	250 (51)	145 (30)	95 (19)	0.027	136 (27)	169 (34)	185 (38)	0.016
	1	32 (50)	21 (33)	11 (17)		13 (21)	25 (39)	26 (40)	
	2	29 (34)	36 (42)	21 (24)		13 (15)	33 (38)	40 (47)	
Colons	0	148 (49)	91 (30)	66 (21)	0.074	79 (26)	104 (34)	122 (40)	0.078
	1	20 (43)	20 (43)	7 (14)		10 (21)	18 (38)	19 (41)	
	2	21 (32)	28 (42)	17 (26)		11 (11)	22 (33)	33 (50)	
Rectals	0	102 (55)	54 (29)	29 (16)	0.606	57 (31)	65 (35)	63 (34)	0.138
	1 - 2	20 (54)	9 (24)	8 (22)		5 (14)	18 (49)	14 (38)	

Abbreviations: AC aortic calcification, mGPS modified Glasgow Prognostic Score.

Table 9-3 - Associations between SIR status as denoted by mGPS and clinico-pathological characteristics in patients undergoing elective curative colorectal cancer resection (n=640).

Characteristic		mGPS 0 n = 490 (%)	mGPS 1-2 n = 150 (%)	p-value
Age	< 65	190 (80)	47 (20)	0.050
	65 – 75	192 (76)	60 (24)	
	> 75	108 (72)	43 (28)	
Gender	Male	268 (76)	86 (24)	0.569
	Female	222 (78)	64 (22)	
ASA grade	1 – 2	345 (79)	94 (21)	0.074
	3 - 4	145 (72)	56 (28)	
BMI[a]	< 30	321 (77)	97 (23)	0.996
	> 30	162 (77)	49 (23)	
Smoking history	No	240 (80)	60 (20)	0.054
	Yes	250 (74)	90 (26)	
Tumour site [b]	Right	160 (70)	71 (30)	0.001
	Left	143 (77)	42 (23)	
	Rectal	185 (83)	37 (17)	

Characteristic		mGPS 0 n = 490 (%)	mGPS 1-2 n = 150 (%)	p-value
T-stage	1 – 2	170 (89)	21 (11)	0.001
	3 – 4	320 (71)	129 (29)	
N-stage	Node-negative	317 (75)	105 (25)	0.230
	Node-positive	173 (79)	45 (21)	
Proximal AC	None	250 (80)	61 (20)	0.090
	Minor	145 (72)	57 (28)	
	Major	95 (75)	32 (25)	
Distal AC	None	136 (40)	26 (16)	0.024
	Minor	169 (74)	58 (26)	
	Major	185 (74)	66 (26)	

Abbreviations: AC aortic calcification, ASA American Society of Anaesthesiology, mGPS modified Glasgow Prognostic Score

Table 9-4 – Cox regression analysis of factors associated with overall survival in patients undergoing curative colorectal cancer resection (n=640).

	Univariate		Multivariate	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Age (<65/65-74/>75)	1.69 (1.39 – 2.06)	<0.001	1.42 (1.14 – 1.77)	0.001
Sex (Female/male)	1.33 (0.99 – 1.80)	0.060	-	-
BMI (<30/>30)	0.73 (0.53 – 1.02)	0.069	-	-
ASA (1 – 2 / 3 – 4)	1.76 (1.31 – 2.35)	<0.001	1.36 (1.01 – 1.84)	0.043
Smoking history (No/yes)	1.39 (1.03 – 1.87)	0.031	1.32 (0.96 – 1.81)	0.085
Tumour site (Colon/rectum)	0.96 (0.71 – 1.31)	0.808	-	-
T-stage (1-2/3-4)	2.12 (1.45 – 3.11)	<0.001	1.49 (0.99 – 2.22)	0.054
N-stage (Negative/positive)	1.71 (1.28 – 2.29)	<0.001	1.69 (1.25 – 2.29)	0.001
mGPS (0/1-2)	1.81 (1.33 – 2.46)	<0.001	1.59 (1.16 – 2.18)	0.004
Proximal AC (None/minor/major)	1.61 (1.34 – 1.93)	<0.001	1.31 (1.07 – 1.62)	0.010
Distal AC (None/minor/major)	1.43 (1.18 – 1.74)	<0.001	0.92 (0.71 – 1.19)	0.507

Abbreviations: AC aortic calcification, ASA American Society of Anaesthesiology, BMI body mass index, mGPS modified Glasgow Prognostic Score.

Table 9-5 - Cox regression analysis of factors associated with cancer-specific survival in patients undergoing curative colorectal cancer resection (n=640).

	Univariate		Multivariate	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Age (<65/65-74/>75)	1.18 (0.89 – 1.57)	0.253	-	-
Sex (Female/male)	1.39 (0.89 – 2.16)	0.146	-	-
BMI (<30/>30)	0.81 (0.50 – 1.31)	0.386	-	-
ASA (1 – 2 / 3 – 4)	1.02 (0.64 – 1.62)	0.937	-	-
Smoking history (No/yes)	0.93 (0.60 – 1.42)	0.721	-	-
Tumour site (Colon/rectum)	1.52 (0.99 – 2.33)	0.056	-	-
T-stage (1-2/3-4)	3.05 (1.62 – 5.75)	<0.001	2.21 (1.15 – 4.25)	0.017
N-stage (Negative/positive)	3.25 (2.10 – 5.04)	<0.001	2.74 (1.75 – 4.31)	<0.001
mGPS (0/1-2)	1.44 (0.90 – 2.32)	0.129	-	-
Proximal AC (None/minor/major)	1.23 (0.94 – 1.62)	0.131	-	-
Distal AC (None/minor/major)	1.10 (0.84 – 1.44)	0.494	-	-

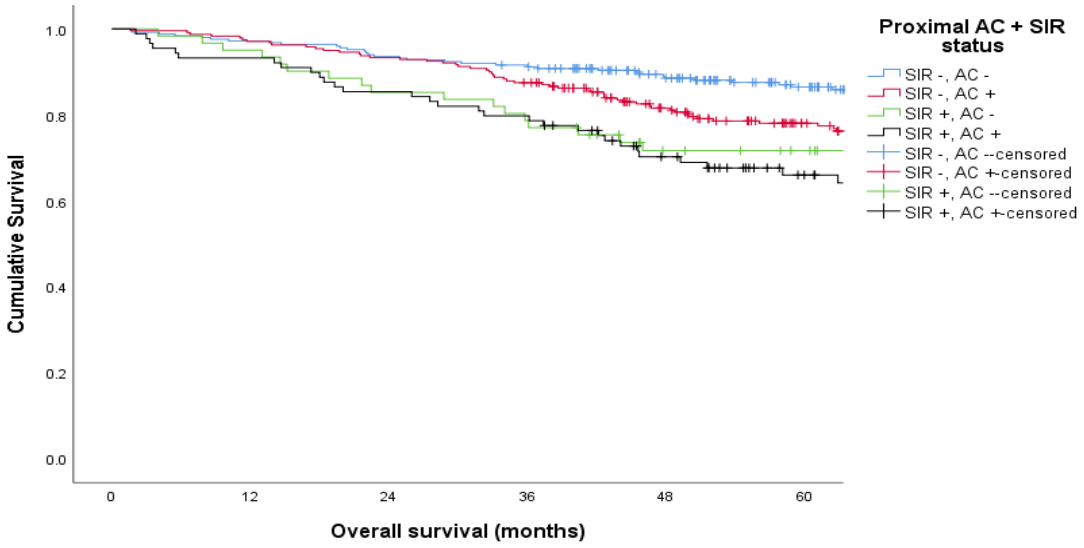
Abbreviations: AC aortic calcification, ASA American Society of Anaesthesiology, mGPS modified Glasgow Prognostic Score.

Table 9-6 - Cox regression analysis of factors associated with cancer-specific survival in patients with resected right colon cancer (n=231).

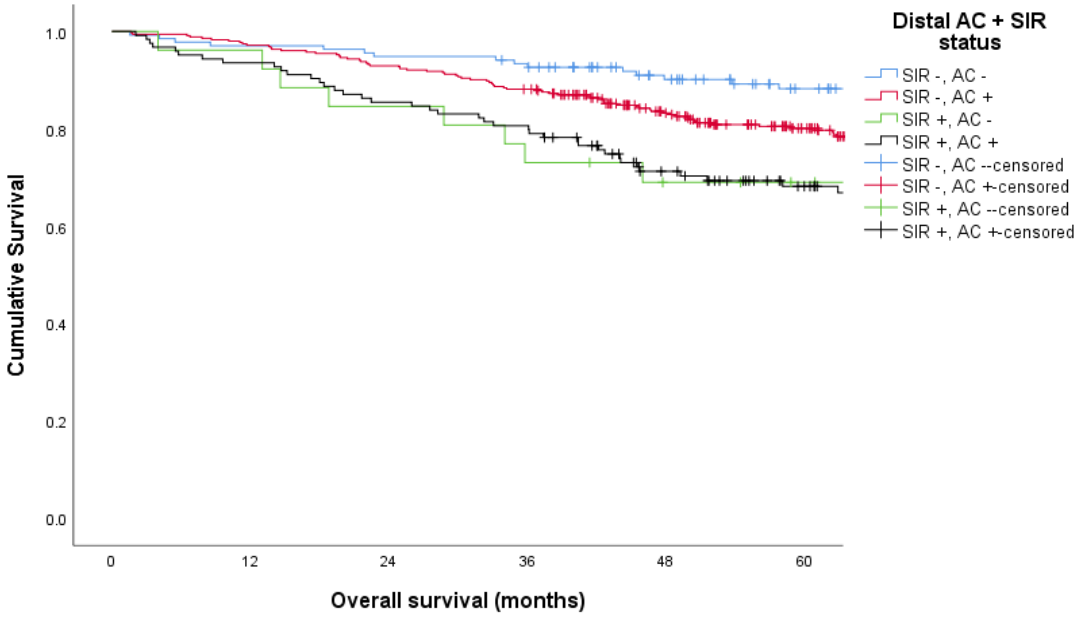
	Univariate		Multivariate	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
TNM stage (I-II/III)	3.59 (1.52 – 8.47)	0.003	4.09 (1.72 – 9.71)	0.001
mGPS (0/1-2)	1.68 (0.73 – 3.89)	0.225	-	-
Proximal AC (None/minor/major)	1.74 (1.03 – 2.95)	0.039	1.92 (1.14 – 3.24)	0.014
Distal AC (None/minor/major)	1.16 (0.68 – 1.99)	0.596	-	-

Abbreviations: AC aortic calcification, mGPS modified Glasgow Prognostic Score, TNM Tumour, Nodes, Metastases.

Figure 9-1 - Overall survival by SIR and AC status in patients undergoing elective curative colorectal cancer resection (n=640).

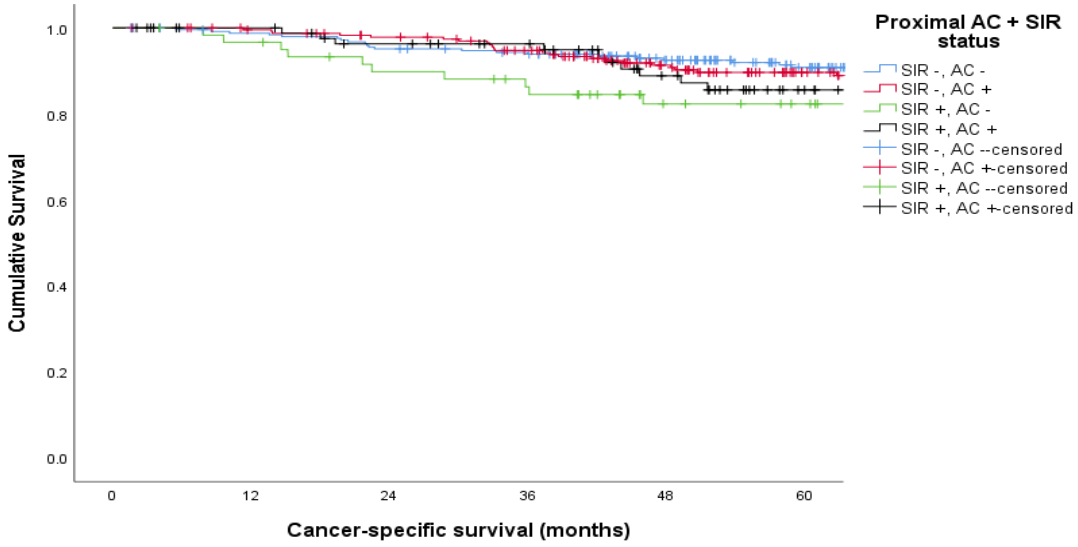


p<0.001

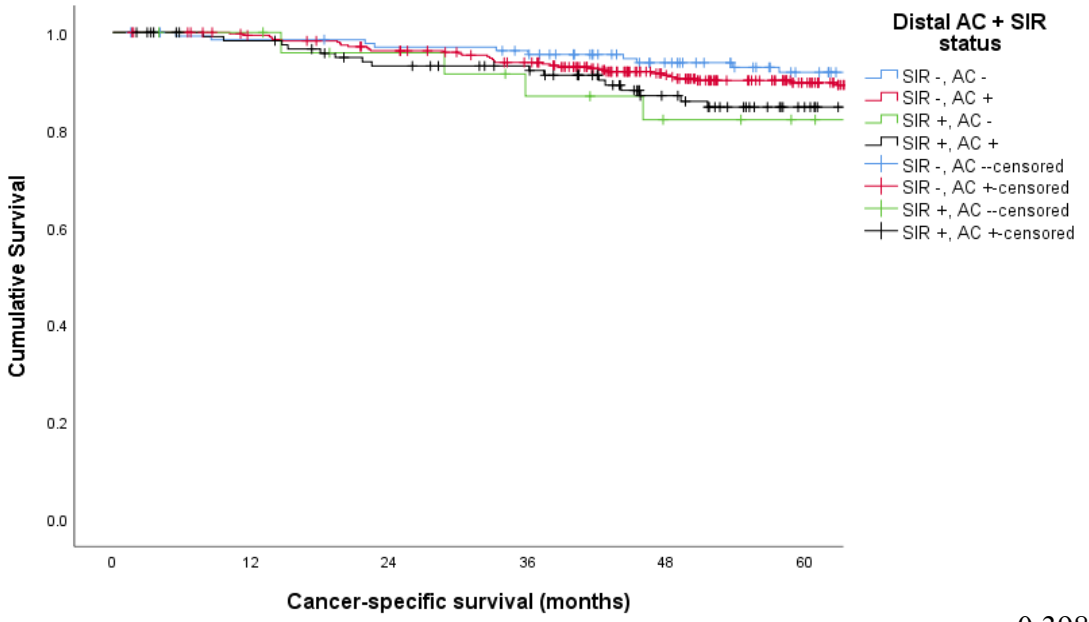


p<0.001

Figure 9-2 - Cancer-specific survival by SIR and AC status in patients undergoing elective curative colorectal cancer resection (n=640).



p=0.368



p=0.398

10 Comparison of the influence of aortic calcification and comorbidity on outcome following colorectal cancer resection

10.1 Introduction

Following the derivation of a reproducible semi-quantitative AC score in Chapter 3, subsequent studies in Chapters 4 and 5 confirmed that patients with an increasing degree of calcification were at greater risk of non-infective complications and inferior overall survival. Furthermore, a trend towards poorer cancer-specific survival in patients with significant AC and right colon cancer was identified. While systemic inflammation plays a role in impaired overall survival, it was shown in Chapters 5 and 9 to be unrelated to cancer-specific survival.

