QUALITY MARKERS IN COLORECTAL CANCER SURGERY: WHAT IS THE EVIDENCE?

By

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STATEMENT OF ORIGINALITY

The author wishes to certify that all of the work presented in this thesis is original in concept, design, and execution. All of the experiments, acquisition and analysis of resulting data, and subsequent production of this thesis were performed by the author unless clearly stated otherwise. Any assistance received in preparing this thesis has been acknowledged.

The author was responsible for recruiting patients, executing studies, and maintaining subsequent contact with patients throughout the duration of the study, as well as liaising with referring consultants about patient progress.

This thesis has not been submitted for any other degree.

Krishanth Naidu

ABSTRACT

It remains crucial to validate metrics of good quality surgery against survival outcomes to accurately reflect a patient's prognosis. This thesis highlights the challenges and complexities of defining, measuring and assessing the quality of colorectal cancer (CRC) surgery.

The assessment of the quality of CRC surgery with respect to oncological outcome is seen to focus on three considerations (Figure 1): the (i) plane of excision, (ii) extent of lymphadenectomy (EoL), and (iii) 'appropriateness' or 'correctness' of a lymphadenectomy (i.e., in areas of watershed blood supply). Whilst all these aspects are likely of equal importance to oncological outcome, most early work appraising CRC surgical quality was centred on correct plane of excision to ensure intactness of the enveloping mesorectal / mesocolic fascia and avoidance of tumour transection[1-4].

The consideration for surgical quality *viz*. EoL has arguably received less attention. This may have partly resulted from challenges in objectively measuring EoL, which has thus far been largely limited to rudimentary assessment of lymph node (LN) harvest. That said, recent interest in central vascular ligation (CVL), also referred to as D3 lymphadenectomy, as a counterpart to complete mesocolic excision (CME), suggests a need for broadening the way that quality of CRC surgery will be contemporarily assessed.

CVL surgery is anatomically based on high vascular ligation at the origin of the principal feeding arterial vessels to maximise central LN clearance, and interest in its routine application has been piqued by the results of several cohort studies demonstrating its association with mesocolic plane surgery[5], decreased local recurrence rates[6], and improved survival[7-9]. While

there has been some controversy regarding routine application of CVL, largely based on concerns surrounding its technical demands and potential complications (especially vascular), the appeal of CVL as a goal for 'good quality' CRC surgery is accepted. However, the determination of whether CVL surgery has been performed remains largely dependent on corroboration by the surgical team, as the level of pedicle transection (and by inference, *unresected* LNs) cannot be assessed by *ex vivo* pathological assessment alone.

The appropriateness of a lymphadenectomy based upon which named vascular pedicles are targeted, particularly in areas of watershed blood supply, has also received little attention. Additionally, while there is a tendency towards CME and CVL surgery in current times, applying such principles to areas of watershed blood supply and mixed lymphatic drainage is challenging. This is likely due to the difficulties in defining dominant lymphatic drainage pedicles in these areas e.g., splenic flexure (SF).

Therefore, **Chapter II**, **Chapter III** and **Chapter IV** explore the aspect of lymphadenectomy extent while **Chapter V** and **Chapter VI** investigate the aspect of '*appropriate*' or *'correct'* lymphadenectomy at the SF.

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To My Family

ARTIFICIAL INTELLIGENCE ATTRIBUTION STATEMENT

During the preparation of this thesis, Microsoft Copilot and ChatGPT were used for the purposes of text enhancement, including sentence structure, grammar checking and spelling correction. Where any text was modified by generative AI, the author then reviewed the resulting content for any errors, inaccuracies or biases, and modified it as required. The author takes full responsibility for the submitted thesis, ensuring the work is their own, and has used generative AI within the parameters of use as per the *University of Sydney generative AI guide for researchers.*

AUTHOR ATTRIBUTION STATEMENTS

CHAPTER II: **Naidu K**, Chapuis PH, Yang J, Koneru S, Chan C, Rickard MJFX, Ng KS. Is computed tomography assessment of residual arterial pedicle length following colorectal cancer surgery a useful marker of surgical quality? Tech Coloproctol. 2025 Apr 12;29(1):101. doi: 10.1007/s10151-025-03130-6. PMID: 40220058; PMCID: PMC11993473.

The undersigned certify that the following is an accurate description of the contributions made by KRISHANTH NAIDU:

Study concept and design

Literature review

Collection of data

Data analysis and interpretation

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The results presented in this thesis have already been published or presented, in part, in the following outputs:

PUBLICATIONS

PAPERS

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3. **Naidu K**, Chapuis PH, Brown K, Chan C, Rickard MJFX, Ng KS. Splenic Flexure Cancer Survival: A 25-year Experience and Implications for Complete Mesocolic Excision (CME) and Central Vascular Ligation (CVL). Meeting Abstracts from the 2023 Annual Scientific Meeting of the American Society of Colon and Rectal Surgeons. Dis Colon Rectum. 2023;66(6):e352-e748. doi:10.1097/DCR.0000000000002917.
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PRESENTATIONS TO LEARNED SOCIETIES

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ABBREVIATIONS

3-D – 3 dimensional

5-FU - 5-fluorouracil

ACPGBI - Association of Coloproctology of Great Britain and Ireland

aHR – Adjusted hazard ratio

AI – Artificial intelligence

AJCC – American Joint Committee on Cancer

APE – Abdomino perineal excision

AR – Anterior resection

ASA – American Society of Anaesthesiologist

ANZ - Australia and New Zealand

BCOR - Bowel Cancer Outcomes Registry

BMI – Body mass index

CC – Colon cancer

ChC – Charles Chan

CME – Complete mesocolic excision

CRC – Colorectal cancer

CRGH – Concord Repatriation General Hospital

CT – Computerised tomography

CVL – Central vascular ligation

CSS - Cancer specific survival

DFS – Disease-free survival

DICOM - Digital imaging and communications in medicine

DLP – Dominant lymphatic pedicle

ERH- Extended right hemicolectomy

HR – Hazard ratio

HVT – High vascular tie

ICA – Ileocolic artery

ICC – Intra-class coefficient

ICG – Indocyanine green

IMA – Inferior mesenteric artery

IQR – Inter-quartile range

IV – Intra-venous

JY – Jessica Yang

KN – Krishanth Naidu

LCA – Left colic artery

LH – Left hemicolectomy

LN – Lymph node(s)

LNY – Lymph node yield

LNR – Lymph node ratio

LODDS – Log odds of positive lymph nodes

LOS – Length of stay

LPLND – Lateral pelvic lymph node dissection

LR – Loco-regional recurrence

LVI – Lympho-vascular invasion

mCRC – Metastatic colorectal cancer

MRI – Magnetic resonance imaging

MSI – Micro-satellite instability

NBOCAP - National Bowel Cancer Audit Project

NCCN - National Comprehensive Cancer Network nCT – Neo-adjuvant chemotherapy

OS – Overall survival

PC – Pierre Chapuis

PiP – Picture in picture

PNI – Peri-neural invasion

RAPL – Residual arterial pedicle length

RC – Rectal cancer

RH – Right hemicolectomy

RT – Radiotherapy

SC – Sub-total Colectomy

SD – Standard deviation

SF – Splenic flexure

SgR – Segmental resection

SFC – Splenic flexure cancer

SLN – Sentinel lymph node(s)

SMA – Superior mesenteric artery

SMV – Superior mesenteric vein

SOC – Standard of care

SPECT – Single photon emission computerised tomography

SPSS – Statistical package for social sciences

SR – Systemic recurrence

SJUH – St. James's University Hospital

Tc-99m – Technetium-99m

Tis – Tumour in-situ

TM – Tissue morphometry

TME – Total mesorectal excision

TNM – Tumour Nodes Metastasis

UHE – University Hospital of Erlangen

UK – United Kingdom

VOI - Volume of interest

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CHAPTER I: INTRODUCTION

CRC surgery has long been governed by principles aiming to completely excise the primary tumour with clear margins, preserving the lymphovascular package's integrity, and ensuring adequate lymphadenectomy[12,13]. However, defining "*good quality*" CRC surgery and its associated metrics remains complex due to the inherent challenge of establishing a clear conceptual and practical definition of what is understood of "*quality*".

Perhaps the earliest documented definition of quality in healthcare was offered by Lee and Jones[14]. In their article, the former authors elude to "*articles of faith*" which describe aspects of the care process a patient receives[14]. They loosely describe quality measures or metrics based on value judgments, defining them as a universally applicable mechanism which enables individuals or institutions to quantify the quality of a selected aspect of care by comparing it to a criterion[11,15,16].

However, whilst important for benchmarking, these structural characteristics and outcomes are not usually actionable for quality improvement, highlighting the necessity of the identification and validation of actionable process measures. This has prompted benchmarking efforts and the creation of international quality assurance databases including the UK's NBOCAP in 2003[17]. These databases aim to collect and analyse data on patients diagnosed with bowel cancer and improve the quality of care by systematically capturing clinical, treatment, and outcomes information, to define best practices and guide the implementation of evidence-based treatment strategies. However, their reporting has been constrained by reliance on verbatim descriptions and subjective impressions of quality care metrics, resulting in a lack of a reproducible framework.

Subsequently, in 2005, Avedis Donabedian described and developed several metrics within a *structure, process,* and *outcome* framework to holistically evaluate the quality of care[18]. While this framework was initially described from a medical point of view, its use has also evolved to include the assessment of the quality of a surgery performed. This was demonstrated by authors such as Faiz *et al.*[11].

In Faiz *et al.*'s[11] study, a *structural metric* was defined as any characteristic of a hospital organization that may influence the quality of care delivered e.g., operative case load. A *process metric* sought to compare current care with treatment standards and protocols, thereby highlighting areas where quality may be lacking that may reflect the interaction that leads to effective care e.g. type of surgery performed and lymphadenectomy rates. Additionally, an *outcome metric* was associated with perioperative measures e.g. anastomotic leak, reoperation rates, mortality rates, and oncological outcomes such as survival or metastatic relapse and local recurrence rates. Table 1 provides a summary of the metrics adapted from Faiz *et al.*[11].

TABLE 1. Summary and Examples of *Structure, Process* and *Outcome* Metrics		
Structural Metrics	Process Metrics*	Outcome Metrics
Operative Caseload/Volume - Surgeon - Hospital - Elective vs. Emergency operation	Surgery Performed - Type of surgery (e.g., RH, AR, etc) - Mode of surgery (i.e., Lap vs. Open vs. Robotic) Pathology Measures - Plane of surgery/dissection (i.e., CME or TME) - Resection Margins - EoL (i.e., LNY, LNR, CVL, HVT, D2. D3 lymphadenectomy) - "Appropriateness" of lymphadenectomy o SF o Rectum	Intra-Operative - Surgical blood loss - Tumour perforation Peri-Operative - Anastomotic Leak/Dehiscence - Return to theatre - Days in intensive care unit - Re-operation - Wound failure Oncological - Mortality rate (e.g., 30-day, 90-day, etc) - Survival (e.g., OS, DFS, CSS, etc) - Recurrence (i.e., LRR, SR)

Table 1: Structure, Process and Outcome Metrics adapted from ***Establishing quality in colorectal surgery***[11]*. * Process metrics are the primary focus of this thesis.*

This thesis focuses on quality markers within the remit of a colorectal surgeon, and its impact on oncological outcome. Specifically, this thesis examines quality metrics through pathology assessment from an oncological perspective. These are process metrics, as seen in a synoptic pathology report, which a surgeon has the capacity to modify intra-operatively (e.g., operating in the correct plane, performing an adequate lymphadenectomy or ligating a particular feeding artery versus another) and in turn is thought to have a direct impact on a patient's cancer outcome.

Accordingly, the pathology quality assessment of CRC surgery is seen to focus on three considerations (Figure 1): the (i) plane of excision, (ii) EoL and (iii) 'appropriateness' or 'correctness' of a lymphadenectomy (i.e., in areas of watershed blood supply). The tables presented below (Table 2, Table 3 and Table 13) aim to synthesize current knowledge and understanding from the literature regarding pathology quality markers and their impact on survival outcomes.

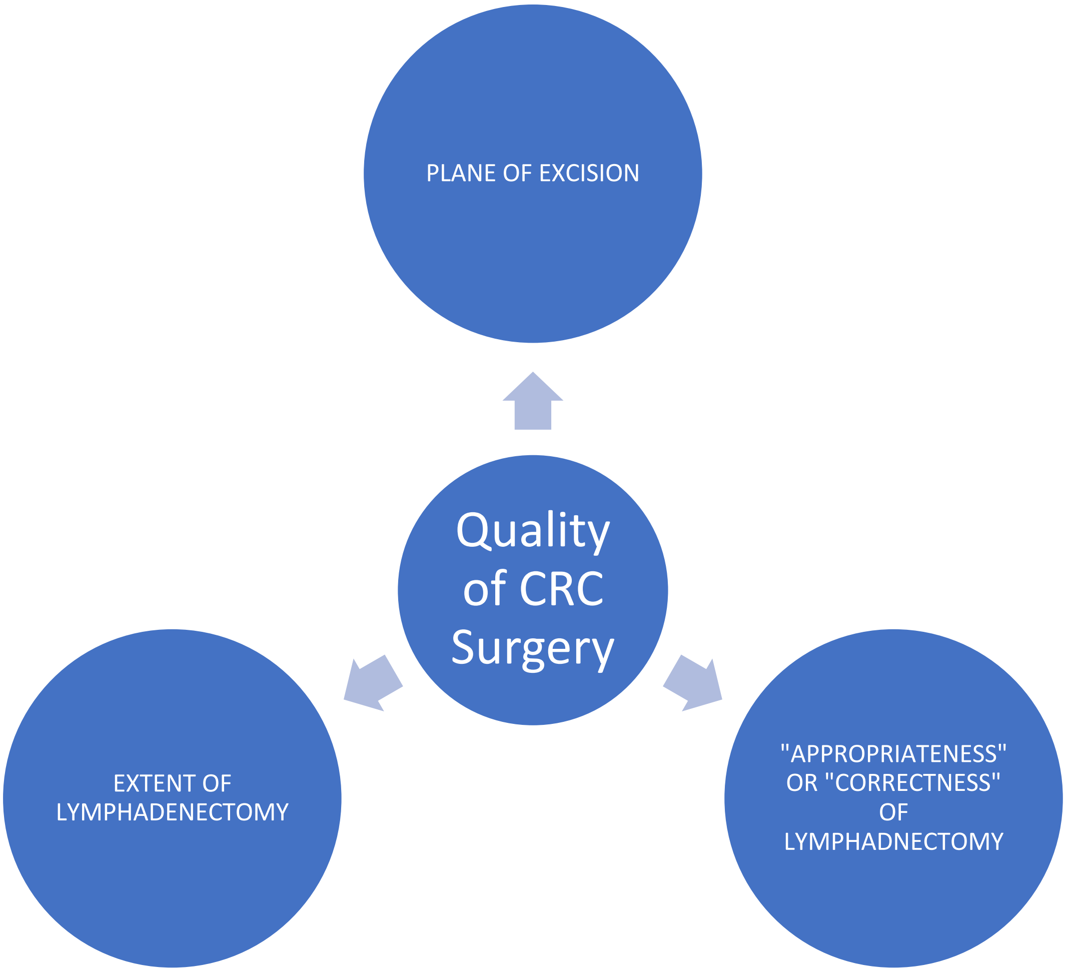


Figure 1: Factors that define good quality CRC surgery.

PLANE OF EXCISION (PLANE OF MOBILISATION)

Most early work appraising surgical quality was centred on the correct plane of excision or mobilisation (i.e. TME and CME) to ensure integrity of the enveloping mesorectal or mesocolic fascia and avoidance of a compromised tumour resection margin. This may come as no surprise given that the early emphasis of good quality cancer surgery was adherence to the extra-fascial ‘holy plane’ which, although first described for the rectum[1,2], is anatomically continuous with the colon.

To this end, comprehensive grading of pathology specimens based on intactness of fascial planes has facilitated research on this aspect of CRC surgical quality[4,19]. For example, in a multi-national RCT that included 80 clinical units, Quirke *et al*[19] reported 3-year LR rate of 4%, 7%, and 13% for mesorectal, intramesorectal, and muscularis propria dissections, respectively[19]. Similarly, in CC patients, West *et al*.[4] found that mesocolic plane surgery was associated with improved survival compared to those who had their muscularis propria plane breached during surgery. Unsurprisingly, these techniques (i.e., TME and CME) are routinely practiced and taught, particularly in ANZ, where formal colorectal surgery fellowship training pathways are available[20].

Given the extensive research and well-established benefits of operating within the correct surgical plane, this thesis will not address this quality marker further.

EXTENT OF LYMPHADENECTOMY

Consideration of EoL as a marker of CRC surgery quality has arguably received less focus. This may partly stem from the difficulty in objectively assessing LN harvest which has traditionally been limited to metrics derived from rudimentary *ex vivo* LN counts (e.g., LNR or LODDS). Consequently, most metrics studied to date focus on LNY and the excised nodal package rather than what remains *in situ* (Table 2).

The current standard, which recommends examining a minimum of twelve LNs, traces back to a guideline issued by the World Congress of Gastroenterology in 1991, partly supported by a subsequent small study of 50 patients aimed at reducing under-staging[21]. This historical benchmark has since been adopted by key organizations, including the AJCC[22] and the NCCN[23,24].

Relying on this historical benchmark has limitations, as it does not fully capture the completeness of lymphadenectomy performed, leaving uncertainty about *residual* nodal tissue. The latter is especially relevant in the context of neoadjuvant treatment regimens, where LN harvest may be artificially reduced due to challenges in LN identification caused by pre-operative radiation-induced tissue fibrosis and reduction in tumour bulk[25,26]. Of note, sampling fewer LNs under such circumstances does not necessarily imply a poor-quality lymphadenectomy or surgery[27]. This quandary highlights an inherent limitation in current EoL metrics, which only assesses excised nodal tissue without providing insight into what may be left behind which, in fact, may prove more relevant.