Complex interactions between host and tumour factors likely influence oncological outcome. The presence of comorbidity has previously been associated with poorer cancer-specific survival in the long-term. As a determinant of treatment suitability and tolerance, whether surgical or cytotoxic, comorbidity is a key consideration in the management of patients with colorectal cancer.

Prior to surgery, the tumour is staged in detail using clinical, endoscopic and radiologic assessment. The patient is also assessed, using predominantly clinical methods based on subjective assessment (ASA grade[406]), self-reported functional indicators (metabolic equivalent tasks, METs[590]) and in selected cases, non-routine tests such as echocardiogram or cardiopulmonary exercise testing. The variability and reliance on subjective measures in staging the patient is clear. Comorbidity scores and indices are available but not routinely used in clinical practice. The Charlson Comorbidity Index represented the first summary measure and was introduced in 1987 to aid comorbidity assessment in clinical trial participants with the aim of increasing the generalisability of trial results[322]. Several scores have since been developed and validated ranging from general summary measures (Elixhauser[324] and ACE-27[330,591] scores) to those derived in populations with cancer (NIA/NCI[328,329]) and cardiovascular-specific scores (Revised Cardiac Risk Index[439,440]).

Aortic calcification is often accompanied by atherosclerotic disease in multiple vascular beds[592]. It is unclear whether AC is a proxy for overall comorbidity or is representative of cardiovascular comorbidity alone. It is likely to be strongly correlated with cerebrovascular and peripheral vascular disease as well as diabetes. The significant overlap between the risk factors for metabolic syndrome (hypertension, hyperglycaemia, dyslipidaemia and central obesity) and cardiovascular disease illustrates the colinearity in comorbidity.

Comorbidity is well recognised as a predictor of postoperative complications [593,594]. In Chapter 4, associations between a higher burden of proximal AC and non-infective complications and distal AC and all complications was demonstrated. However, aside from ASA, comorbidity measures were not included in the regression models. Incorporating comorbidity measures into the analysis of factors associated with complications is required to better define whether AC influences the development of complications in a manner independent of comorbidity, for example, through impaired oxygenation of tissue recently exposed to surgical trauma.

Similarly, it is unclear whether the degree of proximal AC alone influences overall survival or whether it is a function of comorbidity. In Chapter 5, an independent relationship between an increasing degree of proximal AC and inferior overall survival was described. Survival models that include validated comorbidity indices in addition to the degree of AC may facilitate a greater appreciation of their clinical utility.

It was hypothesised that a high burden of aortic calcification would be more closely associated with morbidity and inferior survival in patients undergoing colorectal cancer resection than summary comorbidity measures. This study aimed to assess the relationship between AC and validated comorbidity scores with complications and survival following curative colorectal cancer resection.

10.2 Methods

Consecutive patients who had undergone potentially curative resection of TNM stage I to III colorectal cancer at a single institution between 2008 and 2016 were identified from a prospectively maintained CRC database. Exclusion criteria were similar to Chapters 4 and 5. Tumours were staged according to the fifth edition of the AJCC TNM manual[213] and the third edition of the Royal College of Pathologists dataset for colorectal cancer reporting[537]. Aortic calcification was assessed on abdominopelvic pre-operative CT imaging as described in Chapter 3.

Comorbidity was assessed using the Charlson Comorbidity Index (CCI)[322], the van Walraven modification of the Elixhauser comorbidity score (ECS)[325], the National Institute on Ageing and National Cancer Institute (NIA/NCI) comorbidity index[328] and the Adult Comorbidity Evaluation-27 (ACE-27)[330]. Cardiac risk was assessed using the Revised Cardiac Risk Index (RCRI)[440]. The derivation of these scores and their validation has previously been described. The CCI was derived in patients admitted acutely to general medicine and used the adjusted relative risk of death at 1 year in relation to individual comorbidities to assign a weight to each comorbidity on a scale of 0 (least severe) to 6 (most severe). The weights are then summed to provide a summary score for an individual. The ECS was developed for use in assessing comorbidity associated with length of stay, cost and mortality. The van Walraven modification of the ECS was later used to refine the original list of 30 conditions to 21 comorbidities related to in-hospital death. The comorbidities in the ACE-27 were based on a modification of the Kaplan-Feinstein[595], Charlson and Elixhauser comorbidity indices. The ACE-27 accounts for disease severity by grading pre-specified conditions in to one of three levels of comorbidity: mild (grade 1), moderate (grade 2) or severe (grade 3). The NIA/NCI comorbidity index is a list of 27 comorbidities and was derived to assess the role of comorbidity in predicting the risk of early mortality in patients with 7 tumour types including colon cancer. The RCRI was devised as a tool to aid stratification of patients according to the risk of cardiac events post-surgery using six independent predictors: high-risk surgery (intra-peritoneal, intra-thoracic or supra-inguinal vascular surgery), history of ischaemic heart disease, congestive heart failure or cerebrovascular disease, preoperative

treatment with insulin and preoperative serum creatinine >2.0mg/dL. For all scores, the diagnosis of colorectal cancer for which resection was performed was discounted.

Primary endpoints included postoperative complications and overall survival. These were selected on the basis that some comorbidity scores were originally designed in relation to short-term outcomes such as complications while others were designed in relation to survival at various timepoints. For the analysis of factors related to complications, variables were limited to those available at the time of preassessment. ASA grade assigned on the day of surgery and final TNM stage were therefore not included. This strategy was adopted to enable comparison of variables known to increase the risk of postoperative complications (advanced age, smoking status) with those under investigation (AC and comorbidity measures). Similarly, factors known to influence survival including final TNM stage were included in the survival analysis.

Clinical and pathological data including perioperative complications were recorded prospectively. As for Chapter 4, complications within 30 days were graded using the Clavien-Dindo scale (I to V) according to the treatment required[499]. The follow up schedule described in Chapter 5 was applied to all patients in this cohort. Survival data were censored on 06.09.2019.

10.2.1 Statistical Analysis

Descriptive statistics were used to summarise the baseline characteristics of the cohort. The median and interquartile range (IQR) were used to describe the calcification scores. The median value was used to define the threshold between minor and major calcification categories while patients with no visible calcification were grouped into the 'no calcification' category. Comorbidity scores were grouped into previously published categories.

Associations between comorbidity scores and aortic calcification were assessed using the Mantel Haenszel test for trend. The relationship between clinico-pathologic variables, AC, comorbidity scores and postoperative complications was assessed using binary logistic regression. The relationship between clinico-pathologic variables and overall survival was assessed using Cox regression.

Multivariate analysis of factors with a p-value of <0.05 on univariate analysis was performed. Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA).

10.3 Results

Baseline clinicopathological characteristics of the included patients (n=657) are shown in Table 4.1 of Chapter 4. The distribution of comorbidity as calculated using the ACE-27, CCI, ECS, NIA-NCI and RCRI is displayed in Table 10.1. The majority of patients had no or minimal comorbidity (ACE-27 none or mild (74%), ECS < 2 (56%), NIA-NCI ≤ 3 (81%) and RCRI 1 (74%)). High scores were uncommon across all comorbidity indices/scores: 6% were categorised as having severe comorbidity by the ACE-27 score, 9% had an Elixhauser score ≥4, 3% scored ≥6 on the NIA-NCI and 5% scored ≥3 on the RCRI. Using the age-adjusted CCI, 31% scored ≥4. The difference in proportion of those categorised as having severe comorbidity by the CCI compared to the other scores is accounted for by the incorporation of age as a variable, where each decade over 50 increases the score by 1.

Postoperative complications of any grade were recorded in 282 patients (43%), of which 60 (9%) were major complications (Clavien-Dindo grade III or higher). Median follow-up for survivors was 72 months (minimum 34 months). During follow up, 187 patients died; 101 deaths due to cancer and 86 deaths due to other causes.

The associations between the comorbidity scores and the degree of AC are displayed in Table 10.2. An increasing burden of comorbidity was associated with an increasing degree of both proximal and distal AC across all scores. It was notable that the presence of minor or major distal AC was more frequently associated with low comorbidity scores when compared with proximal AC. For example, 35% and 20% of patients with an ECS of 0 had minor or major distal AC respectively compared with 21% and 6% with minor or major proximal AC for the same ECS.

The relationship between clinico-pathological characteristics including comorbidity scores and all postoperative complications is displayed in Table 10.3. Male gender (OR 1.42, 95% CI 1.03 – 1.96, p=0.032), smoking (OR 1.62, 95% CI 1.18 – 2.23, p=0.003) and increasing RCRI score (OR 1.50, 95% CI 1.12 – 2.00, p=0.006) were independently related to the development of all complications when assessed in a multivariate model including age, sex, smoking history and

degree of AC. Neither the degree of proximal or distal AC was independently related to the development of all complications.

The relationship between clinico-pathological characteristics including comorbidity scores and overall survival is displayed in Tables 10.4. When assessed in a multivariate model including recognised factors related to overall survival (age, sex, smoking history, TNM stage), an increased CCI (HR 1.47, 95% CI 1.13 – 1.91, p=0.004), ECS (HR 1.22, 95% CI 1.02 – 1.46, p=0.027), higher burden of proximal AC (HR 1.37, 95% CI 1.12 – 1.67, p=0.002) and raised mGPS (1.60, 95% CI 1.17 – 2.18, p=0.003) were related to overall survival, independent of TNM stage.

The relationship between clinico-pathological characteristics including comorbidity scores and cancer-specific survival is displayed in Tables 10.5. On univariate analysis, only TNM stage (3.33, 95% CI 2.16 – 5.14, p=0.001) and increasing RCRI score (1.47, 95% CI 1.04 – 2.08, p=0.029) were associated with inferior cancer-specific survival. On multivariate analysis, the RCRI score lost significance (1.38, 95% CI 0.98 – 1.94, p=0.067) while TNM stage remained related to inferior cancer-specific survival (3.19, 95% CI 2.06 – 4.93, p=0.001).

10.4 Discussion

This study confirms that an increasing burden of AC is related to a greater degree of comorbidity in patients undergoing curative colorectal cancer resection. It highlights that proximal AC is more closely associated with the degree of comorbidity than distal AC, supporting the hypothesis in Chapter 4 that the degree of distal AC is more likely to reflect haemodynamic turbulence at the aortic bifurcation than the burden of atherosclerotic disease. The RCRI score was the single comorbidity measure independently related to complications, second only to a history of smoking in terms of effect size. In contrast, the presence of a SIR was most closely associated with inferior overall survival, followed by increasing CCI score and degree of AC. TNM stage remained the sole independent factor associated with inferior cancer-specific survival. It can be seen that no single clinicopathological feature or comorbidity score consistently captured patients at risk of both inferior short and long-term outcome following colorectal cancer resection.

The degree of AC and the comorbidity indices and scores used here are not routinely assessed in clinical practice. The majority of included comorbidity measures are mapped to ICD codes[596,597] and have available algorithms[598] that are frequently used for administrative and billing purposes in regions such as North America. In the UK, such data is not routinely collected or easily accessible. In this study, comorbidity data were ascertained by individual review of the medical records available at the time of surgery for the incident colorectal cancer. This labour-intensive approach represents a barrier to clinical translation. AC, however, can be scored by non-specialists on the staging CT and is reproducible. As shown here, it is associated with the degree of comorbidity, although it is clear that there are caveats. A proportion of patients that have significant AC do not have corresponding levels of comorbidity and vice versa. Of the included comorbidity measures, the RCRI was the simplest to measure, consisting of 6 binary variables that are routinely collected at preoperative assessment. It is possible that in the nurse-led clinic setting, the RCRI score may be more readily utilised than the degree of AC in highlighting patients at increased risk of postoperative morbidity who may benefit from high-risk anaesthetic review.

It is not unexpected that the CCI was demonstrated to be independently related to overall survival. The score was devised with the aim of predicting of 1-year mortality among emergency admissions to general medicine and has been widely validated in varying populations. When considering ease of clinical application, however, the mGPS and degree of proximal AC represent more readily applied methods of highlighting patients at risk of early all-cause mortality. It is possible that their combined use may have complementary value given the finding in the preceding chapter that both remained independently related to inferior overall survival in patients with resected colorectal cancer.

This study is the first to directly compare AC to validated comorbidity measures. Harbaugh and colleagues examined postoperative complications and survival in relation to software-derived AC scores in patients with no known cardiovascular risk factors compared with patients with known cardiovascular risk[489]. The study sample consisted of a heterogenous group of patients undergoing intraabdominal surgery (48% minor abdominal surgery). The degree of AC was reported only in those with no known cardiovascular risk factors, preventing comparison of the rates of AC in those with existing cardiovascular risk factors. As such, the clinical utility of AC as a risk factor for postoperative complications and inferior survival in patients at risk of or with known cardiovascular comorbidity was not assessed. Moreover, the inclusion of patients with preoperative sepsis, disseminated cancer, current steroid use and current systemic chemo- and radiotherapy suggests that emergency surgery was undertaken in a proportion of patients, a recognised risk factor for inferior short- and long-term surgical outcomes.

This study has several limitations. Comorbidity data were abstracted by a single assessor and observer bias may therefore have influenced the results. Comorbidity was limited to that recorded on referral letters from General Practice and the Preoperative Assessment form and may not have captured all aspects of comorbidity. AC data were quantified by two assessors using a visual assessment method. One assessor was blinded to subsequent comorbidity data; the other (KK) was not blinded and this could represent an additional source of bias. However, AC was assessed prior to abstraction of comorbidity data and is therefore unlikely to have influenced the results.

In conclusion, the RCRI score was associated with postoperative complications and could be easily implemented in the preoperative clinic to identify patients at risk of postoperative morbidity, triggering further assessment and optimisation. These results highlight the challenge in identifying patients at risk of inferior overall and cancer-specific survival beyond established measures such as TNM stage. This requires the use of multiple metrics, to which host factors including the degree of AC and systemic inflammation could contribute.

Table 10-1 - Distribution of comorbidity among patients undergoing elective curative resection of colorectal cancer by different indices and scores (n = 657).

Characteristic		n (%)
ACE-27	None	260 (40)
	Mild	226 (34)
	Moderate	128 (20)
	Severe	43 (6)
CCI (age- adjusted)	0 - 1	126 (19)
	2 - 3	326 (50)
	≥4	205 (31)
Elixhauser	0	150 (23)
	1	214 (33)
	2 - 3	231 (35)
	≥4	62 (9)
NIA-NCI	0 - 1	213 (33)
	2 – 3	318 (48)
	4 - 5	107 (16)
	≥6	19 (3)
RCRI	1	485 (74)
	2	142 (21)
	≥3	30 (5)

Abbreviations: ACE Adult Comorbidity Evaluation, CCI Charlson Comorbidity Index, NIA-NCI National Institute on Ageing and National Cancer Institute, RCRI Revised Cardiac Risk Index.

Table 10-2 - Associations between comorbidity scores and aortic calcification among patients undergoing elective curative resection of colorectal cancer ((n = 657).

Score	Category	Proximal AC None	Proximal AC Minor	Proximal AC Major	p-value	Distal AC None	Distal AC Minor	Distal AC Major	p-value
ACE-27	None	163 (63)	69 (26)	28 (11)	0.001	84 (32)	100 (39)	76 (29)	0.001
	Mild	89 (39)	79 (35)	58 (26)		48 (21)	77 (34)	101 (45)	
	Moderate	58 (45)	39 (31)	31 (24)		29 (25)	45 (35)	54 (42)	
	Severe	5 (12)	21 (49)	17 (39)		2 (5)	12 (28)	29 (67)	
CCI (age-adjusted)	0 - 1	112 (89)	11 (9)	3 (2)	0.001	71 (56)	45 (36)	10 (8)	0.001
	2 - 3	160 (49)	110 (37)	47 (14)		73 (22)	130 (40)	123 (38)	
	≥4	43 (21)	78 (38)	84 (41)		19 (9)	59 (29)	127 (62)	
Elixhauser	0	110 (73)	31 (21)	9 (6)	0.001	67 (45)	52 (35)	31 (20)	0.001
	1	101 (47)	65 (30)	48 (23)		48 (22)	83 (39)	83 (39)	
	2 - 3	90 (39)	86 (37)	55 (24)		41 (18)	81 (35)	109 (47)	
	≥4	14 (23)	26 (42)	22 (35)		7 (11)	18 (29)	37 (60)	
NIA-NCI	0 - 1	147 (69)	46 (22)	20 (9)	0.001	95 (45)	70 (33)	48 (23)	0.001
	2 – 3	134 (42)	114 (46)	70 (22)		49 (15)	126 (40)	143 (45)	
	4 - 5	32 (30)	40 (37)	35 (33)		17 (16)	35 (33)	55 (51)	
	≥6	2 (11)	8 (42)	9 (47)		2 (10)	3 (16)	14 (74)	
RCRI	1	276 (57)	142 (29)	67 (14)	0.001	144 (30)	189 (39)	152 (31)	0.001
	2	35 (25)	54 (38)	53 (37)		17 (12)	36 (25)	89 (63)	
	≥3	4 (13)	12 (40)	14 (47)		2 (1)	9 (12)	19 (63)	

Abbreviations: AC Aortic calcification, ACE Adult Comorbidity Evaluation, CCI Charlson Comorbidity Index, NIA-NCI National Institute on Ageing and National Cancer Institute, RCRI Revised Cardiac Risk Index.

Table 10-3 - Relationship between clinico-pathological factors, AC, comorbidity scores and all complications among patients undergoing elective curative resection of colorectal cancer (n = 657).

	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (<65 / 65-74/>75)	1.24 (1.01 – 1.51)	0.040	1.14 (0.90 – 1.45)	0.272
Sex (Female/Male)	1.54 (1.13 – 2.11)	0.007	1.42 (1.03 – 1.96)	0.032
Smoking history (No/Yes)	1.76 (1.29 – 2.42)	0.001	1.62 (1.18 – 2.23)	0.003
BMI (<30/ >30 kg/m^2)	1.36 (0.98 – 1.89)	0.068	-	-
mGPS	1.32 (0.92 - .91)	0.133	-	-
Proximal AC (None / Minor/ Major)	1.16 (0.95 – 1.42)	0.138	-	-
Distal AC (None / Minor / Major)	1.28 (1.05 – 1.56)	0.015	0.97 (0.76 – 1.24)	0.815

	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
ACE-27 (None/mild/moderate/ severe)	1.26 (1.07 – 1.49)	0.007	1.07 (0.86 – 1.33)	0.541
CCI (0-1/2-3/4+)	1.18 (0.95 – 1.48)	0.141	-	-
Elixhauser CI (1/2-3/4-5/6+)	1.23 (1.04 – 1.45)	0.018	0.96 (0.74 – 1.24)	0.747
NIA-NCI (1/2-3/4-5/6+)	1.44 (1.17 – 1.76)	0.001	1.17 (0.84 – 1.63)	0.359
RCRI 1/2/≥3	1.61 (1.21 – 2.13)	0.001	1.50 (1.12 – 2.00)	0.006

Abbreviations: AC Aortic calcification, ACE Adult Comorbidity Evaluation, BMI body mass index, CCI Charlson Comorbidity Index, mGPS modified Glasgow Prognostic Score, NIA-NCI National Institute on Ageing and National Cancer Institute, RCRI Revised Cardiac Risk Index.

Table 10-4 - Relationship between clinico-pathological factors, AC, comorbidity scores and overall survival among patients undergoing elective curative resection of colorectal cancer (n = 657).

	Overall Survival			
	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (<65 / 65-74/>75)	1.73 (1.43 – 2.09)	0.001	1.20 (0.94 – 1.54)	0.146
Sex (Female/Male)	1.38 (1.03 – 1.84)	0.032	1.24 (0.92 – 1.68)	0.155
Smoking history (No/Yes)	1.44 (1.08 – 1.92)	0.014	1.24 (0.91 – 1.67)	0.170
TNM stage (I - II/ III+)	1.72 (1.30 – 2.29)	0.001	1.77 (1.33 – 2.36)	0.001
mGPS (0/1-2)	1.70 (1.26 – 2.31)	0.001	1.60 (1.17 – 2.18)	0.003
Proximal AC (None / Minor/ Major)	1.67 (1.40 – 1.99)	0.001	1.37 (1.12 – 1.67)	0.002

	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Distal AC (None / Minor / Major)	1.49 (1.23– 1.80)	0.001	0.93 (0.72 – 1.20)	0.577
ACE-27 (None/mild/moderate/ severe)	1.37 (1.18 – 1.59)	0.001	0.98 (0.81 – 1.20)	0.856
CCI (0-1/2-3/4+)	2.02 (1.62 – 2.52)	0.001	1.47 (1.13 – 1.91)	0.004
Elixhauser CI (1/2-3/4-5/6+)	1.55 (1.32 – 1.81)	0.001	1.22 (1.02 – 1.46)	0.027
NIA-NCI (1/2-3/4-5/6+)	1.56 (1.31 – 1.85)	0.001	0.89 (0.66 – 1.18)	0.410
RCRI 1/2/≥3	1.28 (1.01 – 1.62)	0.001	1.15 (0.89 – 1.47)	0.288

Abbreviations: AC Aortic calcification, ACE Adult Comorbidity Evaluation, BMI body mass index, CCI Charlson Comorbidity Index, mGPS modified Glasgow Prognostic Score, NIA-NCI National Institute on Ageing and National Cancer Institute, RCRI Revised Cardiac Risk Index.

Table 10-5 - Relationship between clinico-pathological factors, AC, comorbidity scores and cancer-specific survival among patients undergoing elective curative resection of colorectal cancer (n = 657).

	Cancer-specific survival			
	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (<65 / 65-74/>75)	1.23 (0.93 -1.63)	0.145	-	-
Sex (Female/Male)	1.41 (0.91 - 2.20)	0.120	-	-
Smoking history (No/Yes)	0.95 (0.63 - 1.46)	0.827	-	-
TNM stage (I - II/ III+)	3.33 (2.16 - 5.14)	0.001	3.19 (2.06 – 4.93)	0.001
mGPS (0/1-2)	1.37 (0.85 – 2.21)	0.197	-	-
Proximal AC (None / Minor/ Major)	1.28 (0.98 - 1.67)	0.073	-	-

	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Distal AC (None / Minor / Major)	1.13 (0.86 - 1.48)	0.371	-	-
ACE-27 (None/mild/moderate/ severe)	1.06 (0.84 – 1.34)	0.603	-	-
CCI (0-1/2-3/4+)	1.28 (0.94 – 1.74)	0.124	-	-
Elixhauser CI (1/2-3/4-5/6+)	1.20 (0.95 – 1.51)	0.121	-	-
NIA-NCI (1/2-3/4-5/6+)	1.26 (0.96 – 1.64)	0.092	-	-
RCRI 1/2/≥3	1.47 (1.04 – 2.08)	0.029	1.38 (0.98 – 1.94)	0.067

Abbreviations: AC Aortic calcification, ACE Adult Comorbidity Evaluation, BMI body mass index, CCI Charlson Comorbidity Index, mGPS modified Glasgow Prognostic Score, NIA-NCI National Institute on Ageing and National Cancer Institute, RCRI Revised Cardiac Risk Index.

11 The relationship between aortic calcification and cardiopulmonary fitness in patients undergoing colorectal cancer resection

11.1 Introduction

In chapter 4, an increasing burden of AC related to the development of non-infective complications following colorectal cancer resection. Chapters 9 and 10 highlighted that AC is closely associated with comorbidity and systemic inflammation, host factors contributing to higher rates of postoperative morbidity[97,599]. The seminal work of Older and Hall in the 1990s confirmed a higher incidence of morbidity in patients with impaired cardiorespiratory fitness measured by preoperative cardiopulmonary exercise testing (CPET)[414]. It has since become clear that key CPET variables including oxygen uptake at anaerobic threshold and peak oxygen uptake are associated with a higher incidence of postoperative complications following colorectal surgery [309,380,381,600]. Preoperative exercise training programmes have subsequently been shown to improve cardiorespiratory fitness [507,601,602]. A multicentre randomised controlled trial is currently underway evaluating the impact of preoperative exercise training on complications and quality of life in patients undergoing surgery for colorectal cancer [603].

AC represents a static marker of cardiovascular health. It is unknown to what degree vascular calcification correlates with dynamic function. CPET represents one potential mode of dynamic assessment with proven clinical utility in the preoperative assessment of patients with operable colorectal cancer. Furthermore, the availability of preoperative CPET is increasing[416]. However, indications for CPET are not based on the presence of clinical risk factors or high impact comorbidity but centre on risk prediction and postoperative critical care provision[412]. Its use is largely dependent on the desire of the surgeon and anaesthetist to attempt to objectively assess individual risk profile. In some centres, all patients undergoing major abdominal surgery have a preoperative CPET, while in others, it is reserved for patients with significant comorbidity or frailty and used to provide evidence for the need to tailor management to non-operative means. Indeed, defined criteria guiding access to CPET was used in

only 22% of hospitals providing a CPET service in 2013 [415]. Over 75% of units providing CPET in 2018 used surgery type as the main indication for testing, with age, risk score or screening questionnaires used as supplementary factors to support testing in 5 – 16% [416].