Lymph Node Yield

Nodal status (i.e., LN disease positivity) is one of the most important predictors of survival in non-metastatic CRC. Documented 5-year OS rates in node positive CRC patients range from 30-60%, compared to 70–90% in those with node negative disease[28,29]. Additionally, recurrence rates in node-positive CRC patients are approximately 30–35%[28,30,31], with the majority occurring within the first three years following tumour resection[28,31]. To improve survival and reduce recurrence, patients with node-positive CRC are typically considered for adjuvant therapy[32-35].

The optimal number of LNs needed to accurately determine LN status in CRC remains debated. While the historical standard is to harvest and analyse at least twelve LNs[21], recommendations for the optimal number of LNs to harvest have varied widely from six to over 40[21,28,33,34,36-50]. Despite this, studies, as summarised in Table 2, have overwhelmingly (yet, not universally) demonstrated that cancer survival improves as the number of harvested LN increases. This finding underscores a widely accepted notion that a higher LN harvest may improve the detection of micro-metastatic disease, skip metastases, and/or identification of positive LNs, in a manner that reduces under-staging and stage migration[33-35,46,51-54].

Lymph Node Yield and Stage Migration

Proponents of the stage migration theory argue that examining a small number of LNs may miss metastatic nodes, leading to false classification of patients as harbouring AJCC Stage I/II disease. By analysing more LNs and increasing the likelihood of detecting positive LNs, it allows for accurate re-classification of staging e.g., to AJCC Stage III. This in turn allows for improved prognostic accuracy[46].

Prognostic Influence of Lymph Node Yield on Survival Outcomes in Patients with Colon Cancer

Evidence For an Association between Lymph Node Yield and Survival

Since 1998, as shown in Table 2, numerous studies have demonstrated the prognostic value of increased LNY in CC[37,38,50,55,56]. However, these studies reveal a lack of a standardized LNY cut-off, making it difficult to draw uniform conclusions. The heterogeneity in LNY cut-offs may be attributed to variations in LN sampling that are influenced by: variations in patient characteristics (e.g. operation acuity – elective vs. emergency), tumour biology, anatomical variations and differences, the use of neoadjuvant therapy, and pathologist-related challenges (e.g., available personnel and time required in retrieving LNs and detecting nodal metastatic disease)[25-27,48,55-58].

Additionally, variability in surgeon-related factors may influence the adequacy of a nodal harvest. These factors include: (i) a lack of standardisation in cancer resection and lymphadenectomy practices, particularly as CVL surgery has yet to be widely adopted; (ii) differences in surgical competence[48,59] and skill[48,59] amongst and within units; and (iii) inadequate surgical case volumes[48,59]. Moreover, some studies inappropriately include rectal resections in CC cohorts without addressing the use of neoadjuvant therapy or providing a rationale for inclusion, leading to confusion and inconsistency[56]. Finally, while large cancer databases offer substantial data, they often lack granular information on pathology (e.g., MSI status) or technical-related factors such as the level of vascular ligation[56,57].

Evidence Against an Association between Lymph Node Yield and Survival

While a large volume of evidence exists describing an association between LNY and survival, several studies have not demonstrated such a relationship regardless of the cut-off value used[60-71]. For example, Tsikitis *et al.*[70] conducted a study on 249 AJCC Stage III CC patients who underwent standardized surgery and pathology assessments performed by a large group of surgeons and pathologists, respectively, at a high-volume centre. That study found that neither the total number of LNs retrieved, nor the number of negative LNs, significantly influenced CSS (P=0.08) or DFS (P=0.59). This conclusion persisted even when the LN count was dichotomized into groups of more or fewer than twelve (P=0.11).

Storli *et al.*[66] reported similar findings in patients undergoing CME surgery. Firstly, their study observed no significant difference in LNY between laparoscopic and open surgical groups (mean=18 LNs vs. 16 LNs; P=0.09). Also, no association with either OS or DFS were demonstrable when LNY was assessed as a continuous variable in either of these groups. It is noteworthy that, Storli *et al.*[66] proposed a similar explanation to that offered by Tsikitis *et al.*[70] by suggesting that the lack of association between LNY and survival likely stems from the standardization of operative quality. That is, factors such as training, hospital volume, and good quality surgical techniques—like those used in radical resections and CME—may make nodal count alone an unreliable indicator of surgical quality, as most (if not all) resections would be of 'high-quality' and with good LNY.

Wong *et al.*[68] studied 30,625 non-metastatic CC patients using the SEER database and found no evidence of improved OS at hospitals with higher LN harvest rates. Furthermore, despite higher LN harvest rates, these hospitals demonstrated no increase in the rates of AJCC Stage III disease. These authors suggested that hospitals examining more LNs may not necessarily detect more nodal metastases due to variations in surgical practices. For example, some surgeons may perform limited resections but ensure inclusion of the primary vascular pedicle and its associated lymphatics (which typically harbour positive nodes but yield fewer LNs overall) while others may opt for wider resections yielding a higher LN count overall but not necessarily more positive (or *oncologically relevant*) LNs.

Impact of Neoadjuvant Therapy on Lymph Node Yield in Patients with Rectal Cancer

In RC patients who received pre-operative RT, with or without sensitizing chemotherapy, an impact on LNY is evident. While neoadjuvant therapy is known to induce tumour shrinkage and improve local control[3,72], several studies including a meta-analysis[26] have shown a reduction in the number of LNs examined in pre-treated *ex vivo* mesorectal specimens[25,61,73-75]. For example, in a systematic review and meta-analysis by Mechera *et al.*[26], published in 2017, 14 analysed studies involving patients who received neoadjuvant chemo-RT reported a mean LNY of fewer than twelve. On average, neoadjuvant chemo-RT resulted in a mean reduction of approximately four LNs compared to those who did not receive neoadjuvant therapy, along with a mean reduction of 0.7 positive LNs. Similarly, patients receiving only neoadjuvant RT had a mean difference reduction of 2.1 LNs compared to those who did not receive any pre-operative RT[26].

Prognostic Influence of Lymph Node Yield on Survival Outcomes in Patients with Rectal Cancer

Accepting that neo-adjuvant therapy has consequences on LNY raises the question regarding survival implications that LNY has in RC patients. Several studies, including the previously mentioned meta-analysis[26], have shown no association between LNY and survival with or without neoadjuvant therapy. Specifically, Engel *et al.*[67], Kim *et al.*[67] and Rullier *et al.*[61] reported no association between the number of LNs retrieved and recurrence, OS, or DFS in cohorts of 884, 150 and 198 RC patients, respectively. Engel *et al.*[67] suggest that factors beyond surgeon-related variables may contribute to the lack of association between LNY and survival. For example, variations in neoadjuvant chemo-RT and RT-only practices for RC across institutions, countries, and between the East and West may influence the observed results and should be considered when interpreting these studies[64].

Notably, in Mechera *et al*.'s[26] meta-analysis, which set out to understand the survival implications of harvesting twelve or more LNs in patients who had received neoadjuvant therapy, seven studies[60,61,75-79] failed to demonstrate a significant association between the twelve LN benchmark (and other cut-offs) and OS or DFS, despite observing a reduction in LNY of 10% to 40% following neoadjuvant therapy. Additionally, Persiani *et al*.[73] attempted to define a new cut-off value for retrieved LNs in RC patients to predict survival differences but found no statistically significant variation between sub-groups.

Limitations in Relying on Lymph Node Yield to Assess Extent of Lymphadenectomy

Having described the heterogenous data regarding the association between LNY and survival following CRC surgery, and the further artefactual bias imposed by neoadjuvant therapy in RC patients, a call for a more robust assessment of EoL seems justified. Irrespective of the differences observed across various studies regarding LNY and survival, a common thread exists: conclusions about a survival benefit (or lack thereof) based on LN harvest focuses solely on the LNs that are excised without considering what remains *unresected* and its impact on survival. For example, in Figure 2, which illustrates a RH for an ascending CC, the dotted line indicates the conventional (i.e., D2 or non-CVL surgery) limit of mesenteric resection. Medial to this boundary, nodal tissue remains unresected along the residual vascular pedicle (i.e., ICA and ICV) up to the SMA and SMV.

Therefore, assessing the *unresected* LNs following CRC surgery may provide a more comprehensive measure of the quality of the lymphadenectomy performed, as it accounts for residual nodal tissue. Notably, this *unresected* package of nodal tissue has been reported to harbour skip metastases in 0.8%–25%[80], micro-metastases in up to 8.1%[12], and central nodal metastases in up to 11%[12] of patients.

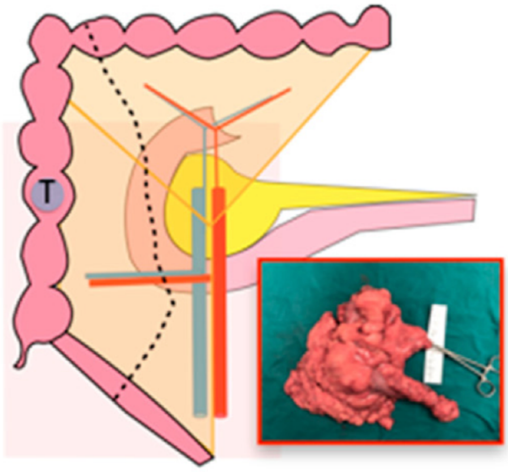


Figure 2: Diagrammatic representation of a conventional RH for an ascending CC (marked by T). The dotted line marked the medial limit of mesenteric resection. Adapted from Balciscueta et al.[10]. Reproduced with permission from Elsevier.

To this end, there is a paucity of prospective evidence with respect to the EoL metrics that focus on *unresected* LNs following lymphadenectomy and their associated long term survival outcomes. Although some studies have described *in vivo* radiological markers[81] and *ex vivo* histopathological assessments[82,83], these studies lack generalisability. For example, the role of *in vivo* radiological markers as surrogates for the EoL and quality measures for survival outcomes have only been studied in a limited number of centres that practice routine CVL surgery. In one such study by Vogelsang *et al.*[81], involving 127 sigmoid cancer patients where routine CVL surgery was intended, a survival benefit was observed in association with a shorter RAPL. Similarly, the influence of *ex vivo* histopathological markers on survival outcomes in CRC remains poorly understood[84,85]. For example, Ng *et al.*[84] demonstrated the feasibility of performing *ex vivo* TM measurements post-CC surgery[84]. However, successful application of these measurements for RC resections have been inconsistently reported[85]. Furthermore, the prognostic significance of *ex vivo* TM measurements in relation to survival outcomes remains unclear, which limits their potential as reliable quality metrics in CRC surgery[84,85].

Consequently, *in vivo* radiological markers and *ex vivo* histopathological assessments will be further studied and discussed in **Chapters II to IV**.

TABLE 2. Overview of Publications on LNY and Their Associations with Survival (up to 2023)											
Study	**Sample size (n)**	**Study Type**	**Colon or Rectum**	**LNY**	**LNY cut offs**	**Neoadjuvant Therapy**	**Laparoscopic or Open Surgery**	**Emergency or Elective**	**AJCC Stage**	**Follow-up (months)**	**Oncological Outcomes**
Becerra *et al.*[56] 2016 USA	360,846	RSCS	Colon	NR	LNY<12 (Suboptimal) vs LNY≥12 (Optimal)	NA	NR	NR	I to III	NR	**5-yr OS** (P<0.01) 64% vs 71 % Suboptimal vs Optimal
Belt *et al.*[40] 2012 Netherlands	332	RSCS	Colon	NR	LNY<10 (Low) LNY≥10 (High)	NA	NR	NR	II to III	Median = 57	Overall, patients with high LNY tended to have fewer recurrences compared with patients with low LNY (28.6% vs 37.7%, P=0.09) **Recurrence Rate** Stage II - 16.4% vs 29.7% (High vs Low; P=0.05) ***5-yr DFS*** Stage II – Low vs High:<70% vs >80% (P=0.04) Stage III – Low vs High: <50% vs <60% (P=0.2)
Bilimoria *et al.*[86] 2008 USA	142,009	RSCS	Colon	<67yrs: Median=11 (IQR 6-17) 67-78yrs: Median=10 (IQR6-16) >78yrs: Median=10 (IQR 6-16)	LNY<12 LNY≥12	NA	NR	NR	I to II	Median = 40	**5-yr OS** LNY≥12 vs LNY<12 - <67yrs: 83.4% vs 79.7%; P<0.001 - 67-78yrs: 68.5% vs 67.1%; P<0.001 - >78yrs: 41.7% vs 36.2%; P<0.001
Bilimoria *et al.*[87] 2008 USA	142,009	RSCS	Colon	Right Colon: Median=12 (IQR 8-18) Left Colon: Median=8 (IQR 4-13)	LNY<12 LNY≥12	NA	NR	NR	I to II	Median = 40	**5-yr OS** Right Colon: <80% vs >80%; P<0.0001 (LNY<12 vs LNY≥12) Left Colon: 80% vs >80%; P<0.0001 (LNY<12 vs LNY≥12)

											LNY≥12: HR 0.8 (95%CI 0.74-0.86; P<0.05)
Bilchik *et al.*[57] 2010 USA	253	Recruited from 2 Prospective Multi-centre Clinical Trials	Colon Rectum	Mean = 20 (SD12)	LNY<12 LNY≥12	17/36 rectal cancers	Lap = 92.9% Open = 7.1%	NR	I to III	Median = 38	**4-yr DFS** (LNY<12 vs LNY≥12) Stage I: 90.5% vs 97.7%; P=0.22 Stage II: 67.5% vs 94.7%; P=0.0036 Stage III: 61.0% vs 61.0%; P=0.61
Bogner *et al.*[88] 2023 Germany	24,085	RSCS	Colon Rectum	NR	LNY<12 LNY≥12	NR	Lap = 18.2% Open = 59.6%	NR	I to IV	NR	LNY≥12: HR = 0.78, 95% CI = 0.70–0.87; P <0.001)
Booth *et al.*[89] 2016 Canada	2,488	RSCS	Colon	Mean = 16	LNY<12 LNY≥12	NA	NR	NR	II	NR	**5-yr CSS MV** LNY≥12 vs LNY<12 = 85%vs76%; P<0.001 LNY<12 LNs: HR1.49(95%CI 1.22-1.83) **5-yr OS MV** LNY≥12 vs LNY<12 = 78%vs66%; P<0.001 LNY <12 HR1.37(95%CI 1.19-1.58)
Budde *et al.*[35] 2014 USA	147,076	RSCS	Colon	2004: Median 12 (IQR 7-18) 2010: 17 (IQR 12-23)	NR	NR	NR	NR	I to IV	Median = 26	**OS MV** LNY (continuous variable): HR 0.987 (95% CI 0.986 to 0.988; P< 0.001)
Bui *et al.*[54] 2006 Canada	960	RSCS	Colon	NR	LNY 1-3 LNY 4-6 LNY 7-9 LNY 10-36	NA	NR	NR	I to III	NR	LNY 10–36 versus LNY 1–3 – HR=0.6 (95%CI 0.4–1.0, P=0.03)
Caplin *et al.*[50] 1998 Switzerland	377	RSCS	Colon	Dukes B Mean = 9.8 (Range 0-57) Dukes C Mean = 9.8 (Range 1-66)	LNY≤6 LNY≥7	NR	NR	NR	II to III	NR	Dukes' B (TNM Stage II) patients with LNY≤6 examined had significantly poorer overall survival than those with LNY≥7 examined (P=0.0014). Such a significant difference was not observed among Dukes' C (TNM Stage III) patients (P=0.7).

Chang *et al.*[63] 2012 China	297	RSCS	Colon Rectum	Mean = 14.5 (SD8.2)	LNY<12 LNY≥12	NA	NR	NR	I	Median = 60 (Range 6-130)	**5-yr DFS (Univariate)** LNY<12 = 89.2% LNY≥12 = 87.3% P=0.72 **5-yr DFS (MV** LNY<12 vs LNY≥12 = HR 1.24 (95%CI 0.65-2.35;P=0.51)
Chang *et al.*[90] 2012 Taiwan	9,644	RSCS	Colon	Stage I Mean = 18 (SD12.4) Stage II Mean = 18.5 (SD12) Stage III Mean = 19.3 (SD13)	LNY≤11 LNY 12-16 LNY 17-23 LNY≥24	NA	NR	NR	I to III	Mean = 49.4 (SD 18.4)	**LNY (AUC)** -5-year OS 0.58 (95%CI 0.56–0.60) -5-year DFS 0.56 (95%CI 0.54–0.58) -5-year DDS 0.57 (95%CI 0. 0.55–0.59)
Chen *et al.*[91] 2006 USA	82,896	RSCS	Colon	Median = 9	LNY 0 LNY 1-7 LNY 8-9 LNY 10-11 LNY 12-14 LNY≥15	NA	NR	NR	I to III	NR	**OS** **Stage I** LNY 0 = 132 months (P<0.001) LNY 1-7 (Ref) LNY 8-14 = 131 months (P>0.05) LNY≥15 = 149 months (P<0.001) **Stage II** LNY 0 = 45 months (P<0.001) LNY 1-7 (Ref) LNY 8-14 = 99 months (P<0.001) LNY≥15 = 131 months (P<0.001) **Stage III** LNY 1-7 (Ref) LNY 8-14 = 52 months (P<0.001) LNY≥15 = 67 months (P<0.001)
Chen *et al.*[92] 2011 USA	36,712	RSCS	Colon	LNY<12 Mean = 7.2 (SD2.7) LNY≥12 Mean = 19.8(SD 8.9)	LNY<12 LNY≥12	NA	NR	NR	I to III	NR	**OS (P<0.001)** LNY<12 = 53 months LNY≥12 = 66 months
Chang *et al.*[93]	10,517	RSCS	Colon Rectum	Mean = 16.5 (SD 11.9)	LNY<12 LNY≥12	1,128/10,517 had	NR	NR	I to III	NR	5-year risk adjusted overall mortality were lower for LNY≥12 than LNY<12 among

2012 Taiwan						preoperative radiotherapy					Stage II (24.3% vs. 31.1%, P=0.012) and Stage I (20.8% vs. 23.6%, P=0.003), but insignificant for Stage III (40.2% vs. 45.6%, P=0.073)
Cho *et al.*[94] 2015 South Korea	556	RSCS	Rectum	Lap Mean = 16.2 (SD8.1) Robot Mean = 15 (SD8.1)	LNY<12 LNY≥12	NR	NR	NR	I to IV	Mean = 51.8 (SD15.3)	**LR, SR, OS** LNY≥12 not associated with LR (P=0.61), SR (P=0.24) or OS (P=0.28)
Choi *et al.*[95] 2010 Hong Kong	664	RSCS	Colon Rectum	Median = 12 (Range 1-58)	LNY<12 LNY≥12	NA	NR	Elective = 84.8% Emerg = 15.2%	II	Median = 44 (Range 12-104)	The **5-yr DFS** rate was significantly higher for patients with LNY≥12 compared to those with LNY<12. The significant difference in 5-year DFS persisted if the dividing number increased progressively from 12 to 23. However, the difference in survival was most significant for LNY=21. The **5-yr DFS** rate of patients with LNY ≥21 was 80% whereas that of patients with LNY<21 examined was 60% (P=0.001, HR 2.08). The **5-yr DFS** also increased progressively up to LNY=21.
Cianchi *et al.*[96] 2002 Italy	140	RSCS	Colon Rectum	Median = 12 (Range 3-38)	LNY≤8 LNY≥9	NA	NR	NR	II	Mean = 66.7 (Range 29-110)	**5-yr OS** LNY≤8 = <65% LNY≥ 9 = >80% P<0.001 **MV** LNY≤8 vs LNY>9: HR 2.7 (1.3-5.7; P=0.01)
Cserni *et al.*[34] 2002 Belgium	8,574	RSCS	Colon Rectum	Median = 10 (Range 1-87)	NR	NA	NR	NR	II	NR	Both OS rates displayed an improvement with an increase of number of LNs examined. The smoothed plot of the martingale residuals against the number of negative LNs was reasonably linear.
Damin *et al.*[77] 2012 Brazil	162	RSCS	Rectum	Whole group Mean = 17.1 (range 3–51)	LNY<12 LNY≥12	71 patients (43.8%) Neoadjuvant treatment consisted of	NR	? 100% elective	II to III	Median = 61 IQR (2-78)	**Whole group** **5-yr OS (P=0.69)** LNY<12 = 71.3% LNY≥12 = 74.8% **5-yr DFS (P=0.11)**

				Pre-op CRTx: Mean = 14.2 No Pre-op CRTx: Mean = 19.4		5,040 cGy delivered to the pelvis in fractions of 180 cGy/day, 5 days per week and fluorouracil, given in bolus IV infusion at a dose of 425 mg/m^2 of body surface area per day (for 5 days) during the first and fifth weeks of radiotherapy					LNY<12 = 67.8% LNY≥12 = 67.2% **Pre-operative chemoradiotherapy group** **5-yr OS (P=0.37)** LNY<12 = 77.8% LNY≥12 = 58.6% **5-yr DFS (P=0.08)** LNY<12 = 85.0% LNY≥12 = 55.2%
Desolneux *et al*.[97] 2010 France	362	RSCS	Colon Rectum	Mean = 12 (Range 6-42)	LNY>8 LNY>12	NA	NR	NR	I to II	Median = 140	Number of LNs removed: HR 0.948 (95%CI 0.905–0.993; P=0.023)
Dillman *et al*.[98] 2009 USA	574	RSCS	Colon	1989-1997 Mean = 8 (SD6.9) 1998-2005 Mean = 14.5 (SD10.2)	LNY<12 LNY≥12	NA	NR	NR	I to IV	NR	**5-yr OS** Stage I (P=0.02) LNY<12 =70% LNY≥12 = 82% HR=0.60 Stage II (P=0.02) LNY<12 =51% LNY≥12 = 71% HR=0.59 LNY=12 was associated with better survival for patients with Stage I or Stage II disease but not Stage III or IV.
Dolan *et al*.[71] 2018 UK	896	RSCS	Colon	Median = 17 (Range 1-71)	LNY<12 LNY≥12	NA	Lap = 18.5% Open = 79.8%	Elective = 86.8% Emerg = 13.2%	I to IV	NR	**LNY** LNY<12 vs LNY≥12 - CSS: 77% vs 78% (P=0.18) - OS: 64% vs 67% (P=0.30)
Doll *et al*.[78] 2009	216	RSCCS	Rectum	Neoadjuvant CRTx:	LNY<12 LNY≥12	102 patients	NR	Elective = 100%	I to IV	NR	**Neoadjuvant therapy** **5-yr OS (P=0.52)** LNY<12 = 77.2%

Germany				Mean = 12.9 (SD5.1) Upfront surgery: Mean = 21.4 (SD 10.8)		Neoadjuvant chemoradiotherapy group: Total of 45 Gy delivered in 5x5 fractions of 1.8 Gy 5 times a week. During the radiation period, chemotherapy with 5-FU was given as a continuous infusion of a dose of 250 mg/m^2 per day 7 days a week. Surgery was performed 4–5 weeks after completion of neoadjuvant therapy. In upfront surgery group = Postoperative chemoradiotherapy given. They underwent the same radiation Protocol as above up to 45 Gy plus an additional 5.4 Gy boost delivered to the tumour bed.					LNY≥12 = 77.3%

						Postoperative adjuvant chemotherapy was scheduled for 4–8 weeks after surgery, followed by radiotherapy together with the third and fourth chemotherapy cycles. After chemoradiotherapy, two further cycles of chemotherapy were planned.					
Durakar *et al.*[99] 2014 Turkey	461	RSCS	Colon Rectum	Median = 11 (Range 1-50)	LNY 1-7 LNY 8-11 LNY≥12 LNY 1-11 LNY≥12	NA	NR	Elective = 87.9% Emerg = 12.1%	I to III	Mean = 130.1	**CSS (P=0.01)** LNY 1-7 = 60.9% LNY 8-11 = 63.9% LNY≥12 = 75.1% **CSS (P=0.004)** LNY 1-11 = 62.3% LNY≥12 = 75.1%
Edler *et al.*[38] 2007 Sweden Denmark	1,025	Prospective RCT	Colon Rectum	Median = 5 (Range 0-32)	LNY<12 LNY≥12 Continuous	5x5Gy 113/298 rectal cancer patients received CRTx	NR	NR	II to III	Median = 60	**5yr OS – Univariate** (P=0.007) LNY<12=64% LNY≥12=77% **5yr OS (MV)** LNs (P=0.009)
Engel *et al.*[67] 2005 Germany	884	RSCS	Rectum	NR	LNY<12 LNY≥12	17/884 patients received CRTx	NR	NR	I to IV	Median = 68.4	**5yr OS (MV) P >0.05** LNY≥12: HR 0.8 (NS)
Evans *et al.*[52] 2007 UK	380	POCS	Colon Rectum	Median = 13 (Range 0-42)	LNY<12 LNY≥12 LNY<10 LNY≥10	145 rectal cancer patients received CRTx	NR	Elective = 78% Emerg = 22%	I to IV	NR	**5-yr OS** **Duke C – 42%** **Duke B** LNY<12 (65%) vs LNY≥12 (70%); P=0.58

					LNY<9 LNY≥9						LNY<10 (62%) vs LNY≥10 (71%); P=0.30 LNY<9 (48%) vs LNY≥9 (72%); P=0.04
Fahim *et al.*[36] 2021 Netherlands	4,531	RSCS	Colon Rectum	NR	LNY<10 LNY≥10	Neoadjuvant Tx (not specified) = 24.3%	Lap = 28.6% Open = 71.4%	Elective: 100%	I to IV	Median = 43 (IQR 23-71)	**Mortality** **Univariate Cox** LNY<10: HR 0.92 (95%CI 0.76–1.11; P=0.39) **MV Cox** LNY<10: HR 1.00 (95%CI 0.81–1.22; P=0.99
Fahim *et al.*[100] 2020 Netherlands	1,139	RSCS	Colon	NR	LNY<10 LNY≥10	NA	Lap = 57.2% Open = 42.8%	Elective: 86% Emergency = 14%	I to IV	Median = 41 (IQR 23-65)	**Mortality** **Univariate Cox** LNY≥10: HR 1.47 (95%CI 1.07–2.01; P=0.02) **MV Cox** LNY≥10: HR 1.29 (95%CI 0.93–1.81; P=0.13
Fiorillo *et al.*[101] 2020 Italy	397	RSCS	Rectum	Mean = 11 (SD5.3)	LNY≤11 LNY>11	59.8% had neoadjuvant therapy - 61 patients had LCCRT - 11 patients had SCRTx - 1 had CT alone	NR	NR	I to III	Median = 30.5 (IQR 5.9-86.1)	**5-yr OS** (P=0.3) LNY≤11 = 92.6% LNY>11 = 86.6 **5-yr DFS** (P=0.23) LNY≤11=83.5% LNY>11 = 82.4%
Fortea-Sanchis *et al.*[102] 2018 Spain	584	RSCS	Colon	Median = 10 (IQR 7-15)	LNY<12 LNY≥12	NA	NR	NR	I to III	Median = 51 (Range 0-99)	**Cusum curve and Mortality** The CUSUM curve for overall mortality was calculated according to the number of LNs analysed mortality initially tended to increase (downward trend) until approximately 12 LNs were analysed; after this, there was a trend of falling risk (upward curve) until approximately 20 LNs were analysed; at this point, the risk slowly increased (the curve rises slowly). These trend changes translate into a significant increase in the probability of death when fewer than 12 LNs were analysed. From a practical standpoint,

											these results indicate that analysing fewer than 12 LNs favours worse outcomes; the intensity of this correlation subsequently decreases (but is not null) and stabilises at around 20 LNs
Fretwell *et al.*[103] 2010 UK	351	RSCS	Colon Rectum	Median = 15 (Range 0-80)	LNY<9 LNY≥9 LNY<10 LNY≥10	Of 115 patients with rectal cancers, 37 received neoadjuvant radiotherapy	NR	NR	II to III	NR	**5-yr OS** Duke's B patients (LNY<9 vs LNY≥9, 45.2% vs 68.4%; P=0.0043) and Duke's C patients (LNY<10 nodes vs LNY≥10 nodes, 25.6% vs 48.8%; P=0.0099). There was a significant reduction in the relative risk of 2.8% in mortality for each additional node sampled in Duke's B and C patients (RR 0.972, 95% confidence interval 0.949–0.994, P = 0.0102).
George *et al.*[53] 2006 UK	3,592	POCS	Colon Rectum	NR	LNY 0-4 LNY 5-10 LNY>10	NR	NR	Elective = 87% Emerg = 12%	I to IV	NR	The HR for LNY 5–10 compared to LNY 0–4 is 0.88 (95% CI 0.80–0.98, P=0.018) and that for LNY 4-10 compared to LNY 0–4 is 0.78 (95% CI 0.68–0.89, P<0.001)
Govindarajan *et al.*[104] 2011 USA	708	RSCS	Rectum	Mean SURG group was 15.5 NEO group, mean = 10.8	LNY 0-3 LNY 4-7 LNY 8-11 LNY 12-15 LNY 16-19 LNY≥20	LCCRT was employed in 96% of patients and involved a median total dose of 50.4 Gy delivered in 26 fractions with 5 FU-based chemo	NR	NR	II to III	NR	The **5-yr DSS** was 89.2%, with no significant association seen based on LNY (P=0.59) There was no association between LNY and DSS in univariate (P=0.19) or MV analysis (HR=0.94; 95% CI, 0.88 to 1.01;P=0.09).
Ha *et al.*[75] 2010 Korea	615	RSCS	Rectum	Whole group Mean = 16.9 (Range 1–57) Preoperative chemoradiotherapy compared with those who did not have	LNY<12 LNY≥12 **Pre- op Chemo radio therapy group** LNY<8 LNY≥8	Preoperative radiotherapy (N=399; 64.9%) was delivered to the whole pelvis at a dose of 45 Gy in 25 fractions, followed by a boost to the primary	Lap = 85.9% Open = 14.1%	Elective = 100%	I to III	Median = 58 (IQR 1–92)	**Whole group** **5-yr OS (P=0.68)** LNY<12 = 81% LNY≥12 = 82.8% **5-yr DFS (P=0.73)** LNY<12 = 76.9% LNY≥12 = 77% **Pre- op Chemoradiotherapy group** **5-yr OS (P=0.33)** LNY<8 = 79.1%

				neoadjuvant therapy =14.5 vs. 21.5; P<0.001).		tumour of 5.4 Gy in 3 fractions over 5.5 weeks. One of 4 preoperative chemotherapeutic regimens was delivered concurrently with radiotherapy.					LNY≥8 = 85.1% **5-yr DFS (P=0.38)** LNY<8 = 76.6% LNY≥8 = 79.3%
Hashiguchi *et al.*[105] 2010 Japan	859	RSCS	Colon	Median = 19 (Range 0-42)	Continuous The ROC analysis identified LNY as a significant prognostic factor with cutoff value of 18 for node-negative and 20 for node-positive patients.	NA	NR	NR	I to III	NR	A MV analysis with these cutoff values identified LNY as a significant prognostic factor independent of tumour depth and the number of LNs involved. The **5-yr CSS** of Stage IIB patients was 96.5% with LNY≥18 and 67.5% with LNY<18 (P=0.0067). Similarly, a LNY=20 cutoff for node-positive patients separated the 5-year cause-specific survival of Stage IIIB patients into 79.3% with LNY≥20 and 63.3% with LNY<20 (P=0.0052).
Hohenberger, *et al.*[7] 2008 Germany	1,329	POCS	Colon - CME Surgery	Median = 32 (Range 2 to 169)	LNY<28 LNY≥28	NA	Open = ?100%	Elective = 1,219 Emerg = 110	I to III	Median = 103 (Range 1- 335)	**5-yr CSS** LNY<28 = 90.7% LNY≥28 = 96.3% (P=0.02)
Iachetta *et al.*[106] 2013 Italy	657	RSCS	Colon Rectum	Median = 19 (Range 1-68)	LNY<12 LNY≥12	Preop RTx = 5%	NR	NR	II	Median = 63 (Range 27-100)	**5-yr RFS** Significant positive correlation was observed between the LNY and RFS (P=0.015). **5-yr CSS** LNY≥20 showed a lower risk of dying of the disease (P=0.013) (HR 0.43, CI 95% 0.22 – 0.85). **MV analysis:**

											LNY=20 as the optimal cut-off, with a 45% reduction in CRC-related death (HR 0.55, 95% CI 0.30 – 0.98; P=0.045).
Ishizuka *et al.*[107] 2011 Japan	205	RSCS	Colon Rectum	NR	LNY≤9 LNY≥10 LNY≤14 LNY≥15 LNY≤18 LNY≥19	NR	Lap = 98% Open = 2%	NR	II	Median = 48.7	**5-yr OS** LNY≤9 = 48 months LNY≥10 = 70.8 months (P=0.003) LNY≤14 = 58.4 months LNY≥15 = 68.5 months P=0.17 LNY≤18 = 59.1 months LNY≥19 = 71.6 months P=0.09
Jestin *et al.*[108] 2005 Sweden	3,735	RSCS	Colon	Median = 8	LNY<12 LNY≥12	NA	NR	NR	I to IV	NR	**5-yr OS (P<0.001)** LNY<12 = <70% LNY≥12 = <80%
Jiao *et al.*[109] 2023 China USA	4,575	RSCS	Colon Rectum	SEER Median = 17 (IQR 13-23) China Median = 18 (IQR 13-26)	LNY<12 LNY≥12	NR	NR	NR	I to IV	NR	In the SEER and Chinese cohort, both before and after PSM, survival analysis showed that patients with LNY≥12 was associated with better CSS (P<0.001) compared with patients with LNY<12.
Johnson *et al.*[39] 2006 Canada	20,702	RSCS	Colon	Median number of negative nodes = 7 Median number of positive nodes = 2	Continuous	NA	NR	NR	III	Mean = 61.2	For Stage IIIB and IIIC patients, there was a significant increase in DSS as the number of negative nodes increased. 5-yr CSS = 73% for Stage IIIB with 13 or more negative LNs versus 55% in those with three or fewer negative LNs evaluated (P<0.0001). In patients with Stage IIIC cancer, those with 13 or more negative nodes had a 5-yr CSS of 58% versus 35% in those with three or fewer negative LNs evaluated (P=0.0001). There was no association between the number of negative LNs and DFS for Stage IIIA patients. After controlling for positive LNs, a higher number of negative

											LNs was found to be independently associated with improved DFS.
Ju *et al.*[110] 2007 China	5,474	RSCS	Colon Rectum	1980s Mean = 7.3 (SD5.9) 1990s Mean = 12.4 (SD8.4)	LNY<12 LNY≥12	NR	NR	NR	I to IV	NR	**5-yr OS** LNY<12 = 57% LNY≥12 = 57% **10-yr OS** LNY<12 = 44% LNY≥12 = 44% **5yr vs 10 yr (P=0.21)**
Kelder *et al.*[111] 2009 Netherlands	2,281	RSCS	Colon	Median = 6(IQR 4-11)	LNY<6 LNY6-11 LNY12-15 LNY≥16	NA	NR	NR	I to III	Median = 51.6 (42-82.8)	**5-yr OS – Univariate (P=0.02); MV (P=0.0002)** LNY<6 = 59.3% LNY6-12 = 64.3% LNY≥12 = 66.5% **5-yr RFS – Univariate (P=0.31); MV (P=0.03)** LNY<6 = 76.6% LNY6-12 = 80.6% LNY≥12 = 79.4%
Kidner, *et al.*[112] 2012 USA	6,214	RSCS	Rectal	NR	LNY 1-4 LNY 5-11 LNY 12-20 LNY≥20	Those who received neoadjuvant therapy was not included	NR	NR	I to II	NR	**Univariate (P<0.0001)** **5-yr OS** LNY1-4=48% **5-yr OS** LNY≥20 = 65% **5-yr OS (MV)** positively correlated with more LNs examined.
Kim *et al.*[69] 2009 South Korea	900	RSCS	Rectal	Stage II Mean= 24.4 (SD14.7) Stage III Mean= 24.8 (SD13.6)	LNY 1-15 LNY16-22 LNY 23-31 LNY 32-111	Those who received neoadjuvant therapy was not included	NR	NR	II to III	Median = 60.8 (Range, 12–199)	CSS of Stage II patients with less than 15 LNs was not different from Stage III patients, but CSS was better in Stage II patients with LNY>15. When using cutoff values of the 25th and 50th percentiles (22 and 31 nodes), RFS was statistically different among subgroups of Stage II and III patients. **MV analysis** Stage II disease with LNY<15 = reduced CSS and RFS.