It is evident that preoperative CPET use is variable and indication for testing is largely subjective. However, in a cohort of over 1,300 patients undergoing major non-cardiac surgery, subjective assessment was associated with a 19% specificity when anaesthetists were asked to predict the functional capacity of patients prior to CPET [405]. It is possible that certain host characteristics in conjunction with subjective assessment may improve patient selection for CPET and pre-operative optimisation. Indeed, aortic calcification may form a component of the frailty phenotype that could be used clinically to identify high risk patients who may benefit from more detailed preoperative assessment.

It was hypothesised that the degree of AC would show a close association with cardiovascular fitness as measured by CPET. The aim of this study was to assess whether an increasing degree of AC correlated with inferior CPET results in patients undergoing curative colorectal cancer resection. Furthermore, this study examined the potential of AC to act as a screening tool to identify patients who would benefit from additional investigation with preoperative CPET leading to optimisation where necessary.

11.2 Methods

This multi-centre retrospective study included consecutive patients undergoing elective resection of stage I to III colorectal cancer with prior cardiopulmonary exercise testing between 2016 - 2018. Several NHS hospitals contributed data: Frimley Park, Frimley Health NHS Foundation Trust; Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde; University Hospital Crosshouse, NHS Ayrshire and Arran; University Hospital Hairmyres, NHS Lanarkshire. Patients were identified retrospectively from prospectively-maintained lists of the weekly colorectal cancer MDT meetings. The medical e-record was then checked to identify patients who had undergone preoperative CPET. Exclusions included patients who underwent emergency, palliative or local resection and those without preoperative CT imaging for evaluation of AC.

CPET was performed in a respiratory lab with full resuscitation equipment using an electromagnetically-braked cycle ergometer. The metabolic cart used at each site varied: GRI ZAN 600 (nSpire Health), UCH Vyntus CPX (Vyaire Medical), UHH Vmax 229 (Vyaire Medical), FP Ultima Cardio2 (MCG Diagnostics). Heart rate, peripheral oxygen saturation, non-invasive blood pressure and 12 lead ECG trace were monitored throughout. Test procedure consisted of 3 minutes at rest, 3 minutes unloaded cycling followed by an incremental ramp protocol. Cessation of the test was determined by the patient; encouragement to exercise to capacity was provided by the testing clinician or physiologist. CPET concluded with a minimum recovery time of 3 minutes during which the patient continued to pedal at a self-determined pace against no resistance. CPET variables including oxygen uptake at anaerobic threshold, peak oxygen uptake, ventilatory efficiency and oxygen pulse were extracted from reports issued at the time of testing. CPETs were interpreted and reported by trained staff with several years' experience.

Aortic calcification was assessed as described previously on anonymised jpeg files extracted from preoperative CT images according to a standard operating procedure. Transverse images were selected by radiologists at each site using anatomical landmarks and files transferred securely to the data centre at GRI for evaluation. AC was scored independently by two study team members (KK and

WH) who were blinded to clinicopathological characteristics at the time of scan evaluation.

Clinical and pathological data including perioperative complications were recorded prospectively. Pathological tumour stage was reported using the TNM staging system [213]. Postoperative complications were classified according to the Clavien-Dindo scale (I to V) [499], with complications requiring surgical intervention are regarded as major and classified as grade III and above.

Ethical approval was provided by the Health Research Authority and Health and Research Wales (reference 20/LO/0370).

11.2.1 Statistical analysis

Descriptive statistics were used to summarise baseline characteristics. CPET variables were grouped according to published thresholds [381]. Calcification scores were described using the median and interquartile range (IQR) and then grouped into categories (no/minor/major calcification) as described in Chapter 4. The intraclass correlation coefficient (ICC) was used to compare inter-rater reliability. ICC estimates and their 95% confident intervals were calculated based on a mean-rating, absolute-agreement, 2-way mixed-effects model and categorised according to Chapter 3. Associations between AC and CPET variables were assessed using the Chi-squared or Mantel-Haenszel test. The relationships between clinicopathological and CPET variables were investigated using logistic regression. Variables with a p-values of <0.05 on univariate analysis were entered into the multivariate model. Statistical analysis was performed using SPSS software (version 27.0; SPSS Inc., Chicago, IL, USA).

11.3 Results

Between 2016 and 2018, a total of 304 patients underwent elective resection of stage I to III colorectal cancer with prior CPET across the participating institutions: Frimley Park (n=159); Glasgow Royal Infirmary (n=48); University Hospital Crosshouse (n=28); University Hospital Hairmyres (n=69). Following exclusion of patients who had significant missing clinical (1) or CPET data (5), surgery for non-colorectal cancer pathology (5), emergency or palliative procedures (2) and distant metastatic disease at time of surgery (1), 290 patients were included. Pre-operative CT imaging was not available in 7 patients and unsuitable for analysis for technical reasons in 2 patients including incomplete coverage of abdominal aorta and significant intra-aortic contrast obscuring calcification. The baseline demographic and clinicopathological characteristics of the remaining 281 patients are presented in Table 11.1.

Most patients were male (74%), aged over 65 years (69%) and ASA grade I or II (64%). The median age was 71 years (range 42-90). The majority of patients had rectal cancer (75%), pathological T-stage 3 or 4 (64%) and node-negative (64%) disease. Laparoscopic surgery was undertaken in 217 patients (77%). Postoperative complications occurred in 114 patients (41%), of which 6% were major (Clavien-Dindo grade III or above).

For proximal AC, 172 (61%) patients had no calcification and 109 (39%) had visible calcification; the median proximal AC score in those with calcification was 1 (range 1 to 4). For distal AC, 77 (27%) patients had no calcification and 204 patients (73%) had visible calcification; the median score was 4 (range 1 to 12). Proximal AC was minor in 70 patients (25%) and major in 39 patients (14%). Distal AC was minor in 78 patients (28%) and major in 126 patients (45%). Inter-rater reliability was excellent: the ICC was 0.97 (95% CI 0.93 – 0.98) for proximal AC and 0.92 (95% CI 0.85 – 0.96) for distal AC.

Four patients terminated the CPET prior to protocol completion due to leg pain and/or dyspnoea, three of whom had reached the anaerobic threshold (AT) prior to cessation. Median oxygen uptake at AT was 11.9 (5.9 – 37.9) ml/ kg/ min.

Median peak oxygen uptake was 17.0 (7.9 – 48.2) ml/ kg/ min. Using previously reported thresholds, 109 patients (39%) had AT less than 11.1 ml/ kg/ min and 158 patients (56%) had a peak VO_2 below 18.2 ml/ kg/ min.

To assess the relationship between CPET variables and postoperative complications within the cohort, receiver operating characteristic (ROC) curves for oxygen uptake at estimated anaerobic threshold (VO_2 at AT) and oxygen uptake at peak (peak VO_2) were constructed, using postoperative complications as the positive state variable (Figures 11.1 and 11.2). The area under ROC curve was 0.52, 95% CI 0.45 – 0.59, p=0.653 for VO_2 at AT and 0.54, 95% CI 0.47 – 0.61, p=0.301 for peak VO_2. Given the poor discriminatory ability of these variables in relation to prediction of complications in this cohort, those previously derived by West et al in a contemporary cohort of patients undergoing major colorectal surgery were used: VO_2 at AT 11.1 ml/kg/min and peak VO_2 18.2 ml/kg/min [381].

Associations between clinical and pathological characteristics and the primary CPET variables of interest (VO_2 at AT and peak VO_2) categorised according to West et al [381] are shown in Table 11.2. The proportion of patients with VO_2 at AT <11.1 ml/kg/min and peak VO_2 <18.2 ml/kg/min increased with increasing age (48% vs 30%, p=0.014 and 77% vs 33%, p<0.001 respectively), ASA grade (61% vs 27% and 76% vs 46%, both p<0.001), BMI (52% vs 33%, p=0.002 and 74% vs 48%, p<0.001 respectively), degree of proximal AC (54% vs 33%, p=0.004 and 72% vs 48%, p<0.001 respectively) and distal AC (46% vs 27%, p=0.009 and 63% vs 42%, p=0.003 respectively). Patients with higher TNM stage (III vs I-II) tumours were also more likely to have poorer VO_2 at AT and peak VO_2 (47% vs 30%, p<0.001 and 70% vs 40%, p<0.001).

The relationship between clinical characteristics and VO_2 at AT is shown in Table 11.3. On univariate analysis, increasing age (1.47, 95% CI 1.08 – 2.00, p=0.014), ASA grade (4.21, 95% CI 2.51 – 7.08, p=0.001), BMI (2.24, 95% CI 1.34 – 3.74, p= 0.002), TNM stage (2.02, 95% CI 1.24 – 3.31, p=0.005), degree of proximal AC (1.61, 95% CI 1.16 – 2.25, p=0.005) and distal AC 1.48, 95% CI 1.10 – 2.00, p=0.009) were associated with VO_2 at AT below the threshold of 11.1 ml/kg/min. On multivariate analysis, only higher ASA grade (4.08, 95% CI 2.32 -7.18, p<0.001), BMI (1.93, 95% CI 1.09 – 3.41, p=0.024) and TNM stage (1.90, 95% CI

1.10 – 3.28, p=0.022) were independently associated with VO_2 at AT <11.1 ml/kg/min.

The relationship between clinical characteristics and peak VO_2 is shown in Table 11.4. On univariate analysis, increasing age (2.64, 95% CI 1.89 – 3.68, p<0.001), ASA grade (3.74, 95% CI 2.17 – 6.44, p=0.001), BMI (3.14, 95% CI 1.81 – 5.45, p= 0.002), degree of proximal AC (1.81, 95% CI 1.27 – 2.58, p=0.001) and distal AC 1.53, 95% CI 1.15 – 2.05, p=0.004) were associated with VO_2 at AT below the threshold of 18.2 ml/kg/min. On multivariate analysis, increasing age (3.51, 95% CI 2.23 - 5.54, p<0.001, ASA grade (2.85, 95% CI 1.43 – 5.67, p=0.003), BMI (6.27, 95% CI 2.93 – 13.40, p<0.001) and TNM stage (4.56, 95% CI 2.40 – 8.65, p<0.001) were independently associated with peak VO_2 <18.2 ml/kg/min.

11.4 Discussion

This study suggests that the degree of aortic calcification visible on preoperative CT is associated with, but not independently related to, cardiopulmonary fitness in patients undergoing elective, curative-intent colorectal cancer resection. Established preoperative risk factors including higher ASA grade and BMI were consistently associated with both lower oxygen uptake at anaerobic threshold and peak exercise, while age was independently related only to lower oxygen uptake at peak exercise. Since ASA is not routinely assessed at nurse-led preoperative assessment and the proportion of patients with BMI above the normal limit of 25 kg/m^2 continues to increase, the need for more specific objective markers that could be used to screen for high-risk patient status persists.

The absence of an association between oxygen uptake both at anaerobic threshold and peak exercise and postoperative complications in this cohort could be attributed to several factors. The study sample was small (n=281) in comparison to that reported by West and colleagues in a validation study of 703 patients undergoing major colorectal resection from six centres [381]. However, similar rates of postoperative complications occurred in both cohorts (37% vs 41% in the present cohort). Differences in patient characteristics including median age (69 (61-76) years [381] vs 71 (42 – 90) years in the present study) and paucity of comorbidity data (ASA not reported [381]) may be underlying contributors. Moreover, while 617 patients (88%) had colorectal cancer, 311 patients (44%) had either missing or unknown TNM staging [381]. Patients with more advanced disease may have formed part of this cohort, with the potential for advanced cancer-associated syndromes such as fatigue and cachexia to influence cardiorespiratory fitness.

Indeed, the finding from this study that patients with more advanced TNM stage tumours have increased odds of suboptimal cardiorespiratory fitness requires consideration. It is possible that once colorectal cancer breaches its local environment, an inflection point is reached where cancer-associated catabolism impacts on the cardiometabolic reserve of the host. While poorly characterised at present, this nexus between tumour and host is likely to be influenced by

multiple factors, including systemic inflammation and toxicity associated with neoadjuvant therapies. Whether such effects can be limited or overcome in the short- and long-term by tailored perioperative nutrition and exercise programmes remains unknown.

CPET has become synonymous with preoperative risk assessment. It was, however, originally described exclusively in relation to postoperative cardiovascular events and mortality [414]. In a global randomised control trial of anaesthetist's subjective assessment compared to self-reported functional capacity measured by the Dukes' Activity Status Index (DASI) and cardiac health represented by serum N-terminal pro-B-type natriuretic peptide levels, only DASI scores were associated with the primary outcome of myocardial injury or death within 30 days [405]. It is notable that coronary artery calcification was originally derived as a non-traditional risk factor for future cardiovascular events or death in asymptomatic populations. Aortic calcification alone is unlikely to accurately identify the high-risk patient who merits further preoperative assessment. Moreover, CPET is only one aspect of the comprehensive characterisation of patient readiness for colorectal cancer resection.

Limitations of this study include those inherent to retrospective multi-centre studies. In addition, selection bias was evident in this cohort. This resulted from differing CPET referral criteria in each centre (e.g. patients planned for rectal cancer resection only underwent preoperative CPET at Frimley Park hospital) and resulted in a high proportion of male patients and rectal resections within the study sample, limiting the use of gender and tumour site as variables. It is also plausible that the presence of CPET proponents within the perioperative medicine department in some centres resulted in more widespread use of CPET whereby all patients undergoing elective colorectal resection were recommended for CPET compared to other centres where only patients subjectively perceived to be of borderline fitness were referred. This represents a significant limitation, although it reflects current differences in perioperative practice and CPET availability throughout the UK. The absence of data on patients' functional capacity such as the number of metabolic equivalent tasks achievable further limits interpretation of the potential clinical utility of aortic calcification as a screening tool for pre-operative frailty. Furthermore,

examining the correlation between AC and fitness assessed by simple field tests such as the 6-minute walk test is required to define whether AC could be used to identify patients who should undergo preoperative fitness assessment using alternative methods to CPET.