											In Kaplan-Meier survival analysis, using cutoff values, the CSS was not significant with LNY of 22. Difference for RFS was not observed with LNY of 23.
Kim *et al.*[37] 2018 Korea	7,880	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	Elective = 92.2% Emerg = 7.8%	II	Median = 38 (Range 1-63)	LNY<12 vs LNY≥12 HR 0.63, 95%CI 0.50-0.79; P< 0.001
Khan *et al.*[113] 2014 USA	194,459	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	NR	III	NR	**5-yr OS** LNY≥12 = 81.1% (95% CI, 80.6-81.6) LNY<12 = 76% (95% CI, 75.5- 76.5)
Klos *et al.*[60] 2010 USA	390	RSCS	Rectum	Neoadjuvant Mean = 13 (SD7) Surgery Mean = 14 (SD7)	LNY<12 LNY≥12	221 patients had neoadjuvant therapy. Received a fractioned dose of 45 or 50.4 Gy over a period of 6 weeks. During the course of radiation, patients received 5-FU CT	NR	NR	I to IV	Median = 36 (18-63.6)	Survival and recurrence were not significantly different between patients with LNY<12 or LNY≥12
Kotake *et al.*[114] 2012 Japan	16,865	RSCS	Colon Rectum	Mean = 20 (SD)	LNY 1-9 LNY 10-16 LNY 17-26 LNY≥27	NR	Open = Nearly 100%	NR	II to III	NR	**5-yr OS** LNY≤9 vs LNY≥27 differed by 6.4% (Stage II CC), 8.8% (Stage III CC), 12.5% (Stage II rectal cancer) and 10.6% (Stage III rectal cancer). With one increase in the LN retrieved, mortality risk decreased by 2.1% for Stage II and by 0.8% for Stage III.
Kritsanasakul *et al.*[115] 2011 Thailand	533	RSCS	Colon Rectum	Median = 10	LNY<12 LNY≥12	NR	Open = 100%	Elective = 90.1% Emerg = 9.9%	I to III	Median = 86 (Range 41–162)	**Univariate** **5-yr OS (P<0.01)** LNY<12 = 73% LNY≥12 = 62.7% **MV analysis**

											LNY≥12 and LNR were independent factors predicting survival probability.
La Torre *et al.*[116] 2012 Italy	204	RSCS	Colon	LNY<12 Median. = 9 LNY≥12 Median = 21	LNY<12 LNY≥12	NA	NR	NR	II	Median = 42 (Range 16-96)	**5-yr OS (P=0.001)** LNY<12 = 50.4% LNY≥12 = 72.8% **5-yr DSS (P=0.001)** LNY<12 = 56% LNY≥12 = 82% **5-yr DFS (P=0.001)** LNY<12 = 53.1% LNY≥12 = 78.5% LNY≥12 was the only independent predictor influencing **DFS HR**= 2.3 (95%CI 1.3–4.9; P=0.02), **DSS HR**=2.7 (95%CI 1.3–6.3;P=0.04) **OS HR**=1.7 (95%CI 1.1–7.3;P=0.03) LNY<18 LNY18-24 LNY>24 To establish whether an extensive nodal dissection would improve survival. The univariate analysis failed to demonstrate a difference between sub-groups in terms of **DFS, DSS, and OS** (5-year DFS rate was, respectively, 87%, 75%, and 79%, P=0.53; 5-year DSS rate was, respectively, 87%, 81%, and 79%, P=0.19; 5-year OS rate was, respectively, 81%, 77%, and 79%, P=0.09)
La Torre *et al.*[79] 2013 Italy	508	RSCS	Rectum	Neoadjuvant group: Mean = 15.2 (SD3.1) Primary surgery group:	LNY<12 LNY≥12	123 (24.2%) received neoadjuvant chemoradiotherapy Radiotherapy	NR	Elective = 100%	I to III	Median = 50.4 (Range 9–120)	**Neoadjuvant group** **5-yr DFS (P=0.48)** LNY<12 = 73% LNY≥12 = 78% **5-yr OS (P=0.26)** LNY<12 = 69% LNY≥12 = 72%

				Mean= 19.7 (SD4.5)		delivered was 45 Gy to the entire pelvis in 25x1.8-Gy fractions over 5 weeks. A concurrent boost of 1 Gy twice a week was delivered to the mesorectum to a total of 10 Gy in 10 fractions resulting in a total dose of 55 Gy. From 2003 to 2008 concomitant chemotherapy was administered with a continuous infusion of 5-FU in a dose of 225 mg/m^2 administered Monday to Friday on the days of radiation. Since January 2009, all patients received oral capecitabine in a dose of 825 mg/m^2 twice a day from Monday to Friday on						

						the days of radiation.				
Law *et al.*[117] 2003 Canada	115	RSCS	Colon	Mean = 8.5(SD 5.5)	LNY<6 LNY≥6	NA	NR	NR	II	Median = 39	**5-yr OS (P=0.03)** LNY<6 = 62% LNY≥6 = 86% **5-yr DFS (P=0.03)** LNY<6 = 69% LNY≥6 = 89%
Le Voyer *et al.*[33] 2003 USA	3,411	RSCS	Colon	Median = 11 (Range 1-87)	LNY<12 LNY≥12	NA	NR	NR	II to III	Median = 79 (Range 1-131)	OS and CSS improved as more LNs were analysed (P=0.0005 and P=0.007, respectively).
Lee *et al.*[51] 2009 USA	4,538	RSCS	Colon		LNY<12 LNY≥12	NA	NR	NR	I to III	Median = 61	**5-yr OS (P<0.001)** LNY<12 = 76% LNY≥12 = 81%
Lewis *et al.*[118] 2012 USA	353	RSCS	Colon	Mean = 24 (SD 15)	LNY<12 LNY≥12	NA	Lap = 50.9% Open = 49.1%	Elective = 71.7% Emerg = 28.3%	I to III	NR	Patients with a LNY≥12 had a mean survival of 54.0 (SD2) months vs 36.3 (SD 5.2) months in those with LNY<12 (P < 0.0001). **Stage II (P<0.0001)** LNY≥12 = 54.7 (SD3.4) months LNY<12 = 27.3 (SD7.3) **Stage III (P=0.14)** LNY≥12 = 44.7 (SD2.8) months LNY<12 = 34.2 (SD7.7) **Stage I (P=0.52)** LNY<12 vs LNY≥12 did not show difference in survival when comparing LN harvest groups
Li *et al.*[119] 2022 UK	36,116	RSCS	Colon Rectum	2015/2016 Median. = 17 2016/2017 Median = 18	NR	Preop radiotherapy >30%	Lap = >60%	Elective = 69.1% Emerg = 30.9%	NR	NR	As the median number of LN excised increases, 2 Yr and 90 Day mortality decreases
Li Destri *et al.*[120] 2017	432	RSCS	Colon Rectum	Median = 13 (Range 1 – 77)	LNY<12 LNY≥12	Long-course neo-adjuvant radio-	NR	NR	II to III	NR	**5-yr DFS (P=0.11)** LNY≥12 = 72% LNY<12 = 63%

Italy						chemotherapy was proposed for all T3 and/or N1 rectal cancer patients					
Lin *et al.*[121] 2021 China	4,344	RSCS	Rectum	Median Neoadjuvant Therapy = 12 (Range 8-17) No neoadjuvant therapy = 17 (Range 13-24)	LNY<12 LNY≥12	NR	NR	NR	I to III	Median = 38.3	LNY>12 had higher OS probability than LNY<12 (OR:1.33; 95% CI 1.1–1.7;P=0.01) The predictive accuracy for survival was greater for LNY≥10 (AUC = 0.78) than cut-offs of 12, 8 or 6 especially in those with N0 disease (AUC =0.77).
Maggard *et al.*[122] 2009 USA	61,237	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	NR	I to IV	NR	**5-yr OS** Increased with greater LNY - T1 survival rate was 70% when LNY<5 and 76% when LNY ≥12. - This minimal difference is contrasted by the results for T4 tumours, which had 43% survival with LNY<5 and 60% when LNY ≥12. For T1 tumours, there was a statistically significant difference in survival at the cut point of LNY=4, with better survival noted for patients with LNY >4 as compared with those with LNY<4 - T1 patients with LNY>4 had a 5-year survival of 74.1% as compared with 68.9% for LNY<4 (P = 0.008). - LNY>5 had a 5-year survival of 75.1% as compared with 68.3% for LNY<5 (P=0.005).

											Examination of LNY>6 did not show a statistically significant improvement in survival. T2 tumours, there was a statistically significant difference in survival noted at the cut point of 4 nodes (better survival for patients with LNY>4 versus LNY<4). However, with stepwise increase in the number of nodes examined the survival difference was greater. The lowest number of nodes for which the P-value was maximized occurred at the cut point of 9 nodes (P<0.0001). The maximum chi-square value occurred at the cut point of 13 nodes. Specifically, T2 patients with LNY>13 had a 5-year OS of 75.4% as compared with 68.9% for LNY<13 (P=0.001).
Mammen *et al.*[123] 2007 USA	5,823	RSCS	Colon	Mean = Stage I vs Stage II vs Stage III = 9.6 vs 12.9 vs 13.6	LNY 0-5 LNY 6-10 LNY 11-16 LNY>16	NA	NR	NR	I to III	NR	**5-yr OS** **Stage II**(P=0.007) 34%, 43%, 47%, and 55% for the lowest to highest quartiles **Stage III (**P>0.05) 31%, 27%, 38%, and 53% for the lowest to highest quartiles
Mason *et al.*[124] 2017 USA	218,186	RSCS	Colon	NR	Continuous	NR	NR	NR	I to III	NR	Increasing LNY was associated with better survival: HR0.95 (0.88–1.04)
Maughan *et al.*[125] 2007 UK	5,947	RSCS	Colon Rectum	NR	Varied	NR	NR	NR	I to III	NR	Patients with LNY>12 had significantly higher survival (53.0%: 95% CI 50.7–55.2%) compared to those with the lowest nodal yield (45.4%: 95% CI 43.1–47.7%; P<0.01). Patients in whom the number of nodes retrieved failed to be reported, had the worst survival (38.2%: 95% CI 27.8–48.7%).
McFadden *et al.*[76] 2013	159	RSCS	Rectum	Neoadjuvant group	LNY ≥7 vs.<7 LNY ≥10 vs.<10	Neoadjuvant therapy in 26 patients	NR	NR	I to III	NR	**Whole Group** **5-yr OS** LNY≥7 = <70%

USA				Median = 7 (IQR 3-10) Surgery group Median = 7 (IQR 4-10)	LNY ≥12 vs.<12 LNY ≥14 vs.<14						LNY<7 = >70% (P=0.63) HR:0.99 (95%CI 0.48-2.04); P=0.97 LNY≥10 = ~70 % LNY<10 = >70% (P=0.63) HR:1.13 (95%CI 0.54-2.38); P=0.74 LNY≥12 = <70% LNY<12= <80% (P=0.79) HR:0.93 (95%CI 0.39-2.26); P=0.88 LNY≥14 = <70% LNY<14 =>70% (P=0.50) HR:0.98 (95%CI 0.38- 2.49); P=0.96
Morris *et al.*[126] 2007 UK	2105	RSCS	Colon Rectum	Mean varied between 3 populations Petersen = 21.3 Yorkshire Colon = 11.5 Yorkshire rectum = 11.8	Varied	NR	NR	NR	II	NR	**5-yr OS** **Dukes B (P=0.01)** LNY 0-3 = 45.4% (95% CI 36.9% to 53.5%) LNY>15 = 79.3% (95% CI 74.8% to 83.1%) The survival of Dukes C patients with one positive node identified was 57.9% (95% CI 53.3% to 62.3%). Dukes B patients with LNY<4, have poorer survival than the best prognosis Dukes C patients.
Morris *et al.*[127] 2007 UK	7,062	RSCS	Colon Rectum	Median LNY = 7 (IQR 4-11) in 1995 to 13 (IQR 8 - 19) in 2003	LNY<12 LNY≥12	Neoadjuvant therapy patients were excluded	NR	NR	I to III	NR	**5-yr OS** Lower in those patients who had LNY <12
Mor-valdezate *et al.*[41] 2013 Spain	1,166	RSCS	Colon	Mean = 9.2 (SD 6.2)	LNY<12 LNY≥12	NA	NR	Urgent: 202 (17.3%) Elective: 964 (82.7%)	I to IV	Mean=85.6; (SD 53.5)	**≥12 LNs vs < 12 LNs** **- 5 yr OS (P=0.09) and 5 yr DFS (P= 0.32)** **- 5 yr OS (NS)** Stage I: 97.4% vs 93.7%

											Stage II:94.1%vs 87.7% Stage III:75.9%vs73.3% StageIV:34.7%vs 23.6% Stages I and II were analysed for the influence of LN ≥ 12 - 2yr OS (P=0.05) - 2 yr DFS (P=0.028). - 5 yr OS (P=0.30) 5 yr DFS (P= 0.054).
Mukai *et al.*[128] 2003 Japan	434	RSCS	Colon Rectum	Mean = 14.2 (SD 11.5)	LNY </= 16 LNY≥17	NR	NR	NR	I to IV	NR	There was a significant difference between patients with retrieval of >17 LNs and <16 LNs = 80.8% vs. 64.0%; P=0.0374, P=0.0106 respectively (adjusted by Dukes' clinical stage)
Nagasaki *et al.*[129] 2015 Japan	446	RSCS	Colon	Median = 17 (Range 4-66)	LNY<12 LNY≥12	NA	Lap = 73.5% Open = 26.5%	NR	III	Median = 60.2 (Range 1-103.9)	**5-yr RFS (Univariate)** LNY<12 = 71.4% LNY≥12 = 76.5% P=0.51
Narayanan, *et al.*[130] 2020 USA	56,812	RSCS	Rectum	NR	LNY<12 LNY≥12	53.5% were administered Neoadjuvant therapy (nCR) 90.6% patients in the nCR group received 4000-6000 cGy radiation. 53.7 % of these patients also received a boost dose of radiation.	Lap = 25% Open = >50% Robot = 18.3%	NR	I to IV	NR	In examining survival for these patients, we found that patients who received nCR had significantly improved OS compared to the surgery alone cohort, regardless of their LNY. LNY≥12 was also associated with improved survival in both nCR and surgery alone groups. P<0.001 LNY<12 - Surgery: 60% - nCR : >60% LNY≥12 - Surgery: >60% - nCR : 70%
Nir *et al.*[131] 2010 Israel	173	RSCS	Colon Rectum	NR	LNY<12 LNY≥12	NR	Lap 100%	Elective = 100%	III	Median 19.1 (Range 3-68)	**5-yr DFS** (P=0.15) LNY<12 = <80% LNY≥12 = >90% **5-yr OS** (P=0.39) LNY<12 = >80% LNY≥12 = >75%

Norwood *et al.*[132] 2010 UK	2,449	RSCS	Colon Rectum	Median = 13 (Range 0-136)	LNY<12 LNY≥12	Of all patients, 0.9% (n = 21) underwent preoperative chemotherapy, 3.1% (n = 77) underwent preoperative chemo-radiotherapy, 0.6% (n = 14) underwent preoperative long course radiotherapy alone and 7.1% (n = 173) underwent preoperative short-course radiotherapy.	NR	Elective = 81.9% Emerg = 18.1%	I to II	NR	Patients with Dukes' A and B cancers with LNY<12 retrieved had a significantly shorter survival compared with LNY≥12 harvested (P=0.001). This significant difference was maintained when analysing both Dukes' A and B patients individually. Subdividing all Dukes' A and B patients further into three groups with LNY0–9, LNY10–15 and LNY≥16, Cox regression analysis revealed that Dukes' A and B patients with nine or less nodes sampled had a significantly reduced survival (P < 0.001) compared with the other two groups. There was, however, little evidence to suggest that the presence of LNY≥16 positively influenced survival over patients with LNY10–15. These differences were maintained on further multivariate analysis, adjusting for age, sex, initial treatment modality, operation type and mode of surgery.
Ogino *et al.*[133] 2010 USA	716	RSCS	Colon Rectum	Median = 10 (Range 0-55)	LNY 0-3 LNY 4-6 LNY 7-12 LNY≥13	NR	NR	NR	I to IV	NR	In univariate Cox regression analysis, compared to patients with 0 – 3 negative LNs, patients with 7 – 12 and ≥ 13 negative LNs experienced low CSM (HR 0.42; 95 %CI, 0.28 – 0.62; and HR 0.28; 95 % CI, 0.18 – 0.44, respectively; P< 0.0001) Compared to patients with 0 – 3 negative LNs, patients with 7 – 12 and ≥ 13 negative LNs experienced an improved CRC – specific c mortality (multivariate HRs 0.56 (95 % CI, 0.36 – 0.87) and 0.43 (95 % CI, 0.27 – 0.71), respectively; P= 0.0002)
Onitilo *et al.*[134] 2013 USA	1,397	RSCS	Colon Rectum	LNY<12 : Mean = 7.3 (Range 1-11)	LNY<12 LNY≥12	NR	NR	Elective = 88.4% Emerg = 11.6%	I to III	Median = 63 (Range 47-118)	There was no difference in unadjusted OS between patients with and without adequate LN recovery. However, in the adjusted Cox proportional hazards analysis adequate LN recovery was

				LNY≥12 Mean = 23.5 (Range 12-90)							associated with a reduced risk for death (HR: 0.71; 95%CI 0.57–0.88; P=0.002
Osterman *et al.*[30] 2018 Sweden	14,325	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	Elective = 85% Emerg = 15%	I to III	Median = 77 (Range 47-118)	**TTR (P=0.67) and OS (P=NA)** Each LN not associated with oncological outcome Low LNY associated with poor OS
Parsons *et al.*[135] 2012 USA	17,906	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	NR	III	NR	Adequate LN evaluation was associated with lower all-cause mortality (HR=0.88; 95% CI0.85 to 0.91), but among 3-year survivors, the impact of adequate LN evaluation on lower mortality was diminished (HR0.94; 95% CI 0.88 to 1.01).
Parsons *et al.*[136] 2011 USA	86,394	RSCS	Colon	NR	LNY<12 LNY≥12 Continuous	NA	NR	NR	I to IV	NR	Patients with high rates of LNY experienced significantly lower relative hazard of 5-year death compared with those with fewer nodes evaluated (aHR 30-39 nodes vs 1-8 nodes, 0.66; 95% CI, 0.62-0.71; unadjusted 5-year mortality, 35.3%).
Persiani *et al.*[73] 2014 Italy	440	RSCS	Rectum	Neoadjuvant group Median = 7 (Range 1–33) Surgery group Median = 12.5 (Range 0–44)	LNY<8 LNY≥8 LNY<9 LNY≥9 LNY<10 LNY≥10 LNY<11 LNY≥11 LNY<12 LNY≥12	Patients who received preop neoadjuvant therapy = 345 External-beam radiotherapy consisted of a total dose of 50.4–55 Gy. All patients received 45 Gy with a conventional fractionation of 1.8Gy/day to the pelvic LNs; booster doses changed	NR	NR	I to III	Neoadjuvant group Median = 87 (range 5–235)	**Neoadjuvant group** **5-yr OS** LNY<8 = 94.6% LNY≥8 = 95.2% (P=0.78) LNY<9= 94.6% LNY≥9= 95.2% (P=0.79) LNY<10= 95.1% LNY≥10= 94% (P=0.37) LNY<11= 95.5% LNY≥11= 94% (P=0.43) LNY<12 = 95.7%

						according to tumour stage. The surgical exploration was planned not earlier than 8 weeks after the end of CRT.					LNY≥12= 93% (P=0.38) **5-yr DFS** LNY<8= 85.1% LNY≥8= 79.3% (P=0.29) LNY<9= 83.8% LNY≥9= 79.9% (P=0.41) LNY<10= 83.6% LNY≥10= 80% (P=0.33) LNY<11= 84.1% LNY≥11= 78% (P=0.21) LNY<12 = 84.9% LNY≥12 = 75% (P=0.18)
Porter *et al.*[137] 2012 Canada	2,250	RSCS	Colon Rectum	Mean = 9.9 (IQR 4-13)	LNY 0-3 LNY 4-7 LNY 8-12 LNY>12	Patients with rectal cancer who received preoperative, long-course radiotherapy, with or without chemotherapy (N = 80)	NR	Elective = 81.5% Emerg = 18.5%	I to III	NR	**4-yr OS (P<0.001)** LNY 0-3 = 66% LNY 4-7 = 65.8% LNY 8-12 = 67.9% LNY>12 = 70.4%
Raoof *et al.*[138] 2016 USA	2,704	RSCS	Rectal	NR	LNY<9 LNY≥9	100%	NR	NR	III	Median = 49 (IQR 32-71)	The proportion of ypN0 patients with adequate LN evaluation after surgery at a particular hospital is an independent predictor of OS for all patients undergoing rectal cancer surgery after neoadjuvant therapy. More importantly, in this population, this metric is a stronger predictor of long-term survival than hospital volume or teaching hospital status as demonstrated in the multivariate models

Read *et al.*[62] 2002 USA	316	RSCS	Colon	Mean = 14 (SD 12)	Continuous	NA	NR	NR	I to III	Median = 63 (IQR 38-88)	**aHR** LNY HR 1.03(95%CI 0.99-1.08; P=0.14)
Rullier *et al.*[61] 2008 France	495	RSCS	Rectum	Mean = 15 (SD 9)	Continuous	332 received long course preoperative radiotherapy Locally advanced rectal carcinomas received long course preoperative radiotherapy (45 Gy in 5 wk) according to the French consensus guidelines. Some of them also received preoperative concomitant chemotherapy with 5-fluorouracil or a boost of radiation on the tumour bed. Surgery was performed 6 weeks after radiotherapy	NR	NR	II to III	Median = 35 (Range 1-135)	In patients treated by preoperative chemoradiotherapy, the **5-yr OS** (71%) and **5-yr DFS** (60%) survival was not associated with the number of LNs retrieved.
Seishima *et al.*[64] 2014 Japan	263	RSCS	Rectum	High BMI Mean = 16 (SD9.6) Low BMI	Continuous	10/263 patients received pre-operative CRTx	Lap = 25.1% Open = 74.9%	NR	I to III	Mean = 56	**5-yr DFS - Univariate** Number of retrieved LNs HR1.01 (95%CI 1.00–1.03); P=0.06 **5-yr DFS - MV**

				Mean = 22.4 (SD14.6)							Number of retrieved LNs HR 1.01 (95%CI 0.99–1.03); P=0.39
Shanmugam *et al.*[65] 2011 USA	490	RSCS	Colon	NR	LNY 1-6 LNY 7-11 LNY12-19 LNY ≥20	NA	NR	NR	II to III	I	**LR** The rates of recurrence decreased with increases in LNY for Stage II (P=0.0004)and III (P <0.0001)patients. Stage II and III there was no statistically significant difference in the rates of recurrence after the collection of 6 – 19 LNs **2-yr DSS (MV)** LNY=12 for Stage II (HR = 0.61; 95% CI, 0.37 - 1.00) or Stage III (HR = 0.97; 95% CI, 0.64 - 1.46) LNY 1-6 vs LNY(7-11, 12-19, ≥ 20) exhibited an improved 5-year and overall DSS. The LNY≥20 had significantly better survival than those with LNY<6 in Stage II (5 years-HR= 0.42; 95% CI,0.20-0.90; overall- HR = 0.45; 95% CI, 0.23 - 0.87) but not for Stage III (5 years-HR = 0.74; 95% CI, 0.39 - 1.40; overall-HR = 0.69; 95% CI, 0.38 - 1.26)
Shulman *et al.*[139] 2019 USA	544,018	RSCS	Colon	NR	LNY<12 LNY≥12	NA	NR	NR	I to III	NR	LNY≥12 had better survival than LNY<12 (HR 0.78, 95% CI 0.74–0.81).
Sjo *et al.*[46] 2012 Norway	950	PSOC	Colon	Median = 12 (Range, 0–73)	LNY 0-7 LNY 8-11 LNY ≥12	NA	NR	Elective = 85% Emerg = 15%	I to III	NR	**5-yr OS** Stage I (P=0.08) - LNY 0-7 = 70% - LNY 8-11 = 86% - LNY ≥12 = 83% Stage II (P=0.004) - LNY 0-7 = 57% - LNY 8-11 = 61% LNY ≥12 = 71% Stage III (P=0.006) - LNY 0-7 = 38% - LNY 8-11 = 60% LNY ≥12 = 54%

											5-yr TTR Stage I (P=0.089) - LNY 0-7 = 83% - LNY 8-11 = 94% - LNY ≥12 = 96% Stage II (P=0.03) - LNY 0-7 = 68% - LNY 8-11 = 71% LNY ≥12 = 81% Stage III (P=0.02) - LNY 0-7 = 46% - LNY 8-11 = 61% LNY ≥12 = 69%
Soriano *et al.*[140] 2022 USA	38,927	RSCS	Rectum	Mean = 14.9 (SD 9.6)	LNY<12 LNY≥12	31,418/38,927 received preop RTx 4,302/38,927 received total neoadjuvant therapy	Lap = 31.2% Open = 50.9% Robotic = 17.9%	NR	II to III	NR	**5-yr OS** - Univariate LNY <12 (70.9%) vs LNY ≥ 12 (74.5%); HR 1.19 (95%CI 1.12-1.25;P<0.001) **5-yr OS - MV** LNY <12 (70.9%) vs LNY ≥ 12 (74.5%); HR 1.26 (95%CI 1.17-1.36 ;P<0.001)
Stelzner *et al.*[141] 2010 Germany	578	RSCS	Rectum	NR	LNY<12 LNY≥12	NR	NR	NR	I to III	Median = 54.4 (Range 1-116)	**5-yr CSS** LNY <12 (88.9%) vs LNY ≥ 12 (83.2%); P=0.42)
Stochi *et al.*[142] 2011 USA	901	RSCS	Colon	<1991 Median = 17) >1991 Median = 22	LNY ≤ 11 LNY 12 – 14 LNY 15 – 17 LNY ≥ 18	NA	Lap = 5.5% Open = 94.5%	NR	II	Median = 116.4 (IQR 67.2-190.8)	**OS** LNY 12-14 associated with longer survival (>70%) vs LNY ≤11 (<60%); P<0.0001
Stochi *et al.*[82] 2001 USA	673	RSCS	Rectal	NR	NR	NR	NR	NR	II to III	Median = 80.4	LNs not related to LR (P=0.82) or OS (P=0.46)
Storli *et al.*[66] 2013 Norway	251	POCS	Colon	Lap Mean = 15.8 (95%CI 14.6-17.0) Open	LNY 0-11 LNY 12-17 LNY 18-28 LNY 29-65	NA	Lap = 49% Open = 51%	NR	I to III	Mean = 48 (Range 24-72)	**3-yr OS – UNIVARIATE (P=0.88)** **3-yr OS – MV (P=0.86)** **3-yr DFS – UNIVARIATE (P=0.92)** **3-yr DFS – MV (P=0.75)**

				Mean = 17.5 (95%CI 1539-19.0)							
Swanson *et al.*[143] 2003 USA	35,787	RSCS	Colon	Median = 9 (Range 0 to >30)	Various ranges + continuous	NA	NR	NR	II to III	Median = 46.3	**5-yr OS** (P<0.0001) LNY 1-7: 49.8% LNY 8-12: 56.2% LNY>12: 63.4%
Tepper *et al.*[55] 2001 USA	1,664	RSCS	Rectal	NR	LNY 0-4 LNY 5-7 LNY 8-12 LNY>12	Intergroup protocol	NR	NR	II to III	Median = 90	**5-yr OS (P=0.02)** LNY 0-4 = 64% LNY 5-9 = 63% LNY 9-13 = 61% LNY>13 = 67% **5-yr RFS (P=0.003)** LNY 0-4 = 38% LNY 5-9 = 42% LNY 9-13 = 42% LNY>13 = 38%
Testa *et al.*[144] 2022 Italy	51	RSCS	Colon CME + CVL	Open CME: Median = 19 (IQR 14-24) Lap CME: Median = 21 (IQR 14-27)	LNY ≤15 LNY>15	NA	Lap = 47.1% Open = 52.9%	NR	I to IV	Median = 29.2	**3-yr OS (P=0.70)** LNY ≤15 – 70% LNY>15 – 80%
Tsai *et al.*[74] 2011 USA	372	RSCS	Rectal	Median = 7 (Range 0-40)	LNY ≤7 LNY>7	Median radiation dose = 45Gy 245 patients (65.9%) received 45 Gy to the pelvis, and 127 patients (34.1%) received 45 Gr to the pelvis along with a sequential or concurrent boost.	NR	NR	I to III	Median = 91.2 (Range 2-223.2)	**UNIVARIATE** **5-yr OS (P=0.07)** LN >7 vs LN≤7 87% vs 81% **5-yr RFS (P=0.005)** LN >7 vs LN≤7 86% vs 72% **5-yr CSS (P=0.004)** LN >7 vs LN</=7 95% vs 86% **MULTIVARIATE** **5-yr RFS (P=0.003)** LN>7: HR 0.39 **5-yr CSS (P=0.04)**

						68% received chemotherapy					LN>7: HR 0.45 **5-yr OS (P=0.23)** LN>7: HR 0.75
Tsikitis *et al.*[70] 2009 USA	329	RSCS	Colon	Mean = 14.7 (SD 9)	LNY ≤12 LNY>12	NA	NR	NR	III	Median = 62	**CSS univariate** LNY>12 vs LNY≤12 (P=0.87) **CSS MV** LNY>12 – HR 0.63 (95%CI 0.36-1.10; P=0.11) **CSS** LNY as continuous variable (P=0.08) **DFS** LNY as continuous variable (P=0.59)
Vather *et al.*[43] 2009 New Zealand	4,309	RSCS	Colon	Median: 11 (Range 1–99)	1-4 nodes 5-8 nodes 9-12 nodes 13-16 nodes >17 nodes	NA	NR	NR	II to III	NR	**5yr MR:** 31.0% **5 yr OS** Stage II: LNY1-4 - <65% LNY 5-8 - >65% LNY 9-12 – 70% LNY 13-16 - >70% LNY≥17 nodes - >70% Stage III: LNY1-4 - 30% LNY5-8 - >35% LNY9-12 – <40% LNY13-16 - <50% LNY≥17 - <50%
Wang *et al.*[45] 2009 USA	24,477	RSCS	Colon	Median – 11 (Range 1-90)	LNY<12 LNY≥12	NA	NR	NR	III	NR	**5 yr OS**: LNY>12 vs LNY<12 (51.0% vs. 45.0%, P<0.0001)
Williams *et al.*[145] 2020 USA	290,776	RSCS	Colon	Mean = 20.2 (SD 20.8)	LNY <12 LNY≥12	NA	Lap – 45.9% Open – 54.1%	NR	I to III	NR	**OS Univariate** LNY≥12 – HR 0.75(95%CI 0.74-0.77; P<0.001) **OS MV** LNY≥12 – HR 0.70(95%CI 0.68-0.71; P<0.001)
Wulf *et al.*[146]	534	RSCS	Rectum	NR	LNY<12 LNY≥12	NR	NR	NR	I to IV	Median = 47 (IQR 17-91)	**LR MV** LNY<12 HR 1.23 (P=0.003)

2004 Germany											**Distant Mets MV** LNY<12 HR 1.23 (P=0.02) **DFS MV** LNY<12 HR 1.12 (P=0.045)
Won *et al.*[147] 2017 South Korea	874	RSCS	Colon Rectum	Right colon - Mean 25.5 (SD 14) Left colon – Mean 18.4 (SD 11.0) Rectum – Mean 14.7 (SD 10.6)	LNY<12 LNY≥12	NR	Lap = 31.9% Open = 68.1%	NR	I to IV	Median = 63 (IQR 28-92)	**DFS** LNY<12 vs LNY≥12 (P=0.46) Stratified by Stage, LNY≥12 associated with better survival (P=0.01) **OS** LNY<12 vs LNY≥12 (P=0.82) Stratified by Stage, LNY≥12 tendency with better survival (P=0.09)
Wong *et al.*[32] 2005 USA	2,149	RSCS	Colon Rectum	Mean LNs varied according to the site Mean = 18 (SD15) nodes	NR	NA	NR	NR	I to III	Median = 51 (Range 0-164)	**5-yr OS** LNY≤7 vs LNY≥8 – 58.5% vs 84.2% (P<0.001) LNY≤ 8 vs LNY≥9 – 62.3% vs 85% (P<0.001) LNY≤9 vs LNY≥10 – 69.2% vs 84.5% (P<0.001) LNY≤10 vs LNY≥11 – 73.5% vs 84.1% (P=0.04) LNY≤11 vs LNY≥12 – 75.1% vs 84.0% (P<0.001) LNY≤12 vs LNY≥13 – 75.3% vs 84.5% (P=0.01) LNY≤13 vs LNY≥14 – 75.7% vs 84.8% (P=0.02) LNY≤14 vs LNY≥15 – 78.2% vs 83.7% (P=0.18)

Wong *et al.*[68] 2007 USA	30,625	RSCS	Colon	Median: 1st Quartile = 6 (IQR 5-7) 2nd Quartile = 8(IQR 7-9) 3rd Quartile = 10(IQR 9-11) 4th Quartile =13(IQR 12-16)	Quartiles	NA	NR	Elective=58.8%	I to III	Median: 1st Quartile = 3.8 (IQR 1.7-5) 2nd Quartile = 3.9(IQR 1.8-5) 3rd Quartile = 3.9(IQR 2-5) 4th Quartile =3.9(IQR 2.1-5)	At the patient level, examination of LNY≥12 was associated with improved survival, relative to LNY<12 (aHR: 0.83 (95% CI 0.78-0.88).
Xiangmao *et al.*[148] 2013 China	729	RSCS	Colon Rectum	Mean=14.9 (Range 1-58)	LNY≥13 LNY<13	NA	NR	NR	II	Median=48	**5-yr OS** The survival rate of patients increased gradually when LN harvest ranged from 8 to 13. After 13, the survival rates were roughly the same. When the cut-point of LN was 13, the difference of survival rates between the two groups was the most significant (88.7 vs 64.9%, P = 0.0001).
Yoshimatsu *et al.*[149] 2005 Japan	212	RSCS	Colon Rectum	Group B (Dukes B): Mean = 19 (SD 13.5) Group C (Dukes C): Mean =18.4(SD14.8)	LNY≥12 LNY<12 LNY≥11 LNY<11 LNY≥10 LNY<10 LNY≥9 LNY<9	NA	NR	NR	I to III	NR	**5-yr OS** LNY≥12 (87.2%) vs LNY<12 (72.2%) ; P=0.13 LNY≥11 (87.7%) vs LNY<11 (70.5%); P=0.09 LNY≥10 (86.1%) vs LNY<10 (69.6%); P=0.06 LNY≥9 (86.7%) vs LNY<9 (66.7%); P=0.02
Zhang *et al.*[150] 2013 China	265	RSCS	Colon Rectum	Median=7 (IQR 0-34)	LNY<12 LNY≥12	NR	NR	NR	I to IV	NR	**3-yr OS** LNY<12: 68.8% vs LNY≥12: 90.9% LNY≥12 HR: 0.22 (95%CI 0.10-0.46; P<0.0001)

RSCS: **Retrospective Cohort Study;** ***RSCCS:*** **Retrospective Case Control Study;** ***POCS:*** **Prospective Observational Cohort Study;** ***RCT:*** **Randomised Controlled Trial;** ***MR:*** **Mortality Rate;** ***CSS:*** **Cancer-Specific Survival;** ***TTR:*** **Time to Recurrence;** ***DFS:*** **Disease-Free Survival;** ***DSS:*** **Disease-Specific Survival;** ***RFS:*** **Recurrence-Free Survival;** ***OS:*** **Overall Survival;** ***HR:*** **Hazard Ratio;**

***MV:* Multi-Variate; *NA:* Not Applicable; *NR:* Not Reported; *ROC:* Receiver Operating Curve; *AUC:* Area Under the Curve; *SCRT:* Short Course Chemoradiotherapy; *LCCRT:* Long Course Chemoradiotherapy; *and AJCC:* American Joint Committee on Cancer.**

Table 2: Publications on LNY and Survival Associations.