To conclude, an increasing degree of aortic calcification was associated with impaired cardiorespiratory fitness in patients awaiting colorectal cancer resection. However, this was displaced on multivariate analysis by host factors such as increasing age, ASA and BMI as well as tumour factors including TNM stage. This study represents the first step in establishing individual, image-based risk assessment as a method that could be used to bridge the gap between subjective and objective approaches to preoperative evaluation. Further studies assessing the relationship between other image-based risk markers, self-reported measures of functional capacity and simple field tests of fitness are warranted.

Table 11-1 - Baseline characteristics of patients with operable colorectal cancer undergoing preoperative CPET (n = 281).

Characteristic		All (n= 281 (%))
Age (years)	< 65	86 (31)
	65 – 75	104 (37)
	> 75	91 (32)
Gender	Male	208 (74)
	Female	73 (26)
ASA grade	1 - 2	181 (64)
	3 - 4	101 (36)
BMI[a]	< 30	189 (68)
	> 30	90 (32)
Tumour site	Colon	71 (25)
	Rectum	210 (75)
Surgical approach	Laparoscopic	217 (77)
	Open	64 (23)
Clavien-Dindo grade	Grade 0	167 (59)
	Grade I – II	87 (35)
	Grade III+	16 (6)
T-stage	1 – 2	102 (36)
	3 – 4	179 (64)

N-stage	Node-negative	181 (64)
	Node-positive	100 (36)
Proximal AC	None	172 (61)
	Minor	70 (25)
	Major	39 (14)
Distal AC	None	77 (27)
	Minor	78 (28)
	Major	126 (45)

[a] Missing cases – BMI (2)

Table 11-2 - Associations between clinicopathologic characteristics and CPET variables among patients with operable colorectal cancer (n = 281).

		AT <11.1			Peak VO_2 <18.2		
		No n= 171 (61%)	Yes n=110 (39%)	p-value	No n= 122 (43%)	Yes n=159 (57%)	p-value
Age	< 65	60 (70)	26 (30)	0.014	58 (67)	28 (33)	<0.001
	65 – 75	64 (61)	40 (39)		43 (41)	61 (59)	
	> 75	47 (52)	44 (48)		21 (23)	70 (77)	
Gender	Male	134 (64)	74 (36)	0.039	105 (51)	103 (49)	<0.001
	Female	37 (51)	36 (49)		17 (23)	56 (77)	
ASA grade	1 – 2	132 (73)	49 (27)	<0.001	98 (54)	83 (46)	<0.001
	3 - 4	39 (39)	61 (61)		24 (24)	76 (76)	
BMI[a]	< 30	127 (67)	62 (33)	0.002	98 (52)	91 (48)	<0.001
	> 30	43 (48)	47 (52)		23 (26)	67 (74)	
Tumour site	Colon	26 (37)	45 (63)	<0.001	12 (17)	59 (83)	<0.001
	Rectum	145 (69)	65 (31)		110 (52)	100 (48)	
TNM stage	I – II	90 (70)	39 (30)	0.005	77 (60)	52 (40)	<0.001
	III	81 (53)	71 (47)		45 (30)	107 (70)	
Proximal AC	None	116 (67)	56 (33)	0.004	89 (52)	83 (48)	0.001
	Minor	37 (53)	33 (47)		22 (31)	48 (67)	
	Major	18 (46)	21 (54)		11 (28)	28 (72)	
Distal AC	None	56 (73)	21 (27)	0.009	45 (58)	32 (42)	0.003
	Minor	47 (60)	31 (40)		31 (40)	47 (60)	
	Major	68 (54)	58 (46)		46 (37)	80 (63)	

Table 11-3 - Relationship between clinicopathological factors and oxygen uptake at anaerobic threshold among patients with operable colorectal cancer (n = 281).

	Univariate	**p-value**	**Multivariate**	**p-value**
	Odds ratio (95% confidence interval)		**Odds ratio (95% confidence interval)**	
Age (<65/65-75/>75)	1.47 (1.08 – 2.00)	0.014	1.08 (0.71 – 1.64)	0.715
ASA grade (1-2/3-4)	4.21 (2.51 – 7.08)	<0.001	4.08 (2.32 – 7.18)	<0.001
BMI[a] (<30/>30 kg/m^2)	2.24 (1.34 – 3.74)	0.002	1.93 (1.09 – 3.41)	0.024
TNM stage (I-II/III+)	2.02 (1.24 – 3.31)	0.005	1.90 (1.10 – 3.28)	0.022
Proximal AC (None/minor/major)	1.61 (1.16 – 2.25)	0.005	1.10 (0.71 – 1.25)	0.671
Distal AC (None/minor/major)	1.48 (1.10 – 2.00)	0.009	1.24 (0.87 – 1.76)	0.236

Table 11-4 - Relationship between clinicopathological factors and peak oxygen uptake among patients with operable colorectal cancer (n = 281).

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	Univariate	p-value	Multivariate	p-value
	Odds ratio (95% confidence interval)		Odds ratio (95% confidence interval)	
Age (<65/65-75/>75)	2.64 (1.89 – 3.68)	<0.001	3.51 (2.23 – 5.54)	<0.001
ASA grade (1-2/3-4)	3.74 (2.17 – 6.44)	<0.001	2.85 (1.43 – 5.67)	0.003
BMI[a] (<30/>30 kg/m^2)	3.14 (1.81 – 5.45)	<0.001	6.27 (2.93 – 13.40)	<0.001
TNM stage (I-II/III+)	3.52 (2.15 – 5.78)	<0.001	4.56 (2.40 – 8.65)	<0.001
Proximal AC (None/minor/major)	1.81 (1.27 – 2.58)	0.001	1.02 (0.61 – 1.70)	0.947
Distal AC (None/minor/major)	1.53 (1.15 – 2.05)	0.004	1.10 (0.72 – 1.66)	0.663

Figure 11-1 - ROC curve - oxygen uptake at estimated anaerobic threshold and any complication (p=0.653).

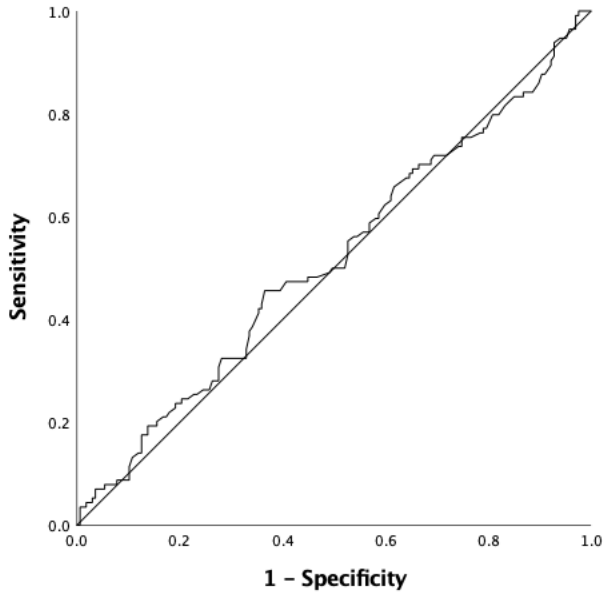
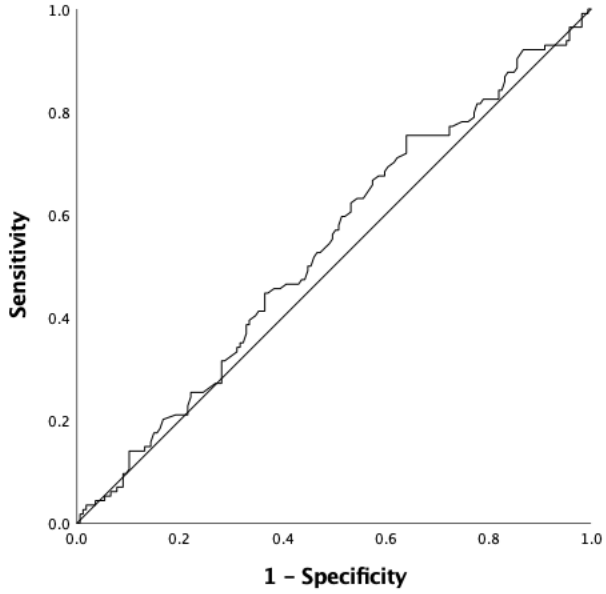


Figure 11-2 - ROC curve - oxygen uptake at peak and complications and any complication (p=0.301).



12 Dynamic imaging of the mesenteric haemodynamic response to exercise

12.1 Introduction

The correlation between vascular calcification - a static measure - and CPET - a dynamic test of cardiopulmonary function, outlined in the preceding chapter supports the hypothesis that further assessment of patients with a significant burden of AC may improve the preoperative identification of patients with limited physiological reserve. While no strong association between AC and anastomotic leak has been demonstrated in this body of work, it remains unclear whether the burden of AC relates to colonic perfusion.

Colorectal cancer resection represents major intra-abdominal surgery and is associated with a stress response. Coupled with preoperative fasting, vasodilation associated with anaesthesia and intraoperative haemodynamic changes create a significant physiological challenge in an untrained subject. The vasculature of the gastrointestinal tract acts as a reservoir, with blood preferentially diverted to critical sites to meet increased demand in situations of stress [604]. Following anastomosis formation, healing is contingent on adequate supply of oxygenated blood. Minimising factors that may restrict blood flow is critical, including tension at the anastomotic site and persistent hypotension.

The intraoperative haemodynamic response of the mesenteric vasculature is poorly characterised. Studies in patients receiving epidural anaesthesia have reported decreased mesenteric flow unresponsive to fluid resuscitation, requiring vasopressors [605,606]. The increased use of minimally invasive surgical approaches has led to declining use of epidural anaesthesia. Other measures of perfusion such as serosal tissue oxygen pressures have been assessed in patients receiving inhalational anaesthesia, with differences noted in tissue oxygenation between desfluorane and isofluorane following resection and anastomosis formation [607]. There is a lack of data on the effect of contemporary anaesthetic techniques and other factors known to influence mesenteric blood flow during colorectal resection, in part due to difficulties in measuring mesenteric flow during surgery.

Exercise similarly results in diversion of blood flow from the colon to meet increased demand from skeletal muscle and facilitate thermoregulation by increasing blood flow to the skin [608]. Gastrointestinal symptoms can result such as abdominal pain and in cases of prolonged endurance exercise, bleeding per rectum. During colorectal surgery, the surgical stress response may contribute to a low-flow state. While temporary, suboptimal blood flow in these situations may form part of a multi-factorial chain of events that results in failure of anastomotic healing.

The effect of exercise on GI tract blood flow has previously been investigated [609-611]. The majority of studies have examined this in healthy volunteers [609-613], athletes [614] or those with autonomic nervous system dysfunction [615-617] using ultrasonography. This modality is operator-dependent and limited by the presence of bowel gas and respiratory motion artefact. Relatively few studies have examined this further despite the development of newer imaging techniques including computed tomography angiography (CTA) and magnetic resonance angiography (MRA). More recently, the development of 4-dimensional flow sequences for use during MRA have made possible combined assessment of vascular anatomy and haemodynamic measurement [618].

A non-invasive imaging technique that requires no contrast and can reliably measure aspects of abdominal blood flow represents a valuable tool. For patients being considered for colorectal surgery, this could be used to investigate mesenteric perfusion, highlighting those at high risk of anastomotic leak due to ischaemia. In such patients, stoma formation may be chosen preoperatively. However, 4D MRA is a new imaging technique for use only in research at present. Its application first requires assessment to establish its clinical value.

It was hypothesised that 4D MRA would be safe in patients awaiting surgery and could be used to assess changes in mesenteric flow following exercise as a surrogate for the stress of surgery. The aim of this pilot study was to assess whether 4D MRA could quantify changes in mesenteric flow in response to exercise in a cohort of healthy volunteers and patients awaiting surgery.

12.2 Methods

Adults over the age of 18 who were able to mobilise independently were eligible for inclusion. Patients with mesenteric vascular or colorectal disease requiring surgery were identified by consultant surgeons providing clinical care. In addition, healthy volunteers recruited from the Glasgow Clinical Research Imaging Facility volunteer bank were eligible to participate in the absence of a history of mesenteric vascular or cardiovascular disease (except hypertension). Exclusion criteria included MR-unsafe devices or implants, significant arrhythmia or previous surgery altering the anatomy of the mesenteric blood supply. Ethical approval was provided by the South West-Central Bristol Research Ethics Committee (18/SW/0166).

Participants attended having fasted for 6 hours from food and two hours from clear fluids and provided written informed consent. Baseline height and weight, medical history and current medications were recorded. Resting heart rate and blood pressure were measured.

Baseline imaging was then obtained. Scans were performed on a 3.0 T Prisma MRI system (Siemens Healthineers) using an 18-channel body array coil. Both 2D and 4D flow data were acquired for each participant as described in Appendices 3 and4. Continuous ECG monitoring was used throughout.

Exercise intervention

The exercise intervention was adapted by specialists in exercise and metabolic health (LS, SG) from that previously demonstrated to be safe in patients with comorbidity [619]. Immediately following baseline imaging acquisition, a familiarisation session was performed in which the exercise intervention, consisting of interval aerobic training using a stepping protocol on an exercise step with adjustable height, was demonstrated and then trialled by the participant. Four intervals of stepping exercise lasting four minutes were performed interspersed with three-minute intervals where the participant walked at a comfortable pace around the room. This was performed under the supervision of an exercise physiologist (LS) and clinician (KK). Continuous heart

rate monitoring was undertaken to ensure the exercise was performed in the range of 70 to 80% of the participant's maximum heart rate (220 minus participant's age). The height of the step and frequency of steps were adjusted to safely meet the target heart rate for each participant.