"APPROPRIATENESS" OR "CORRECTNESS" OF LYMPHADENECTOMY

Having described the role that EoL has as a measure of quality in CRC surgery, the Introduction of this thesis now pivots to focus on a complementary aspect of high-quality CRC surgery: the "appropriateness of lymphadenectomy". Conceptually, an "appropriate lymphadenectomy" involves the ligation of the central tumour-supplying arterial vessel(s) and the accompanying lymphatic tissue, ensuring complete lymphovascular dissection and excision in accordance with oncological surgical principles.

While this concept is generally straightforward for most parts of the colorectum, it becomes more complex in regions with watershed blood supply due to the difficulties in defining the dominant tumour supplying vessel(s). Classically, watershed areas in the colon and rectum are located at the SF and the distal rectum. This thesis (**Chapters V and VI**) will focus specifically on the SF, guiding the reader through the unique considerations associated with addressing the "appropriateness" or "correct" lymphadenectomy practices when managing patients with SFC. It is beyond the scope of this thesis to look at the watershed area of the rectum.

The Clinical Relevance of Performing An Appropriate Lymphadenectomy in Splenic Flexure Cancers

In an era where there is an increasing push towards CME and CVL surgery, applying such radical lymphadenectomy principles to the SF poses a unique technical challenge. This challenge arises from the SF being a watershed area with heterogeneous blood supply and shared lymphatic drainage patterns between the IMA and MCA pedicles. As a result, when dealing with SFCs, defining which lymphovascular pedicle to ligate, so as to perform a "correct" lymphadenectomy according to CVL principles is difficult. It is therefore unsurprising that there is currently no consensus regarding the optimal technical approach to

SFC - whether through extended or non-extended colonic resection[7,151,152]. This lack of consensus is concerning as it may lead to an *inadequate* resection, potentially compromising oncological outcomes.

Splenic Flexure Anatomy

The fact that SF, located between the terminal portion of the midgut and the beginning of the hindgut, receives its blood supply from both the SMA and IMA, is naturally an important consideration in the management of SFCs[153-157].

During week six of development, the midgut rapidly begins to form. The midgut extends from the duodenum, distal to the entrance of the bile duct, and to the junction between the proximal two-thirds and distal one-third of the transverse colon[158]. Immediately distal to this junction, the hindgut begins. By week ten of development, the primary intestinal loop rotates 270° counterclockwise around an axis formed by the SMA. Then, the mesentery of the transverse and descending colon fold and partially fuse. The left edge of this attachment is at the lower pole of the spleen. The fusion of the posterior leaf of the transverse mesocolon with the descending mesocolon and the retroperitoneal wall, fixes the descending colon to the left abdominal wall in a retroperitoneal position. This process leads to the formation of the SF[158].

Splenic Flexure Cancers

Table 3 summarises the current understanding and knowledge of SFCs and their management.

SFCs are considered rare, accounting for only 2% to 8% of all surgically treated CRCs[159-161]. Studies investigating the prognosis of SFCs have yielded conflicting results, with reported 5-year OS rates ranging widely between 28% to 84%[151,162]. Several studies have reported poorer survival outcomes in patients with SFCs when compared to those with non-SFCs[162-165]. For example, Aldridge *et al.*[163] noted a LR rate of 4% and 5-year OS rate of 50% in SFC patients, but as 94 surgeons were involved in this study, there were concerns as to whether a standardised operation was performed routinely. Additionally, Lykke *et al.*[164] reported a poorer prognosis (a 5-year OS rate of 56.6%) in SFC patients. However, in this study, the anatomical limits of SF were not clearly defined and the study did not include to AJCC Stage IV patients or recurrence data[164]. In addition, the authors hazarded that the worse survival was likely due to the challenges posed with central ligation of the branches of the MCA. Be that as it may, the watershed bloody supply of the SF suggests that central ligation of the IMA should receive a comparable emphasis[166].

Contrarily, while SFC patients are more likely to present urgently with obstruction and/or perforation and require an open operation compared to their non-SFC counterparts[165,167,168], some studies have reported similar survival outcomes between SFC and non-SFC patients[152,160,167-170]. This is despite the aforementioned clinicopathological factors being associated with poorer survival outcomes[167,168,171]. The similar survival outcomes between SFC and non-SFC patients may be because the most important determinant of oncological outcome, i.e. the propensity to metastasize may be similar between the SFC and non-SFC groups. For example, Kim *et al.*[169] reported in their retrospective study of 167 SFC patients, with a near

80% 5-year OS rate, that there was no difference in survival (P=0.13) and metastatic rates between the SFC and cancer at other sites (i.e., right sided CC [P=0.97] and left sided CC [P=0.68]) despite a higher rate of obstruction seen in the SFC group. Similarly, in a study by Nakagoe *et al.*[160], a 5-year OS rate between 69.2% (AJCC Stage III) to 100% (AJCC Stage 0-II) was reported in a cohort of 26 SFC patients where there was no significant difference in AJCC Stage between the SFCs and the CCs at other sites (P=0.45). Additionally, Reddavid *et al.*[152] found no significant difference in 5-year OS between cancers in the SF and transverse colon (*n*=32 patients) compared to other sites (73.1% vs. 82.3%; P=0.29), while also noting a homogeneous distribution of AJCC stages throughout the colon and rectum (P=0.48). However, the interpretation of all these studies is confounded by inconsistencies in the definition of the SF[169]. There are no objectively reproducible boundaries (e.g., distal third of the transverse colon to the descending colon, without excluding the sigmoid-descending junction[169]) and there is a lack of standardization in defining the type of resection performed. Additionally, there is a lack of clarity on the vascular pedicles divided in specific SFC operations, including mixed reports on IMV and IMA ligation[169], as shown in Table 3.

<u>What Surgical Resection Should be Performed for Splenic Flexure Cancers?</u>

Currently, there is no standard recommendation for the type of surgery to be performed for a patient presenting with an SFC. For example, an anonymous survey of members of the ACPGBI revealed that 63% of members preferred performing an ERH, followed by LH (23%) and SgR (14%)[172], whereas, in France a SgR was preferred (70%), followed by LH (17%) and SC (13%)[173]. The lack of consensus may be largely a consequence of the lack of clarity as to whether SF lymphatic drainage preferences the MCA or the IMA nodal basins, or both[156,157,174]. Based on early cadaveric studies and histological examinations[156,157], most of the LN metastases seen in patients with SFCs were located along the paracolic arcade and the LCA, a tributary of the IMA[175]. Additionally, others have indicated the presence of positive LNs along the SMA and specifically at the root of the MCA or its left branch[160]. More recently, LNs in the infra-pancreatic and the gastroepiploic stations have been implicated[160,176].

The combination of these random anatomical incongruences with the various definitions of SFC as well as the lack of standardization regarding the extent of resection (Table 3), have contributed to the absence of dedicated CME and CVL guidelines for SFCs[12]. Accordingly, while most surgeons ascribe the extent of bowel resection to the vascular pedicle(s) ligated, others define a SFC resection solely by the section of bowel divided proximally and distally without defining the ligation of the supplying vascular pedicle[177,178]. Invariably, this has led to different surgical approaches and present a fundamental challenge with managing those with SFCs when a personalised approach is most indicated in these patients.

The surgical approaches for SFCs can be broadly classified into two categories: extended and non-extended resections. Traditionally, extended resections have been performed on the premise that a longer colonic resection and wider mesenteric excision improves oncological outcome by removing all potentially involved LNs (that would otherwise be inaccessible through non-extended resections), thereby addressing the variations in SF vascularization and its lymphatic drainage[178]. In addition, an extended resection avoids the need for a colo-colic anastomosis, which has been suggested to be associated with increased risk of anastomotic leak[179]. Thus, extended resections include operations such as performing an ERH, an ELH or a SC with the consideration of a Deloyer's procedure in some situations[151].

Conversely, non-extended resections include procedures such as a LH or SgR of the SF. In the former, aside from resecting the distal third of the transverse colon, SF and proximal descending colon, the left branch of the MCA and LCA is ligated. In the SgR technique, a dedicated pedicle ligation is not performed but principles of proximal and distal resection margins are respected[180]. The rationale for non-extended techniques are based on the preservation of resection margins and the adequacy in nodal harvest (i.e., LNY of at least twelve)[178,181,182]. Furthermore, the preservation of larger segments of colon may reduce the post-operative risks of diarrhoea and malnutrition[170]. Alongside this, some argue that preservation of the rectosigmoid junction may reduce disruption to the inherent defecatory mechanism and preserve the storage capacity of this area[170]. Other described advantages include a lower risk of ureteric and para-aortic nerve injuries[170].

At CRGH, SFC patients are managed with either an ERH, LH, or a SgR. The definition of these procedures are detailed in **Chapter V**. However, as indicated in Table 3, there is a significant lack of standardization in the nomenclature of surgical options for SFCs particularly regarding the division of vascular pedicles and the extent of colon resected. This lack of uniformity, combined with variations in CME and CVL practices, complicates the interpretation and comparison of oncological outcomes following SFC resections[151,160,169,170,176-178,182-198].

Should Splenic Flexure Cancers be Managed with a Limited Lymphadenectomy?

Setting aside their limitations, studies reporting survival outcomes between SFCs and non-SFCs have variably questioned a trend toward limited resection and lymphadenectomy for SFCs[178,190,196,198,199]. This is particularly interesting given the increasing adoption of CME and CVL as standard practices for other CCs[7,12,200]. Indeed, amidst contemporary moves towards more radical lymphadenectomy for cancers of non-watershed areas, why should the approach to lymphadenectomy for SFCs be different to their non-SFC counterparts, particularly since some of the studies report no difference in rates of metastasis between SFC and non-SFC patients[152,160,169]. **Chapter V** of this thesis aims provide context regarding the outcomes of patients following SFC surgery by appraising the "Concord experience", with a view to establish a SFC phenotype and understand long-term oncological outcomes in reference to clinicopathological factors.

Towards an Individualised Understanding of Splenic Flexure Cancer Lymphatic Drainage

To robustly understand the appropriate management of a SFC, a way forward may be to map an individual's SF lymphatic outflow and subsequently define the dominant lymphatic drainage pedicle of individuals with an SFC. While current understanding of SF lymphatic drainage is based on historical cadaveric and non-physiological experimentation[84,201,202], understanding an individual's specific lymphatic drainage at a watershed area, such as the SF, may facilitate a tailored treatment approach.

A physiological means of mapping the native SF lymphatic system and identifying the preferential pedicle may be through *in vivo* intraluminal submucosal delivery techniques. The *in vivo* technique is preferred due to its advantage of identifying aberrant lymphatic drainage, particularly when confronted with advanced-stage pathologies[203]. It also has the theoretical prospect of adjusting for a planned resection when required[203]. The feasibility of mapping an individual's lymphatic drainage from the SF using *in vivo* intraluminal submucosal delivery of a radioisotope is explored in **Chapter VI** of this thesis. Such a tailored approach, applied to individual patients (in recognition of the heterogenous and disparate drainage patterns between different patients) may facilitate a targeted lymphadenectomy that conforms to CME and CVL principles without incurring excessive colonic devascularisation that may ultimately prove unnecessary.

TABLE 3. Overview of Publications on SFCs, Their Vascular Ligations and Their Associations with Survival (up to 2023)									
Study	**SFC definition**	**Sample size (n)**	**Elective Operation**	**Procedure Performed**	**Vessel ligated**	**Laparoscopic Resection**	**AJCC Stage**	**Follow-up (months)**	**Oncological Outcomes**
Ardu *et al*.[170] 2020 Italy	Steffen et al[174]	53	100%	Segmental	MCA_L+LCA	100%	I-IV	Mean=43.5	**MR**: 0% **OS**: 69.8% Survival data compared to ERH for TC (67.5%; P=0.92) and HAR for RC (78.1%; P=0.65) **DFS**: 69.8% Survival data compared to ERH for TC (62.5%; P=0.74) and HAR for RC (71.5%; P=0.92) **Recurrence**: 18.8% Recurrence data compared to ERH for TC (15%; P=0.68) and HAR for RC (15%; P=0.0.56)
Bademci *et al*.[198] 2019 Spain	Steffen et al[174]	124	100% 100% 100%	Segmental LHC STC	MCA_L+LCA MCA_L+IMA ICA+RCA+MCA+IMA	93% 58% 61%	I-III	Mean = 35 (SD24) Mean = 54 (SD39) Mean = 84 (SD64)	**MR**: Segmental -2/28; LHC -5/55; STC - 3/41; 30-day - 1/41 **OS**: NR **DFS**: NR **LR**: LHC – 2/55 **SR**: Segmental -3/28; LHC - 3/55; STC - 2/41
Beisani *et al*.[197] 2018 Spain	Steffen et al[174]	144	100%	LHC STC	MCA_L+IMA ICA+RCA+MCA+IMA	38% 32%	I-III	Median = 46	**30-day MR**: LHC – 5%; STC – 6% (P=0.9) **5-yr OS** (NS): LHC - 84%; STC – 85% **DFS**: NR **LR** (NS): LHC - 4%; STC – 1% **SR** (NS): LHC - 16%; STC – 22%
Binda *et al*.[196] 2019 Italy	Steffen et al[174]	324	89.2% - - 77.2%	LCR - Segmental - LHC ERH	- MCA_L+LCA MCA_L+IMA ICA+RCA +MCA+LCA	37.9% - - 29.1%	I-IV	NR	**30-day MR**: LCR – 3.6%; ERH – 5.1% **OS**: NR **DFS**: NR **LR**: NR **SR**: NR
Bracale *et al*.[195] 2019 Italy	Between the distal third of the TC and the first part of the DC	112	100%	NR ?Segmental	MCA_L+LCA+IMV	100%	I-IV	Median = 43 (range 12–149)	**MR**: 13/112 **OS**: 51% at 148 months **DFS**: NR **Recurrence** (not specified):11.6%
Carlini *et al*.[182] 2016 Italy	Between the distal third of the TC and the first part of the DC	20	NR	NR	MCA_L±MCA +LCA	100%	I-III	Mean = 58 (SD 31)	**MR**: NR **OS**:NR **DFS**: NR **LR**:0% **SR**: 2/20 at 14 and 19 months
Ceccarell *et al*.[194] 2010 Italy	Steffen et al[174]	15	NR	NR ?Segmental	LCA+LCV+ MCV_L	100%	I-III	Mean = 29 (SD16)	**MR** (30-day): 0% **OS**:NR **DFS**: NR **LR**:NR

									SR: NR
Chenevas-Paule *et al.*[193] 2020 France	Steffen et al[174]	28	100%	Segmental	MCA_L+LCA+ IMV	100%	I-III	Median = 50.9 (range 7-138)	**MR** (90-day): 3.5% **5-yr OS**: 46.3% **5-yr DFS**: 39.2% **LR**: 2/28 **SR**: 7/28
de'Angelis *et al.*[192] 2021 Italy	Steffen et al[174]	90	0%	Segmental LHC ERH	MCA_L+LCA MCA_L+IMA ICA+RCA+MCA+LCA	29.4% 33.3% 10.9%	I-IV	Mean = 41.6 (SD33.5)	**MR**: NR **OS** (P=0.70): Segmental – 1yr (93.3%), 2yr (77%), 5yr (63.4%); LHC - 1yr (87.4%), 2yr (79.4%), 5yr (58.9%); ERH - 1yr (86.2%), 2yr (78.6%), 5yr (62.6%) **DFS** (P=0.40): Segmental – 1yr (78.7%), 2yr (69.6%), 5yr (58%); LHC - 1yr (84.6%), 2yr (75.2%), 5yr (60.2%); ERH - 1yr (80.3%), 2yr (74.3%), 5yr (57.5%) **LR**: 5/90 **SR**: 15/90
de'Angelis *et al.*[191] 2016 Italy	Steffen et al[174]	54	100%	LHC ERH	MCA_L+IMA ICA+RCA+MCA+LCA	100%	I-III	Mean = 70.9 (SD 46.1)	**MR**: NR **OS** (P=0.85): LHC – 1yr (96.3%), 3yr (91.9%), 5yr (75.1%); ERH - 1yr (92.6%), 3yr (85.8%), 5yr (72.8%) **DFS** (P=0.64): LHC - 1yr (96.2%), 3yr (75.5%), 5yr (66.7%); ERH - 1yr (85.2%), 2yr (76.7%), 5yr (67.1 %) **Recurrence**(P=0.76): LHC – 22.2%; ERH - 29.6%
de'Angelis *et al.*[190] 2021 Italy	Steffen et al[174]	399	74.4%	Segmental LHC ERH	MCA_L+LCA MCA_L+IMA ICA+RCA+MCA+LCA	68.8% 78.6% 75.5%	I-IV	Mean = 41.7 (SD 37.3)	**MR:**NR **OS** (P=0.26): Segmental – 1yr (97.8%), 2yr (95.2%), 5yr (76.3%); LHC - 1yr (97.5%), 2yr (92.2%), 5yr (74.3%); ERH - 1yr (93.3%), 2yr (87.9%), 5yr (66.2%) **DFS** (P=0.56): Segmental – 1yr (86.2%), 2yr (78.8%), 5yr (70.3%); LHC - 1yr (90.9%), 2yr (83.4%,), 5yr (76.3%); ERH - 1yr (85.5%), 2yr (82.8%), 5yr (73.9%) **LR** (P=0.95): 2.6% **SR** (P=0.94): 18.4%
Degiuli *et al.*[151] 2020 Italy	Steffen et al[174]	1,304	94.6% 88% 95.2%	Segmental ERH ELC STC - excluded	MCA_L+LCA ICA+RCA+MCA+LCA MCA_L+IMA	62.1% 39% 53.5%	I-III	Median = 46 (IQR 0–150)	**MR:** <1% (P=0.46) **5 yr OS** (P=NS): Segmental – 0.84 (95% CI, 0.81–0.88); ERH - 0.77 (95% CI, 0.64–0.86); ELC - 0.84 (95% CI, 0.80–0.88)

									5 yr DFS (P=NS): Segmental – 0.85 (95% CI, 0.81–0.88); ERH - 0.78 (95% CI, 0.64–0.87); ELC - 0.86 (95% CI,0.81–0.89) **LR**: NR **SR**: NR
Gravante *et al*.[189] 2016 UK	Between the distal TC and proximal DC	98	73.5% 51.8%	LH ERC	MCA_L+LCA ICA+RCA+MCA+LCA	26.5% 6.2%	I-IV	NR	**MR**: LH - 2.9%; ERH - 1.6%, **OS** (P=0.16): LH - 51.8 months; ERH - 50.4 months **DFS**: NR **LR**: NR **SR**: NR
Han *et al*.[188] 2010 Korea	Proximal splenic flexure and the sigmoid colon-descending colon junction	90	100%	LHC	MCA_L+LCA+IMV±IMA	38.9%	I-III	Lap - Median = 21 (Range 1-60) Open – Median = 26 (range 2-60)	**MR**: 0% **DFS**: NR **LR**: Open – 1 patient **SR**: lap – 3 patients; Open - 7 patients
Huang *et al*.[175] 2022 China	Steffen et al[174]	117	100%	Segmental LHC ELC	LCA+/-MCA_L+/-MCA_A MCA+LCA IMA+/-"other arteries"	84.9% 86.4% 86.4%	I-III	Median = 58.5 months (IQR 6-113)	**MR**: NR **OS** (P=NS): Segmental – 94.0%; LHC – 90.2%; ELC – 94.1% **DFS** (P=NS): Segmental – 88.2%; LHC – 90.2%; ELC – 83.0% **LR** (P=1.00): 4.3% **SR** (P=0.70):11.1%
Kim *et al*.[169] 2010 Korea	Distal third of the TC to the DC, with excluding the sigmoid descending junction	167	NR	LHC STC	MCA_L+LCA NR	NR	I-IV	Median = 82 (range 1-184)	**MR**:NR **OS** (P=0.44): LHC - 143.6±6.0 months; STC - 121.8±10.9 months **DFS**(P=0.66): LHC - 135.3±6.4 months; STC - 117.0±12.3 months **Total recurrence**: 54/167
Kim *et al*.[187] 2017 Korea	Steffen et al[174]	51	100%	NR	MCA_L+LCA+/-MCA trunk + LCV ± IMV	64.7%	I-III	Lap - Median = 59.0 (IQR 50.0–73.5); Open – Median = 61.0 (IQR 27.8–87.0)	**MR**: NR **5 yr OS** (P=0.56): Lap – 84.3%; Open – 76.0% **5 yr DFS**(P=0.08): Lap – 93.8%; Open – 74.5% **LR**: Lap – 2 patients **SR**: Lap – 2 patients; Open – 2 patients
Labiad *et al*.[177] 2022 France	Between the distal third of the TC and the first part of the DC	198	0%	Segmental LHC STC Stoma	*Based on colon resection as opposed to vascular ligation	NR	I-IV	Median = 13.8 [IQR 4.5–31.0].	**MR**:6% **3 yr OS** (P=0.87): Segmental – 71%; LHC – 67%; STC – 73%; Stoma – 63% **3 yr DFS** (P=0.65): Segmental – 58%; LHC – 33%; STC – 51%; Stoma – 54% **LR:**NR **SR:**NR

Manceau *et al.*[178] 2022 France	Between the distal third of the TC and the first part of the DC	313	100%	Segmental LHC STC	MCA_L+LCA MCA_L+IMA ICA+RCA+MCA+LCA*	33%	I-III	Median = 45.1 (range 0.4–162.7)	**MR**:NR **5 yr OS** (P=0.63): Segmental – 80%; LHC – 81%; STC – 78% **3 yr DFS** (P=0.94): Segmental – 72%; LHC – 82%; STC – 72% **LR**:NR **SR**:NR
Martin Arevalo *et al.*[186] 2018 Spain	Steffen et al[174]	170	75% 73% 63.4%	Segmental LHC ERC	MCA_L+LCA MCA_L+IMA ICA+MCA+LCA	8.3% 19% 16.9%	I-III	Mean = 81.1 (SD64.6)	**MR**:6.5% **OS**: ERC vs. LC (P=0.24), ERC vs SLC (P=0.78), or LC vs. SLC (P=0.78) **DFS**: Unclear **5 yr LR**: LHC – 2 patients SR:NR
Matsuda *et al.*[185] 2018 Japan	Steffen et al[174]	17	NR	NR	MCA_L+LCA+IMV++/-MCA_A	100%	I-IV	Median = 16	**MR**: NR **OS**: NR **DFS**: NR **LR**: **SR**: 1/17 (Stage IV)
Nakagoe *et al.*[160] 2001 Japan	Between the distal third of the TC and the first part of the DC	27	22.2%	Segmental LHC	MCA_L or LCA MCA_L+LCA	0%	I-III	Median = 60.9 (range 1.5 - 127.6)	**MR**:3.7% **OS:** Survival stratified by stage **DFS:** NR **LR**: NR **SR**: NR
Odermatt *et al.*[184] 2014 UK	According to surgeon's clinical judgment	68	74%	LH ERC	MCA_L ICA+RCA+MCA	41.1%	I-III	Median = 74.4 (95 % CIs 58.8–132)	**30-day MR** (P=0.62): LH – 3.3%; ERC – 7.9% **5 yr OS** (P=0.20): LH - 60% (95% CIs 44–83); ERC - 49% (95 % CIs 35–68) **5 yr RFS** (P=0.18): LH - 54 % (95 % CIs 39–77); ERC 41 % (95 % CIs 28–61) **LR**: NA **SR**:NA
Okuda *et al.* [183] 2016 Japan	NR	95	NR	LH or Segmental	MCA_L+LCA	64.2%	I-III	Stage II Median = 84 Stage III Median = 61	**MR**:NR **5 yr OS**: →Stage II – Lap LC = 94.6%; Open LC = 85.6% → Stage III – Lap LC = 73.5%; Open LC = 66.2% **5 yr DFS**: →Stage II – Lap LC = 91.1%; Open LC = 85.6% → Stage III – Lap LC = 60.3%; Open LC = 56.7% **LR**:NR **SR**:NR

Ozgur *et al*,[176] 2023 USA	Between the distal third of the TC and proximal third of the DC	142	100%	TC STC ERH ELH LH (Group1-3) Segmental (Group 4)	ICA+RCA+MCA+IMA ICA+RCA+MCA+LCA+SA NR MCA_L+IMA NR MCA_L+LCA+IMV	9%	I-III	Median = 114.9 (IQR 65.5-197.8)	**MR**:2.8% **OS**: HR 1.8 (0.9-3.4); P=0.06 [Group1-3 = Ref] **DFS**: HR 1.6 (0.9-2.9); P=0.10 [Group1-3 = Ref] **LR**: NR **SR**:NR
Pedrazzani *et al*.[204] 2021 Italy	Steffen et al[174]	641	100%	STC LH LHC -Last 2 were combined together	ICA+RCA+MCA+LCA MCA_L+IMA+IMV+ MCA_L+LCA	45.2%	I-III	Lap - Median = 59.9 (IQR 37–98) Open – Median = 60 (IQR 38–98)	**5 yr MR**:21.9% **5 yr multivariate OS** (P=0.44) **5 yr multivariate CSS** (P=0.78) **DFS**:NR **LR**: Stratified by Lap vs Open **SR**: Stratified by Lap vs Open
Pisani Ceretti *et al*.[181] 2015 Italy	Steffen et al[174]	23	NR	NR	MCA_L+LCA + SA_1+IMV	100%	I-III	Mean = 33 (SD17)	MR:0% OS: NR DFS: NR LR: 1/23 SR: 1/23
Reddavid *et al*.[152] 2019 Italy	NR	32	NR	Segmental LHC ERH ELH **Combined TC and SF cancers together as an arm vs other cancer resection**	MCA_L+LCA IMA+IMV+MCA_L NR NR	52.9% 44.4% 30% 33%	I-IV	NR	**MR**:NR 5 yr OS (P=0.33): Segmental – 100%; LHC – 100%; ELH – 44.4%; ERH – 75% **DFS**:NR **LR:** NR **SR:** NR
Rega *et al*.[205] 2019 Italy	Steffen et al[174]	103	100%	Segmental ERH ELH	MCA_L+LCA+IMV ICA+RCA+MCA+LCA IMA+IMV+ MCA_L+LCA	17.5% 13.6% 20.8%	I-III	42 months (IQR 24–70 months).	MR:0% OS(P=0.21) P=0.211 among the three groups DFS (P=0.62): p=0.621 between all three LR: Segmental –2 patients SR: Segmental – 12 patients; ERH – 4 patients; ELH – 5 patients

Secco *et al.*[206] 2007 Italy	Unable to retrieve full paper	129	100%	Unable to retrieve full paper	I-III	Median follow-up was 60 and 56 months for group 1 and 2, respectively	**MR**:NR 5 yr OS (P=0.33): Segmental – 100%; LHC – 100%; ELH – 44.4%; ERH – 75% **DFS**:NR **LR:** NR **SR:** NR
LR: **Local Recurrence;** ***SR:*** **Systemic Recurrence;** ***MR:*** **Mortality Rate;** ***CCS:*** **Cancer-Specific Survival;** ***TTR:*** **Time to Recurrence;** ***DFS:*** **Disease-Free Survival;** ***RFS:*** **Recurrence-Free Survival;** ***OS:*** **Overall Survival;** ***HR:*** **Hazard Ratio;** ***MV:*** **Multi-Variate;** ***ERH:*** **Extended Right Hemicolectomy;** ***ELH:*** **Extended Left Hemicolectomy;** ***MCA*****: Middle Colic Artery;** MCA_L**: Left branch of Middle Colic Artery;** MCA_A**: Middle Colic Artery Accessory branch;** ***LCA*****: Left Colic Artery;** ***LCV*****: Left Colic Vein;** MCV_L**: Left branch of Middle Colic Vein;** ***IMV:*** **Inferior Mesenteric Vein;** ***ICA*****: Ileo-Colic Artery;** ***RCA*****: Right Colic Artery;** ***IMA:*** **Inferior Mesenteric Artery;** MCA_R**: : Right branch of Middle Colic Artery;** SA_1**: Sigmoid Artery 1st branch;** ***NR:*** **Not Reported;** ***ROC:*** **Receiver Operating Curve;** ***AUC:*** **Area Under the Curve;** ***IQR*****: Inter-Quartile Range, and** ***AJCC:*** **American Joint Committee on Cancer** **NB: Extensive variation in vascular pedicle ligation and type of operation performed.**							

Table 3: Publications on SFCs and Survival Associations.

PART I: EXTENT OF LYMPHADENECTOMY

CHAPTER II: Is Computed Tomography Assessment of Residual Arterial Pedicle Length Following Colorectal Cancer Surgery a Useful Marker of Surgical Quality?

This chapter has been prepared, submitted and published in *Techniques in Coloproctology* as **"Naidu K, Chapuis P, Yang J, Koneru S, Chan C, Rickard M, Ng KS. Is computed tomography assessment of residual arterial pedicle length following colorectal cancer surgery a useful marker of surgical quality? Tech Coloproctol. 2025 Apr 12;29(1):101. doi: 10.1007/s10151-025-03130-6. PMID: 40220058; PMCID: PMC11993473".** The published manuscript is included as **APPENDIX I** in **Chapter IX**.

Preamble

Recent interest in CVL (i.e., D3 lymphadenectomy) as a complement to CME, hints at an alternative approach to understanding the quality of lymphadenectomy performed[81,207]. CVL stresses vascular ligation at the origin (i.e., high) of feeding arterial vessels to maximize central LN clearance. The interest in its routine application has been highlighted by the results of several cohort studies demonstrating decreased LR rates[6] and improved oncological outcomes[7-9].

Although the routine use of CVL remains controversial due to its technical demands and complications, the concept of CVL is recognised as a benchmark for quality in CRC surgery[9,12,208]. This recognition stems from CVL's potential role in dealing with skip metastases[80], micro-metastases[12], and central nodal metastases[12]. Moreover, despite the lack of long-term randomized evidence for CVL in CRC surgery, interest in and acceptance of this technique is demonstrated by at least 22 reviews or meta-analyses published on the topic since 2009[10,20,209-219].

When compared with *conventional* colectomy (Figure 2), which generally represents less defined colonic mobilization and more distal (i.e., low) vascular division at a convenient location, oncological outcomes appear superior following CVL surgery, with level 2 evidence for improved 4-year DFS (85.8% vs 73.4%, P=0.0014)[9,12]. Additionally, a meta-analysis by Balciscueta *et al.*[10] which included three studies[207,208,220] and involved 434 patients, found a significant reduction in LR rates among those undergoing CME with CVL/D3 surgery, compared to conventional surgery, with low heterogeneity among the studies. However, long-term randomized data are currently unavailable, and case series have shown a relatively high rate of post-operative complications and mortality, possibly associated with injury to central vascular structures[221]. Recent population data from the Danish Colorectal Cancer Group also found that CME with CVL surgery was associated with a higher rate of intra-operative organ injuries (9.1% vs 3.6% P < 0.001), with a 90-day postoperative mortality rate as high as 6.2%[208]. Additionally, the existing literature is limited by inconsistent and variable terminology, and national guidelines offer widely differing recommendations regarding the role of CME and CVL surgery[20].

In cases where CVL is not strictly practised, as at CRGH, a HVT is typically ensured. This approach aims to maximise the volume of mesentery excised *en bloc* with the tumour and ensure an adequate LN harvest[222,223]. However, an unambiguous consensus of what constitutes ‘as high as possible’ is lacking.

Regardless, in both practices (i.e., CVL and HVT), the quality of lymphadenectomy is likely more accurately be determined by assessment of unresected *residual* LN tissue rather than the number of LNs found in the resected specimen. Naturally, this leads to the question of, “how will the unresected *residual* LN tissue be best assessed?”. While plane of surgery is accurately established by pathology assessment of the resected specimen, the determination of whether CVL or HVT surgery has been performed remains largely dependent on corroboration with the surgical team, and the level of pedicle transection (and by inference, *unresected* LNs) cannot be assessed by *ex vivo* pathological assessment alone.

Alternatively, *in vivo* RAPL measured on post-operative imaging has been proposed as a more robust method to verify CVL surgery[81,202,224]. However, its role as a surrogate of the EoL and as a quality measure of survival outcomes in non-routine CVL practice has not been studied. This will be explored in detail in **Chapter II.**

ABSTRACT

INTRODUCTION: *In vivo* RAPL has been proposed as a quality indicator for CVL (i.e., RAPL ≤10mm) in CRC surgery. However, its survival association in non-routine CVL practice requires clarification. This study aimed to assess the feasibility and reproducibility of measuring RAPL alongside its oncological associations in non-routine CVL surgery.

METHODS: A prospective cohort study at Concord Hospital was conducted on AR or RH patients with Stage I to III CRC (1995-2019). Using surveillance CT, the RAPL of the IMA or ICA pedicle was measured independently by two observers. The intra-class correlation coefficient assessed the reproducibility of the measurements. Kaplan–Meier and univariate Cox regression analyses estimated OS and DFS, while univariate and multivariate linear regression models tested correlations between RAPL and clinicopathological features.

RESULTS: 1,425 patients underwent a CRC operation. Post-operative CTs were reviewed in 424 patients, with 422 (mean age 69.0 years [SD12.3]; 54.0% males) RAPLs measured. The majority studied underwent an AR (59.2%). Excellent inter-rater reliability was noted in AR (ICC=0.97; P<0.001) and RH (ICC=0.89; P<0.001) patients. No association was observed between RAPL and OS or DFS in either group. Also, RAPL lacked association with nodal harvest in either AR (P=0.54) or RH (P=0.16) patients.

CONCLUSION: The value of RAPL as a quality marker of CRC surgery in non-routine CVL practice has not been confirmed. Furthermore, its lack of association with LN harvest emphasizes the importance and need for comprehensive pathology examination of the specimen following resection of CRC.

INTRODUCTION

Central tenets of 'good quality' CRC surgery include operating in the correct anatomical plane, complete primary tumour removal with clear margins and performing an adequate lymphadenectomy. Most early studies appraising surgical quality have centred on the correct plane of excision (TME for RC or CME for CC) and avoidance of tumour transection[1,4,7,12,19]. However, the EoL has perhaps received less focus. This may be due to challenges in its objective measurement which is so far limited to the elementary assessment of LN harvest in the excised specimen[225] when perhaps the assessment of *unresected* LNs may be more meaningful. Indeed, recent interest in CVL as a counterpart to CME suggests that an alternative approach to appraising the EoL would be useful[81,207].

Based on the level of vascular ligation from the origin of the arterial vessel(s) supplying a particular segment of colon, CVL contributes to the completeness of a cancer operation[12]. This demarcates the maximum EoL by completely clearing regional LNs and those located centrally (to minimise any residual LN tissue potentially involved with cancer)[12,84]. In cases where CVL is not strictly practised, pedicle ligation *as high as possible* (i.e., HVT) is the usual aim. In both practices, the quality of lymphadenectomy may more accurately be determined by assessment of unresected *residual* LN tissue rather than the number of LNs found in the resected specimen.