Post-exercise imaging

Post-exercise portal vein 2D and 3D cine phase contrast (PC) images were acquired immediately after the patient completed the exercise protocol.

2D and 4D flow MRI data analysis

2D time-resolved phase contrast MRI data were analysed using Argus Flow software (Siemens Healthcare GmbH, Erlangen, Germany). Anatomical, magnitude and phase images were imported into Argus Flow and background phase and phase anti-aliasing (10% of highest velocity encoding - Venc) corrections were applied. A region of interest was selected over the portal vein. The software then calculated the following waveforms and indices: area, flow, mean velocity and peak velocity.

Siemens prototype software (Flow version 2.4, Siemens Healthcare GmbH, Erlangen, Germany) was used to process and analyse 4D flow images. Anonymised datasets were uploaded and phase anti-aliasing, background phase and motion tracking corrections were applied.

The portal vein (PV) was segmented using a centreline model. The portal venous system was identified from the two data sets, for both pre- and post-exercise, as shown in Figures 1 and 2. Three analysis planes were selected manually and placed in the region of the portal vein for each participant. Quantitative parameters were automatically generated for each of the selected planes. These parameters included a time integrated flow evaluation with maximum peak velocity magnitude, temporal average net flow and temporal average net forward volume within the contour over time. The software also generated 3D streamlines within the segmented vessels as shown in Figure 12.1.

The inter-rater reliability was tested using the intra-class correlation coefficient (ICC) for 4D net PV flow on pre- and post-exercise imaging from all participants. A total of 2 appraisers (KK, PHB) assessed average net flow through the portal vein.

12.2.1 Statistical analysis

Descriptive statistics were used to assess the baseline participant characteristics. Net flow rates were described using the mean and standard deviation. Comparison of 4D flow values pre- and post-exercise was assessed using the Wilcoxon signed rank test (2-tailed). ICC estimates and their 95% confidence intervals were calculated based on a mean rating (k = 2), absolute-agreement, 2-way mixed-effects model and assessed as described in Chapter 3[493]. Analysis was performed using SPSS Statistics for Mac (version 27.0; SPSS Inc., Chicago, IL, USA).

12.3 Results

Between February 2019 and February 2020, 10 participants were recruited, constituting 5 healthy volunteers (3 male) and 5 patients (4 male). Median age of volunteers was 41 (31 – 69) years and of patients 59 (41 – 65) years. For the patients, 2 were recruited from colorectal clinic and 3 from the vascular clinic.

All healthy volunteers completed the exercise intervention. Of the patients, 1 terminated the exercise intervention early, due to leg fatigue and breathlessness. There were no adverse events. Target heart rate was reached and maintained in all 8 patients who completed the intervention.

The basic demongraphics and pre- and post-exercise net PV flow values for all participants are shown in Table 12.1. The 4D portal vein flow results at each pre-specified time point during pre- and post-intervention assessment for all participants is displayed in Table 12.2 A statistically significant difference between pre- and post-exercise net PV flow was noted in 9 participants: increased in 6 participants and decreased in 3 participants. In one participant, no significant difference was evident between pre- and post-exercise net PV flow. The mean net flow for healthy volunteers was 12.62 ± 3.63 mL/s pre-exercise and 13.45 ± 4.32 mL/s post-exercise. The mean net flow for patients was 15.12 ± 6.58 mL/s pre-exercise and 13.13 ± 6.78 mL/s post-exercise. Figures 12.2 and 12.3 display the mean change in net PV flow pre- and post-exercise in healthy volunteers and patients respectively.

Scan acquisition time for the 4D flow sequences was approximately 8 to 10 minutes per patient per phase (pre- and post-intervention) in addition to the acquisition time for standard 2D imaging.

Inter-rater reliability was excellent. ICC for pre-exercise net PV flow was 0.96 (95% confidence interval 0.95 - 0.97) and 0.97 (95% CI 0.96 - 0.98) for post-exercise net PV flow.

12.4 Discussion

This study confirms that 4D flow MRA is feasible and reproducibly measures dynamic changes in portal vein flow in response to exercise. The exercise intervention was safe and well tolerated by both healthy volunteers and patients while effectively eliciting a haemodynamic response in the majority of participants. Scan acquisition time was acceptable and compatible with translation to clinical use. Although a small sample, it appears that net PV flow in healthy volunteers following exercise increased while the converse was true for patients with colorectal or mesenteric vascular disease. This suggests that 4D MRA may offer a safe method of interrogating arterial flow in patients awaiting colorectal cancer resection.

The use of portal vein flow as a surrogate for mesenteric arterial flow was established by recent work from this group confirming pre- and post-prandial PV flow to be comparable to abdominal aortic, coeliac and superior and inferior mesenteric arterial flow [620]. The portal vein as a target vessel provides two advantages: it is not affected by calcification or collateralisation and has a large calibre, avoiding the artefact arising from dense calcium deposits and the technical challenges of measuring flow through small and tortuous vessels such as the IMA. However, the portal vein drains the entire gastrointestinal tract and flow through it may overestimate that of the colorectum. It is worth noting that the superior mesenteric arterial supply extends the length of the small bowel to the transverse colon and as such, it is not possible to precisely measure localised colorectal blood flow.

Several practical aspects of assessing mesenteric flow in response to exercise were raised by this pilot study. Cardiac gating requires ECG pad use and displacement due to sweating during exercise was noted in the initial participants. This was overcome by ensuring optimum skin contact on placement and use of a pressure dressing to secure pads and limit movement. The use of a metronome to guide and progress stepping exercise was necessary to ensure participants exercised at a pace commensurate with their target heart rate. Real time heart rate monitoring was achieved through continuous ECG. In the latter 4 participants, heart rate at cessation of exercise and at commencement of post-

exercise imaging were each recorded and will be reported as a variable in future studies using pre and post intervention 4D flow MR imaging.

It is possible that the earliest changes in portal vein flow in response to exercise were not captured due to the time elapsed between cessation of exercise and transfer to the scanner for post-intervention imaging. This varied between 20 to 60 seconds and was influenced by patient mobility and body habitus. Attempts to limit this included the use of multiple staff members to assist in the transfer process with dedicated individual tasks (coil placement, table controls, safety assurance). In-scanner exercise could overcome such delays and has been reported in patients undergoing cardiac MRI [621]. However, ensuring adequate exercise intensity whilst maintaining abdominal views and limiting movement artefact may be more challenging in abdominal MRI. Moreover, MR compatible exercise devices are not widely available or affordable.

Limitations include the small sample size. The study was originally planned to recruit 20 participants (10 healthy volunteers, 10 patients). The suspension of non-essential studies due to the Covid-19 pandemic prevented further recruitment. Cautious interpretation of the changes reported in portal vein flow in patients and healthy volunteers is required, but the results demonstrate that patients typically had lower pre- and post-exercise portal vein flow. Moreover, from the individual flow profiles pre- and post-intervention (data not shown), it was evident that previous literature suggesting a binary increase or decrease in mesenteric haemodynamics in response to exercise is simplistic. However, validation in a larger cohort is required. Future studies comparing CT-defined atherosclerotic disease and the corresponding MRA flow characteristics are planned. The incorporation of real-time MRI to hybrid operating theatres is currently being realised. While primarily intended for use during endovascular interventional procedures, the potential future availability of non-invasive intraoperative assessment of mesenteric flow during abdominal surgery is a realistic possibility.

In conclusion, this study demonstrated the application of 4-dimensional MRA as a method of quantifying measure portal vein flow in response to exercise in both healthy volunteers and patients. Examining the trends in portal venous flow and

comparing these with mesenteric arterial flow in a larger cohort may enable a clearer picture of the effect of exercise on mesenteric flow.

Table 12-1 - Mean net portal vein flow pre- and post-exercise (n=10).

Participant	Participant details, Age (years), Gender (M/F)	Mean net flow pre-exercise (mL/s)	Mean net flow post-exercise (mL/s)	p-value
01	Healthy volunteer, 69, M	14.7 ± 0.65	13.0 ± 0.66	<0.001
02	Healthy volunteer, 59, F	7.11 ± 0.52	9.22 ± 0.47	<0.001
03	Patient, 53, M	15.1 ± 0.54	10.2 ± 1.55	<0.001
04	Healthy volunteer, 33, M	17.4 ± 1.48	20.5 ± 1.76	<0.001
05	Healthy volunteer, 31, M	12.3 ± 1.56	11.5 ± 3.27	0.191
06	Patient, 41, M	19.4 ± 1.01	9.7 ± 1.55	<0.001
07	Patient, 61, F	2.9 ± 0.88	4.4 ± 0.75	<0.001
08	Patient, 59, M	11.5 ± 1.14	13.0 ± 1.02	<0.001
09	Healthy volunteer, 41, F	17.0 ± 1.22	18.7 ± 0.79	<0.001
10	Patient, 62, M	21.2 ± 1.29	22.8 ± 0.69	0.001

Table 12-2 - 4D MRA portal vein flow pre- and post-exercise (mL/s)

Participant		Timepoint																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	Pre -	14.2	15.5	15.5	15.5	15.1	14.0	13.7	14.1	14.8	16.1	15.6	15.0	14.7	14.3	14.1	14.2	14.4	14.8	14.6	14.2
	Post -	12.7	14.0	14.0	13.1	13.1	14.0	13.3	13.1	13.1	13.2	13.6	13.4	12.8	12.3	12.5	12.3	11.8	11.8	12.9	12.5
2	Pre -	6.6	7.3	7.5	7.3	7.0	6.8	6.4	6.2	6.2	6.6	6.9	7.1	7.2	7.3	7.2	7.4	7.8	8.0	7.9	7.6
	Post -	9.1	9.4	9.9	8.7	8.6	8.4	9.4	9.6	9.5	9.3	9.7	10.2	9.6	8.9	8.7	8.7	9.1	9.5	9.3	9.0
3	Pre -	14.6	14.9	15.3	15.8	16.5	15.9	15.5	15.5	15.3	14.7	14.5	14.3	14.6	14.7	15.2	15.4	15.3	15.0	14.9	15.2
	Post -	11.6	13.1	13.6	12.6	11.9	10.1	9.3	9.8	10.5	10.0	9.8	9.0	9.0	8.6	9.1	9.4	8.8	8.8	8.8	9.7
4	Pre -	15.6	17.6	18.8	19.0	16.9	15.7	14.6	14.6	15.6	16.9	18.9	19.0	18.7	18.4	18.4	18.5	18.4	18.2	17.1	17.1
	Post -	17.9	21.8	24.2	23.1	21.9	20.7	21.4	21.5	20.6	21.0	21.1	20.4	20.8	20.7	20.0	19.7	19.3	18.9	17.9	16.8
5	Pre -	10.4	11.9	13.6	13.1	12.4	11.6	11.5	11.7	12.5	13.6	13.4	14.6	15.1	14.1	13.6	12.2	11.6	11.1	9.7	9.2
	Post -	7.3	15.5	17.8	15.9	13.9	11.9	12.5	13.3	12.2	11.5	12.3	11.6	11.7	12.6	12.1	10.9	9.5	7.6	5.9	4.6

10.4 11.9 13.6 13.1 12.4 11.6 11.5 11.7 12.5 13.6 13.4 14.6 15.1 14.1 13.6 12.2 11.6 11.1 9.7 9.2
10.4 11.9 13.6 13.1 12.4 11.6 11.5 11.7 12.5 13.6 13.4 14.6 15.1 14.1 13.

Participant		Timepoint																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
6	Pre -	17.7	18.0	20.3	19.4	19.1	18.4	19.0	18.9	18.7	18.9	19.3	20.0	20.5	20.6	20.8	21.0	20.6	19.7	18.8	18.0
	Post -	8.7	9.3	11.3	11.9	13.6	11.8	11.0	10.2	8.5	8.8	9.3	10.3	9.7	9.1	9.4	9.0	8.3	8.1	7.2	8.5
7	Pre -	2.6	2.4	2.9	3.4	3.3	2.8	2.4	2.0	1.6	1.6	1.7	1.8	1.8	1.8	1.8	1.8	1.7	1.6	1.5	1.9
	Post -	4.5	4.8	5.2	6.0	5.6	5.0	4.5	3.6	3.2	3.2	3.3	3.6	3.9	4.2	4.2	4.4	4.4	4.4	4.5	4.5
8	Pre -	11.6	12.4	13.4	13.5	13.3	13.1	12.6	11.9	11.4	10.9	10.5	10.4	10.5	10.4	10.5	10.5	10.5	10.6	10.8	11.1
	Post -	12.3	13.9	13.9	14.5	14.3	14.8	13.1	11.4	11.4	11.6	11.9	12.5	13.3	12.9	13.4	13.5	13.3	13.5	12.6	12.2
9	Pre -	15.6	17.6	18.9	18.4	18.6	18.3	18.5	17.8	17.5	17.2	17.5	17.3	16.5	16.3	16.0	16.0	16.0	16.2	15.6	14.6
	Post -	18.8	19.2	19.5	20.2	20.0	19.7	17.7	17.2	17.7	17.9	18.0	18.3	18.4	18.6	18.7	18.6	18.4	18.7	19.0	18.4
10	Pre -	19.9	23.1	22.8	21.5	21.5	22.8	22.8	22.2	21.6	21.4	22.0	20.4	20.8	21.1	21.2	21.2	20.6	19.7	19.4	18.1
	Post -	23.1	23.7	24.1	23.3	22.2	22.1	22.6	22.3	21.4	22.0	22.4	22.5	22.8	22.6	22.7	22.2	22.9	23.5	23.7	23.6

Figure 12-1 – 4D MRA image showing confluence of portal vein (1), splenic vein (2) and superior mesenteric vein (3).

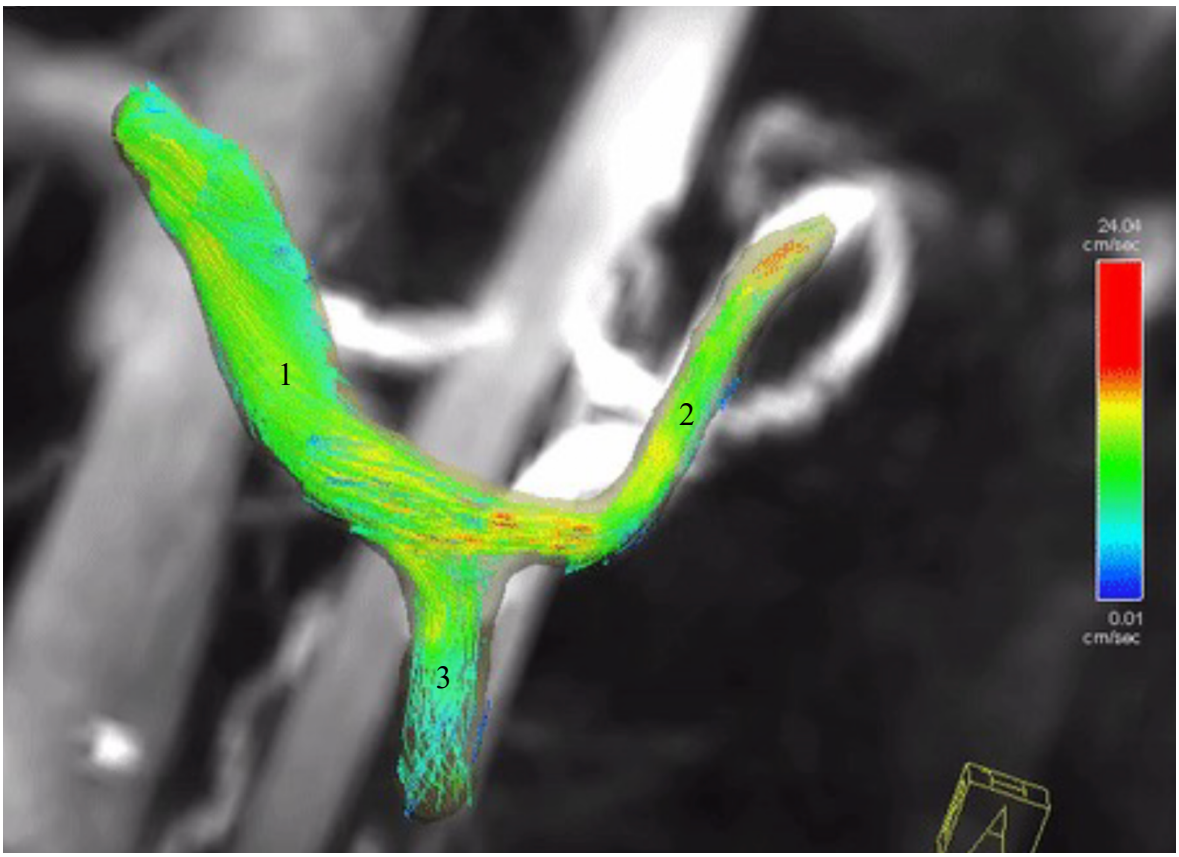


Figure 12-2 - Mean net portal vein flow in healthy volunteers pre- and post-exercise (n=5, error bars indicate standard error).

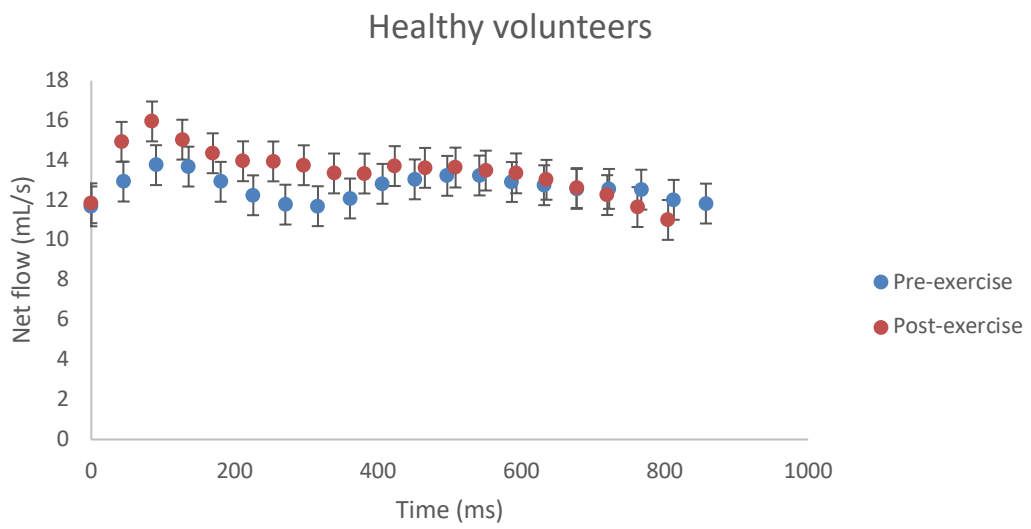
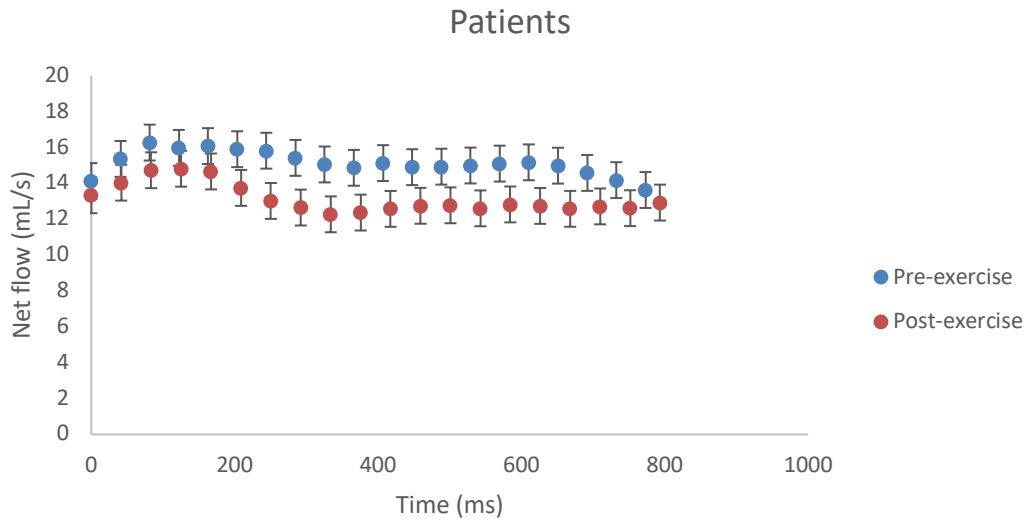


Figure 12-3 - Mean net portal vein flow in patients pre- and post-exercise (n=5, error bars indicate standard error).



13 Conclusions

At the beginning of the research period, it was proposed that aortic calcification may influence the outcome of treatment for colorectal cancer in patients with operable disease. At host level, this was hypothesised to act via increased complication rates resulting from impaired tissue oxygenation compounded by a greater burden of comorbidity, in particular cardiovascular disease. At tumour level, it was hypothesised that patients with a significant degree of AC were more likely to harbour hypoxic tumour microenvironments that predisposed to aggressive tumour biology. The latter was proposed to correlate with more advanced tumour stage, higher rates of recurrence and inferior survival. Moreover, the pro-inflammatory aetiology of atheroma formation was considered to represent an explanatory factor in the aetiology of systemic inflammation in patients with colorectal cancer. It was postulated that the efficacy of radiotherapy and chemotherapy would be reduced in those with significant calcification as a consequence of reduced oxygen delivery and higher rates of toxicity respectively. As a radiographic marker that is readily assessed on CT, AC was hypothesised to be a more clinically applicable comorbidity measure than validated but rarely used comorbidity indices. In this way, AC was thought to have a role in identifying patients who could benefit from more detailed preoperative assessment using tools such as CPET. Finally, the potential for the haemodynamic characteristics of patients with significant AC to be characterised by novel imaging with dynamic flow measurement was to be explored.

The derivation of a semi-quantitative aortic calcification score was described in Chapter 3 and compared with other methods of visual and software quantification. Previous work assessing AC in patients undergoing surgery employed differing approaches to quantification resulting in a lack of standardisation. Reproducibility was excellent both within and between raters using the novel score derived here. Moreover, a clinically relevant method of stratifying patients in relation to the degree of AC present was undertaken, contrasting previous literature in this area that focused on binary approaches denoting absence or presence of calcification. This method allowed for the fact that vascular calcification increases with age. Therefore, a mild degree of calcification was considered separately to a heavy burden of calcification, with

the latter more likely to reflect a pathological degree of atheroma formation. Finally, the score was validated in an external cohort in Chapter 6, representing the first study of its kind in patients with colorectal cancer. In future, the development of dedicated software with the potential for automated analysis would aid clinical translation of AC assessment. This could reduce the burden associated with manual scoring. It has been interesting to observe during this period of research the increased incidence of reporting of coronary artery calcification as an incidental finding on non-dedicated imaging. Indeed, this is now supported by the British Society of Cardiovascular Imaging and the Scottish Intercollegiate Guidelines Network. The development of algorithms that could reliably identify and measure the degree of abdominal AC would enable routine reporting of this data and facilitate its clinical use.

A systematic exploration of the relationship between aortic calcification and postoperative morbidity was undertaken in Chapter 4. The degree of aortic calcification was not independently related to complications considered together or categorised as major, infective or non-infective. Associations with non-infective complications were evident, representing morbidity including cardiac (acute coronary and venous thromboembolic events as well as tachyarrhythmias) and non-cardiac events (ileus, wound dehiscence). This represents an important contribution to the literature as it is the first study in a homogenous patient group with a large sample size in which multivariate analysis was undertaken. A trend towards significance was noted with higher anastomotic leak rate in patients with a significant burden of aortic calcification. Due to its relative rarity in elective colorectal cancer resection, a multicentre prospective study with sample size in the thousands would be required to definitively address whether AC is an independent factor in anastomotic leak. Integrating AC assessment into current trials of intra-operative mesenteric haemodynamic assessment would help to clarify its role in preoperative identification of patients who may be at higher risk of anastomotic leak.

Chapter 5 reported inferior survival following colorectal cancer resection in patients with significant aortic calcification. This was most marked when examining overall survival. However, higher rates of poorer cancer-specific

survival were noted on subgroup analysis of colonic tumour site. Patients with right-sided tumours and significant aortic calcification had the poorest prognosis. Cautious interpretation is necessitated by the limited subgroup sample size (n=223) and the low number of events (23 cancer-related deaths). Higher rates of cardiovascular disease in patients with right colon cancer compared with left have, however, previously been reported. Validation in a large external cohort, along with examination of host-specific (comorbidity, race, cardiometabolic risk factors) and tumour-specific factors (mismatch repair deficiency, degree of tumour necrosis) is warranted. It was notable that 5 year survival in the whole cohort was high at 71%. Factors associated with inferior survival that are not amenable to significant change in the period prior to surgery are unlikely to contribute to a marked improvement in survival rates.

Further examination of the potential mechanisms underlying the relationship between the degree of calcification and survival was demonstrated in Chapters 6 and 7. It was hypothesised that the degree of aortic calcification may correspond to reduced efficacy of neoadjuvant radiotherapy in patients with rectal cancer and to impaired tolerance and completion of chemotherapy in patients receiving adjuvant therapy for colon and rectal cancer. While no association between calcification grade and neoadjuvant therapy response was evident, this study incorporated an external cohort in which similar findings were reported. This provided validation of the technique of AC assessment used in this thesis and confirmed excellent reproducibility. Studies examining patient factors as potential determinants of neoadjuvant response are scarce, in comparison to those assessing tumour and treatment characteristics. Therefore, although negative, publication of this work highlights the need to focus further on host characteristics that may interact with tumour and treatment factors to influence outcome. This may be particularly relevant in patients with locally advanced disease and borderline fitness for neoadjuvant therapy in whom objective pre-treatment markers that correlate with treatment outcome could be used to underpin shared decision-making on treatment intent.

Similarly, the findings of Chapter 7 highlighted that patients with a significant burden of AC are less likely to receive adjuvant chemotherapy and more likely to be undertreated if they do proceed to adjuvant treatment. Although not possible

to characterise, AC likely correlates with the oncologist's subjective assessment of patient fitness for treatment. Future integration of AC assessment to clinical trial protocols as a proxy measure of comorbidity may improve generalisability of trial results. Furthermore, examining recurrence and survival among eligible patients based on receipt of chemotherapy with reference to adverse host characteristics such as AC may highlight whether undertreatment, represented by single agent and reduced dose regimens in contrast to full dose combination chemotherapy, impacts long term outcome. However, the multitude of interacting factors that influence such treatment decisions renders interpretation of this type of data complex.

To investigate the hypothesis that a high burden of aortic calcification may result in tumour hypoxia, immunohistochemical analysis of tissue microarrays available in a subset of patients was undertaken in Chapter 8. The hypoxic marker carbonic anhydrase IX was noted to be low in the cytoplasm of tumour cells in patients with a higher burden of aortic calcification, a characteristic that was associated with poorer cancer-specific survival. Moreover, an association between high membranous CA IX expression and right sided tumour location was clear. This study would have been strengthened by the inclusion of a wider range of hypoxic markers and use of broader scope techniques including omics profiling. This was limited by the restricted access to laboratory space due to the pandemic. A more comprehensive assessment of hypoxic markers including transcriptomic and mutational analysis in a larger sample size is planned. This will facilitate exploration of the associations between AC, molecular subtypes and signalling pathways within hypoxic microenvironments. This work will enable a clearer appreciation of the mechanisms by which tumour hypoxia may contribute to the inferior cancer-specific survival noted in right colon cancer in this thesis.

Chapter 9 focused on clarifying the relative prognostic importance of AC and systemic inflammation. Neither the relationship between AC and survival nor the mechanisms by which they interact have been examined outside the work presented in this thesis. The results of this chapter are particularly relevant as both cardiovascular disease and colorectal cancer share systemic inflammation as an aetiological factor but studies examining this nexus are few. While the

presence of a systemic inflammatory response was more closely associated with inferior overall survival than the degree of AC, it was not related to cancer-specific survival in the whole cohort or in patients with right colon cancer. By contrast, an increasing degree of AC remained independently related to cancer-specific survival in patients with right colon cancer. This contrasts previous literature where a raised mGPS in patients with operable colorectal cancer has been demonstrated to be independently related to poorer overall and cancer-specific survival. Further interrogation of the tumour characteristics is required, particularly as right sided tumours are often associated with differing host (female gender, advanced age) and tumour (MSI-high, higher T stage) characteristics. Factors including the influence of oestrogenic hormones on cardiovascular disease risk and the higher rates of necrosis in large tumours more typical of right colon cancer may contribute. These are two areas among others that are worthy of further examination as potential drivers of the relationship highlighted here.

In order to assess whether AC simply reflects underlying comorbidity or has independent clinical value, a comprehensive investigation of its role in comparison to validated comorbidity indices was undertaken in Chapter 10. Previous studies have focused on single endpoints, overlooking the impact of postoperative morbidity on survival. By assessing the relationship with postoperative morbidity and survival, it was possible to determine that neither the mGPS score nor degree of AC was associated with all complications, but that both were related to overall survival, independent of recognised adverse host factors and the comorbidity measures assessed. No single comorbidity measure was independently associated with both inferior short and long-term outcome. However, the RCRI, a simple 6 item score based on routine clinical data, was independently related to the development of postoperative complications and associated with inferior cancer-specific survival. Its integration into preoperative assessment may highlight high risk patients and trigger further investigation and implementation of preoperative optimisation strategies. This study confirmed that AC alone does not identify patients at risk of poor outcome but may represent a valuable phenotypic feature of the vulnerable patient.

To further characterise the clinical relevance of aortic calcification, Chapter 11 examined the correlation between the degree of aortic calcification and cardiopulmonary fitness in a multicentre UK cohort. The first study of its kind, the results suggest that AC assessment may have a role as a screening tool for preoperative cardiopulmonary exercise testing, a limited resource. Significantly, patients with more advanced tumours had higher odds of suboptimal cardiorespiratory fitness, suggesting that such patients may warrant early identification and more detailed preoperative assessment and intervention. Cardiometabolic factors such as cross talk between circulating inflammatory mediators and skeletal muscle and adipose tissue metabolites may be influenced by regional tumour progression. Exploring these potential underlying mechanisms is an important target of future work. Work to determine the relative importance of aortic calcification, adverse body composition and functional measures of frailty alone and in combination in the preoperative assessment and optimisation of patients with colorectal cancer is in progress.

Finally, the feasibility of using novel dynamic MRA imaging to quantify mesenteric flow was examined in Chapter 12. The sample size was smaller than planned, preventing assessment of the relationship between the degree of calcification and mesenteric perfusion. However, it was possible to characterise changes in mesenteric blood flow in response to exercise, resulting in proof of concept for this prototype imaging technique. Work is underway to restart recruitment following suspension due to the pandemic. The correlation between mesenteric venous and arterial flow in response to exercise in patients with varying degrees of AC will be assessed in the expanded cohort. The role of 4D MRA in preoperative work up of patients considered high risk for anastomotic leak then requires to be investigated in future prospective studies.

Overall, the work presented in this thesis represents a systematic exploration of the relevance of aortic calcification visible on preoperative CT imaging to outcomes both at host and tumour level in patients with colorectal cancer. Potential confounders including comorbidity and systemic inflammation were examined to ensure clinical relevance, as well as attempts to understand the mechanisms by which relationships with complications and survival may have arisen. This adequately meets the aims of the thesis as outlined in Chapter 2.

However, it must be noted that the use of aortic calcification as a measure of cardiovascular health is primitive given that it reflects only the presence of calcified atheromatous plaque and does not capture functional aspects such as pump efficacy and electrophysiological function. This work would have been strengthened by inclusion of such measures to provide a fuller appreciation of cardiovascular status. Only 63 patients (10%) had a history of recent or previous echocardiogram, however, suggesting further clinical data would not have been readily available.

Moreover, the work presented in this thesis does not take account of patient reported outcomes and other important measures such as acceptability and tolerability of more detailed preoperative investigation, factors that require to be addressed as preoperative assessment continues to evolve. Finally, it was originally planned to incorporate a prospective study of a preoperative exercise intervention for high-risk patients to improve postoperative outcome. Ethical approval was gained but funding difficulties compounded by the commencement of a large multicentre randomised controlled trial of preoperative exercise restricted this work. By contributing to the recruitment, supervision and delivery of this trial locally, experience was gained and feasibility demonstrated. Full trial results are awaited.

Future work includes exploring in a geographically distinct population the incidence of aortic calcification and its relevance to outcome in patients with colorectal cancer. This work is currently in progress as a result of collaborations with Sørlandet Hospital, Kristiansand, Norway and Dokkyo Medical University Hospital, Tochigi, Japan. This will provide data on the effect of race and environmental factors on the presence of aortic calcification. A study comparing the value of body composition measures and aortic calcification alone and in combination as preoperative markers of inferior outcome is planned. This is multicentre and will address the potential to use phenotypic features as screening tools for more detailed preoperative investigation and optimisation. Finally, combining self-reported and objectively-measured tests of functional capacity with radiographic markers including aortic calcification and body composition is planned as part of a pilot study examining whether such multimodal approaches can be used at preoperative assessment to highlight

patients for preoperative optimisation and high risk clinic review. The use of these measures as factors by which exercise prescription can be personalised will also be trialled in a subset of the patients recruited.

At the outset of the research period, it was intended that external validation in several large cohorts would be undertaken. Despite forging collaborations with teaching hospitals in the UK, Japan and Norway, the absence of mature and sufficiently detailed data prevented meaningful comparison during the study period. Enhanced data collection continues in collaborating centres in Japan and Norway to facilitate validation of the findings in Chapters 4 and 5.

In conclusion, the work presented in this thesis supports the use of aortic calcification as a measure associated with inferior outcome that may assist in identification of patients who may benefit from further investigation and optimisation. Combining its use with independent risk factors and dynamic measures of function is required to holistically assess relevant host factors influencing outcome among patients awaiting curative colorectal cancer resection.

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Appendix 1

Example search strategy (MEDLINE)

The relationship between aortic calcification and anastomotic leak following gastrointestinal resection: a systematic review.

1. anastomosis, surgical/ or elective surgical procedures/ or colorectal neoplasms/ or esophageal neoplasms/ or gastric neoplasms/ or colectomy/ or esophagectomy/ or gastrectomy/ or digestive system surgical procedures/

2. (colorectal surgery* or colectomy* or colon cancer* or rectal cancer* or esophagectomy* or esophageal cancer* or gastrectomy* or gastric cancer*).mp

3. or/1-2

4. vascular calcification/ or calcinosis/ or arterial occlusive disease/ or atherosclerosis/ or iliac artery/ or aorta, abdominal/ or aorta, thoracic/

5. (aortic calcification* or calcium score* or calcium volume*).mp

6. or/4-5

7. postoperative complications/ or treatment outcome/ or risk factors/ or anastomotic leak/

8. (anastomotic leakage* or prognostic factor* or risk factor*).mp

9. or/7-8

10. 3 and 6 and 9

Appendix 2

Immunohistochemistry protocol – CA IX in Colorectal Cancer Tissue

De-wax and rehydrate

2 x 3 minutes Histoclear

2 x 2 minutes 100% ethanol

2 minutes 90% ethanol

2 minutes 70% ethanol

10 minutes water

Antigen Retrieval

Prepare Citrate buffer pH6 (0.346g citric acid and 2.41g sodium citrate, 1 Litre H_2O)

Heat for 12-14 minutes in microwave

Add slides, seal and lid, heat for 2 minutes/ until yellow button pops up

Heat for 5 minutes under pressure

Remove lid, cool for 30 minutes

Rinse in water 10 minutes

Staining

30 minutes 3% Hydrogen Peroxide

Rinse in water

Block in 10% Casein for 60 minutes

Blot, then add CA IX (1 in 800) Bioscience

Incubate overnight in cold room

Bring slides to room temperature

Wash 2 x 5 minutes in Tris Buffered Saline

Incubate at room temperature with ImmPRESS reagent for 30 minutes

Wash 2 x 5 minutes in Tris Buffered Saline

Add DAB substrate (1 drop in 1mL) for 5 minutes

Wash in water

Counter Staining

30 seconds Haematoxylin

2 minutes water

10 seconds acid/alcohol

2 minutes water

2 minutes Scotts Tap Water

1 minute water

Dehydration and Mounting

1 minute 70% ethanol

1 minute 90% ethanol

2 x 1 minute 100% ethanol

2 x 1 minute Histoclear

Mount using Pertex

Appendix 3

Imaging of the abdominal vasculature was undertaken using True FISP fat saturated breath hold with a Repetition Time/Echo (TR/TE) Time 246/1.31 ms, Field of View (FoV) 340 mm, resolution 1.3 × 1.3 mm, slice thickness 2.4 mm, flip angle 36° and acquisition time of 20 s in the transverse, sagittal and coronal planes. Additional images of the portal and splenic veins (PV and SV) were obtained to facilitate perpendicular planning of 2D flow slices across these vessels using the following sequences:

1. True FISP single shot fat saturated breath hold, orthogonal to the vessel, TR/TE 986/3.26 ms, FoV 400 mm, resolution 1.6 × 1.6 mm, slice thickness 4 mm, flip angle 36°, TA 20 s.

2. Breath hold True FISP cine planned perpendicular to the middle of the imaged vessel, FoV 340 mm, resolution 1.3 × 1.3 mm, slice thickness 7 mm, flip angle 44°, TA 4.7 s.

The cine sequence captured pulsatile motion of the PV and was subsequently used for the positioning of the 2D flow scans.

Half-Fourier Acquisition Single-shot Turbo spin Echo (HASTE) imaging was used to acquire 2D flow data of the aorta at three levels: above the coeliac axis, at the superior mesenteric artery (SMA) and below the renal arteries. Planning of the superior mesenteric vein (SMV), PV and SV was undertaken as previously described.

2-dimensional, time resolved, phase contrast MRI (PC-MRI) were acquired with the following parameters using retrospective ECG gating: resolution 1.8 × 1.8, slice thickness 6 mm, TR/TE 3.7–5.3/2.47–3.1 ms, with a scan time of 14 s (breath-hold) and 30 time frames between each R-R interval. The velocity encoding value (Venc) was set at 50 cm/s for the veins with the option to increase the Venc if aliasing was apparent. The acquisitions used retrospective ECG gating.

The research 4D flow sequence provided by Siemens (WIP 785A, Siemens Healthcare GmbH, Erlangen, Germany) was used with ECG and respiratory gating combined with navigation. Parameters were: imaging volume 288 × 288 × 72 mm, 1.8 mm acquired resolution, 1.8 mm slice thickness, TR/TE = 4.8–5.9/ 2.25–3.19 ms, iPat 3, with a scan time of approximately 8 min and 20 time frames between each R-R interval.

2D flow was acquired initially to help determine the Venc settings for 4D flow of the PV. For 4D flow, the Venc was set at 30 cm/s (pre-exercise) and 40 cm/s (post-exercise) for volunteers and 20 cm/s (pre-exercise) and 30 cm/s (post-exercise) for patients. A lower Venc setting was selected for patients as it was found from 2D acquisitions that these patients typically had a lower peak velocity when compared to the healthy volunteers. It was crucial that the Venc set for venous flow matched the real velocity within the vessel. Mismatch leads to a higher signal to noise ratio in the region of interest [622]. The Venc settings for healthy volunteers and patients were capable of sufficiently visualising flow in the portal vein.

Appendix 4

The Effect of Acute Exercise on Human Colonic Blood Flow in Health and Disease: 4D MRA Scan Protocol

Protocol Version: 1
Date: 12 December 2018
REC Reference Number: 18/SW/0166
Sponsor's Protocol Number: GN18HS661
Sponsor: NHS Greater Glasgow & Clyde
Funder: Royal College of Physicians & Surgeons Glasgow

Healthy volunteers: Baseline scan

- Localisers
- Axial trufisp fs
- Coronal truefisp fs
- 4D flow - venc 30 cm/s

Healthy volunteers: Post-exercise scan

- Localisers
- Axial trufisp fs
- Coronal truefisp fs
- 4D flow - venc 40 cm/s

Patients: Baseline scan

- Localisers
- Axial trufisp fs
- Coronal truefisp fs
- Contrast MRA
- 4D flow - venc 20 cm/s

Exercise: Post-exercise scan

- Localisers
- Axial trufisp fs
- Coronal truefisp fs
- 4D flow - venc 30 cm/s