The determination of whether CVL surgery has been performed remains largely dependent on corroboration by the surgical team. This practice does not allow for the level of pedicle transection to be assessed based upon pathological assessment of the excised specimen. Alternatively, *in vivo* RAPL measured on post-operative imaging has been proposed as a more robust method to verify CVL surgery[81,202,224]. However, its role as a surrogate of the EoL and quality measure of survival outcomes in non-routine CVL practice has not been studied.

Therefore, the primary aim of this study was to assess the feasibility and reproducibility of measuring RAPL utilising surveillance CT in patients who had an AR or RH operation for CRC for which CVL was not routinely practised. A secondary aim of the study was to investigate whether RAPL was associated with survival outcomes or standard clinicopathological variables. We posited two hypotheses: that (i) measuring RAPL would prove to be both feasible and reliably reproducible, and (ii) a longer RAPL was associated with poorer oncological outcome and less radical lymphadenectomy.

MATERIAL and METHODS

A prospective observational cohort study of consecutive patients who underwent a potentially curative resection for a rectal or colon adenocarcinoma was performed. Patients included were those with either an AR or RH performed between January 2009 and December 2019 at CRGH, Sydney, Australia. Patients were identified from a prospectively maintained institutional database. Patients with AJCC Stage IV cancer, inflammatory bowel disease, polyposis coli, or a synchronous or metachronous CRC were excluded (Figure 3).

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sydney Local Health District Ethics Committee (2020/ETH03325 and CH62/62011-136-P Chapuis HREC/11/CRGH206).

Surgical Procedures

An AR operation was performed with adherence to TME principles and ligation of the IMA proximal to the origin of the ascending LCA, without abdominal aorta exposure[226]. A RH operation was performed according to the technique described by Bokey et al.[226], which involved ligation of the ICA without routine exposure of the SMA or SMV. In our unit, some surgeons perform a full lymphadenectomy but preserve the vascular stump by sweeping potential central nodes from the pedicle root distally in patients with suspected central lymphadenectomy. The precise tumour location was marked by the surgeon on a diagram including whether there was distant metastasis or unresected tumour remaining at the completion of the operation.

Standard Clinicopathological Variables

Clinical information, operative details, tumour pathology, and follow-up data were obtained from the database for analysis[227,228]. Specifically, details concerning the method of pathology reporting and staging have been previously described[229,230].

Clinico-pathological Variable of Interest – CT measurement of IMA and ICA RAPL (Figure 4)

The RAPL of the post-AR IMA or post-RH ICA were prospectively measured and recorded in patients identified from the prospective database. The RAPL was positively identified if the location of a visible named vessel correlated to its expected anatomical position, or terminated at a surgical staple, surgical clip, or "radiological granuloma". Patients whose RAPLs measured ≤10mm were regarded as having had a CVL[12].

The IMA and ICA were chosen as the pedicles to measure as they correlated to the main feeding arteries in our two most commonly performed operations. On the left side, the IMA reliably originates from the abdominal aorta and assumes a subtle leftward course, following its origin at the level of the third lumbar vertebral body[224,231]. Contralaterally, the ICA is the most constant collateral of the SMA, emerging usually approximately at the level of the fifth or sixth jejunal artery and assumes an antero-inferior trajectory[224].

Surveillance CT imaging up to three years post-surgery was used to standardize the timeline for RAPL measurement. Helical imaging acquisition of the abdomen and pelvis obtained during either the venous or arterial (if available) phase was used for multiplanar reconstruction or volume-rendered reformat of the mesenteric vasculature, using the syngo®.via (Siemens Healthcare, Germany) platform. The CTs were reviewed by KN and once best displayed, a calliper tool was used to measure the post-resection arterial stump lengths. Patients were excluded from the study if their CT scans had missing or inconclusive results, specifically when the RAPL could not be easily traced due to slice thickness or the absence of IV contrast, as shown in Figure 4. The RAPL measurements were repeated randomly in 10% of the cases by a (blinded) specialist gastrointestinal radiologist (JY), to determine the reproducibility of the measurements and overall inter-observer reliability.

Pathology Reporting and Staging

Specialist pathologists examined resected specimens using a standard synoptic protocol, with pathology data coded by CC. Adenocarcinomas including mucinous and signet ring types were analysed. Fat clearance techniques were not employed in node retrieval. Tumour staging followed the AJCC pTNM system.

Surveillance and Follow-up

Patients were reviewed at least six-monthly for the first two years after resection and followed up yearly thereafter until death or December 2021, unless lost to follow-up[232]. The approach to surveillance and indications for post-operative adjuvant chemotherapy have been previously detailed[230].

The date of resection was the start of the follow-up period. Follow-up times were censored at the last contact for patients who did not experience the terminal event up to December 2021, were lost to follow-up, or remained alive. The approach to identifying the date and cause of death have been described previously[230,233].

Outcome Measures

The primary outcome measure focused on assessing the reproducibility of CT-based RAPL measurements in terms of reliability and agreement. Secondary outcome measures included OS, DFS, and any LR or SR within the peritoneal cavity or elsewhere, and biopsy proven whenever possible[174,234,235].

Statistical Analyses

Continuous variables were reported as mean (SD) for normally distributed variables and as median (IQR or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages. The inter-observer reliability of RAPL measurements was tested using ICC modelling. Univariate and multi-variate linear regression models tested correlation between RAPLs and clinicopathological factors. Survival and recurrence estimates were modelled using the Kaplan-Meier function with log-rank test performed to determine difference in survival distributions. Cox-regression modelling tested for associations between outcome measures and relevant clinicopathological variables, including RAPL measurements. A multi-variate model was not performed because multiple variables violated the proportional hazards assumptions, and alternative stratification of these violating variables rendered too small a case load for analysis. The level for 2-tailed statistical significance was $P<0.05$ with confidence intervals at the 95% level. All the analyses were performed using SPSS® version 29 (IBM, New York, USA).

Sample size

Sample size calculations were made based on a previously reported 5-year DFS of 59.4%[230]. A relative improvement of 10% (attributable to CVL), in the 5-year DFS was deemed clinically relevant. Estimating that 5% of our patients had a CVL resection in our institution and calculating for a two-sided significance of 0.05 (*alpha* of 0.05 and power of 0.85), a minimum of 415 patients were required for adequate power[236].

AJCC Stage III Sub-group Analysis

It was expected that the greatest survival advantage for patients undergoing CVL surgery would be observed in the AJCC Stage III population. To understand the survival association between RAPL and Stage III disease, a sub-group analysis was performed on this population, alongside a separate Cox regression survival analysis.

RESULTS

Study Population

A total of 1,425 patients had a CRC resection during the study period. Of these, 827 were suitable for analysis. We reviewed post-operative CTs for 424 consecutive patients (Figure 3) of which 422 (99.5%) had a recordable RAPL. Of these 422 patients, 250 (59.2%) and 172 (40.8%) patients had AR and RH surgery, respectively. Detailed clinico-pathological characteristics of AR and RH patients are shown in Table 4.

The temporal trend in RAPL measurements is presented in Figure 5. The median RAPL of patients who underwent an AR or RH was 26.4mm (IQR 19.2-34.3) and 29.8mm (IQR 21.6-40.9), respectively. Eleven AR (4.4%) and four RH patients (2.3%) had CVL (i.e., RAPL≤10mm) performed.

The absolute inter-observer agreement for AR and RH RAPLs was 94.6% and 78.9%, respectively. Inter-rater reliability assessed by ICC was 0.97 (95%CI 0.93–0.99; P< 0.001) and 0.89 (95%CI 0.64–0.96; P<0.001) in AR and RH patients, respectively.

Comparison of Survival Outcomes between Clinicopathological Characteristics in AR and RH patients (Table 5)

Table 5 summarizes the associations between clinicopathological characteristics and survival outcomes in patients who underwent AR and RH resections. Figure 6A-D presents the Kaplan-Meier plot of OS and DFS for these patients stratified according to RAPL.

Anterior Resection

For those who underwent an AR, death occurred in 66 patients (26.4%). The 5-year OS and DFS rates were 75.6% (95%CI 72.5-78.7) and 66.6% (95%CI 63.4-69.8) respectively. A LR was diagnosed in 11 patients (4.4%), and SR was diagnosed in 58 patients (23.2%). The mean time to LR and SR was 1.5 years (95%CI 1.0-1.9) and 1.9 years (95%CI 1.5-2.6), respectively.

RAPL was not associated with OS (P=0.14) or DFS (P=0.26), nor was CVL (OS [P=0.31]; DFS [P=0.18]). A poorer OS was associated with increasing age (P<0.001), ASA >2 (P<0.001), AJCC Stage III tumours (P=0.008), poorly differentiated (P=0.04) and high grade (P=0.004) tumours, the presence of LVI (P=0.03), and PNI (P=0.001). Poor prognosis characterised by increased DFS hazards mirrored all of the above factors, in addition to large tumours (P=0.02).

Right Hemicolectomy

Amongst the RH patients studied, death occurred in 57 patients (33.1%). The median DFS was 9.3years (95%CI 5.2-13.4). The 5-year OS and DFS rates were 69.6% (95%CI 65.5-73.7) and 61.8% (95%CI 57.6-66.0) respectively. A LR was diagnosed in three patients (1.7%), and SR diagnosed in 36 (20.9%). The median time to LR and SR was 2.8 years (95%CI 0.3-5.2) and 1.2 years (95%CI 0.7-1.6), respectively.

RAPL was not associated with OS (P=0.15) or DFS (P=0.12). Similarly, CVL was also not associated with OS (P=0.35) or DFS (P=0.54). Poorer OS was associated with an increasing age (P=0.001), ASA score >2 (P=0.03), intra-operative blood loss >500mls (P=0.003), larger tumours (P=0.02), mucinous or signet cancers (P=0.04), and LVI (P=0.005). Regarding DFS, a poorer prognosis mirrored all factors noted above.

Clinico-pathological Characteristics and RAPL Associations in AR and RH patients (Table 6A and 6B)

The association of RAPLs to other clinico-pathological features are presented in Tables 6A (univariate analysis) and 6B (multi-variate analysis).

Anterior Resection

On univariate analysis, a shorter RAPL was associated with female patients (P=0.01), laparoscopic operation (P<0.001), and more recent study years (P=0.03). A longer RAPL was associated with a higher BMI (P=0.04) and ASA grades (P=0.01). RAPL was not associated with the number of LNs examined (P=0.54). On multivariate analysis, only ASA grade (P=0.04) was significantly associated with a longer RAPL, and laparoscopic operation (P<0.001) with a shorter RAPL.

Right Hemicolectomy

RH patients had a longer RAPL than AR patients (Table 6). On univariate analysis, a shorter RAPL was observed in female patients (P=0.002) and laparoscopic operation (P=0.02). A longer RAPL was associated with increasing age (P=0.008) and a high ASA grade (P=0.004). Again, RAPL was not associated with the number of LNs examined (P=0.16). On multivariate analysis, only gender (P=0.01) was associated with a shorter RAPL.

AJCC Stage III Sub-group Analysis

There were 238 AJCC Stage III patients with a recorded RAPL. Of these, 146 had an AR and 92 had a RH. The median RAPLs of patients who underwent an AR and RH were 26.7mm (IQR 19.5-36.3) and 29.8mm (IQR 21.2-40.5) respectively. Within this cohort, six AR patients (4.1%) and three RH patients (3.3%) had a CVL performed.

The survival associations of the sub-cohort are summarised in Table 7. In patients who underwent an AR, RAPL was associated with poorer OS (P=0.04) but not DFS (P=0.10). In those who had a RH, RAPL was neither associated with OS (P=0.27) nor DFS (P=0.64).

DISCUSSION

Over a 10-year period, this large and adequately powered prospective cohort study evaluated the role of RAPL measured on surveillance CT as a marker of surgical quality, and EoL. This was achieved by investigating the association of RAPLs with survival outcomes and other clinicopathological variables in patients who had either an AR or RH for CRC. This study demonstrates the feasibility and reproducibility of measuring RAPLs in patients in whom CVL surgery was not routinely performed. Although some clinicopathological factors were individually associated with poorer survival outcomes, RAPLs showed no influence on these outcomes. Notably, RAPL was not associated with the extent of the LN harvest. These findings suggest that the use of RAPL as a quality marker for CRC when measured in a non-routine CVL population is of minimal value in predicting patient outcome.

The current assessment of the EoL in CRC surgery relies on simple measures, such as LN harvest, and a nodal count of twelve is widely accepted as an adequate lymphadenectomy for staging purposes[21,237]. Measurements based on TM, such as the area of mesentery excised, have been previously described and proposed as objective measures for lymphadenectomy extent[84]. Nevertheless, when considering lymphadenectomy for the purpose of ensuring all potentially involved nodes are excised, of which CVL is a possible 'gold standard', there should be focus on quantifying the *unresected* nodal tissue remaining. Neither LN harvest nor TM measurements of the pathology specimen can provide this assessment. Our study investigated whether *in vivo* measurement of RAPLs using routine surveillance CT could fill this role by providing an objective assessment of residual nodal tissue using RAPL as a surrogate marker.

This study confirmed the feasibility and reproducibility of measuring IMA and ICA pedicle length following AR and RH, respectively. IMA and ICA pedicles were consistently identified in over 400 post-operative scans, with identification aided through recognition of a surgical clip/staple, or "radiological granuloma". The reproducibility of these measurements was confirmed by a second independent observer demonstrating good inter-observer agreement. Notably, IMA pedicle lengths were, on average, shorter than ICA pedicle lengths, likely a reflection of the accepted and inherent complexity seen in the vascular anatomy of the right colon compared to the left. The difference in IMA and ICA pedicle lengths justifies our approach of considering AR and RH patients separately in a study designed to evaluate the association between pedicle length and survival.

The measurement of RAPL following CRC surgery has previously been described, but with heterogenous study populations, study designs and imaging protocols[202,224,238-240]. One previous study measured ICA stump length following RH surgery many months after the index operation[238], but did not investigate its relationship with survival outcomes. In another study, where radio-surgical correlation of IMA ligation was noted in only 41% of patients, no survival difference between the presence or absence of radio-surgical correlation of IMA ligation was observed[240]. In a separate registry-based study, Bostrom *et al.* highlighted no survival difference between the level of vascular tie when it was dichotomised to high and low ligation. These authors did not record individual RAPLs[241]. Only one study has previously investigated the relationship between RAPL and survival outcome, but in a cohort where CVL was routinely intended to be practiced. In that study, a pedicle length of less than 1cm (verifying CVL had been performed) was associated with survival benefit[81]. Our study aimed to determine if a similar survival benefit was demonstrable in a cohort where CVL was not routinely practiced. Our original hypothesis that a shorter RAPL would confer some survival benefit was not substantiated, as there was no association between RAPL and OS or DFS in either AR or RH patients.

The result of our subgroup analysis warrants comment. When confined to Stage III patients alone, there was an apparent association between RAPL and OS, but not DFS, in AR patients. This association, however, was weak, and is difficult to explain. It is challenging to resolve why a shorter RAPL would provide an OS benefit without improvement in DFS. It is noted that this significant association was observed only on univariate analysis.

Our study was unable to demonstrate a clear association between RAPL and LN harvest. A more radical operation has been associated with a larger LN harvest in previous studies[6,207,208], so intuitively, an association between a shorter RAPL and a larger LN harvest should be expected. We suppose two reasons for this observation. The first relates to the surgical technique practiced within our unit, whereby some surgeons perform a full lymphadenectomy but preserve the vascular stump by sweeping central nodes from the pedicle root distally. This may result in a longer RAPL despite a complete lymphadenectomy being performed. Secondly, pursuing a higher vascular tie may not necessarily translate into acquiring a larger nodal harvest. In a previous study from our unit investigating the relationship between apical node positivity and survival outcome[242], over one-third of resected CRC specimens did not have an apical node present (defined as a node within 1cm of the pedicle ligature), suggesting that extending pedicle transection proximally may not necessarily result in a greater (apical) LN yield.

Although this study was unable to demonstrate an association between RAPL and survival outcomes or LN yield, its clinical implications are important. Only one previous study used a methodology comparable to ours, but in a cohort of patients where CVL was routinely intended to be performed, and found a survival benefit with a shorter RAPL, specifically, where CVL was verified[81]. The fact that our study was unable to replicate those results in a cohort of non-routine CVL implies the benefits of a shorter RAPL may only be reaped when a CVL is performed (RAPL≤10mm), and that likely, CVL is an 'all or nothing phenomenon'. Furthermore, the lack of association between RAPL and LN yield serves to emphasise the continuing importance of comprehensive pathology assessment of resected specimens, of which accurate nodal count remains highly relevant to cancer staging and guiding the need for adjuvant therapy.

This prospective study has several limitations. In patients who underwent a RH for a hepatic flexure malignancy, the ICA was regarded as the principal vascular pedicle to standardise the identification of a single pedicle radiologically, despite other pedicles sharing lymphatic drainage in this region. As not all patients had their heights recorded in the database, we were unable to adjust RAPL measurements according to the patients' BMIs. Additionally, in a unit where CVL was not routinely practiced, it is highly probable that aside from simply sweeping the central nodes up into the resection margin of the specimen without a high tie of the vessel, CVL was selectively performed in cases where central lymphadenopathy was suspected. This scenario presents a potential for introducing selection bias. However, the well-powered and large cohort size, long study duration, application of standardized surgery by specialist colorectal surgeons following anatomical planes, and routine detailed generic pathology reporting, are strengths of our study. Additionally, the inclusion of urgent operations and minimally and maximally invasive surgical approaches improves the generalizability of our study.

In summary, this study is the first to demonstrate the feasibility of measuring RAPL using surveillance CTs following non-routine CVL surgery for CRC in AR and RH patients. The null association between RAPL and survival outcomes questions the role of RAPL as a quality marker for CRC surgery in patients in whom CVL is not intended. The role of RAPL measurement may be confined to *post hoc* verification that CVL surgery has been performed, as this appears to be where survival benefits have been documented. Otherwise, accurate and structured pathology assessment of resected specimens remains crucial for disease prognostication and guidance of the need for adjuvant therapy in patients who have had a potentially curative operation for CRC.

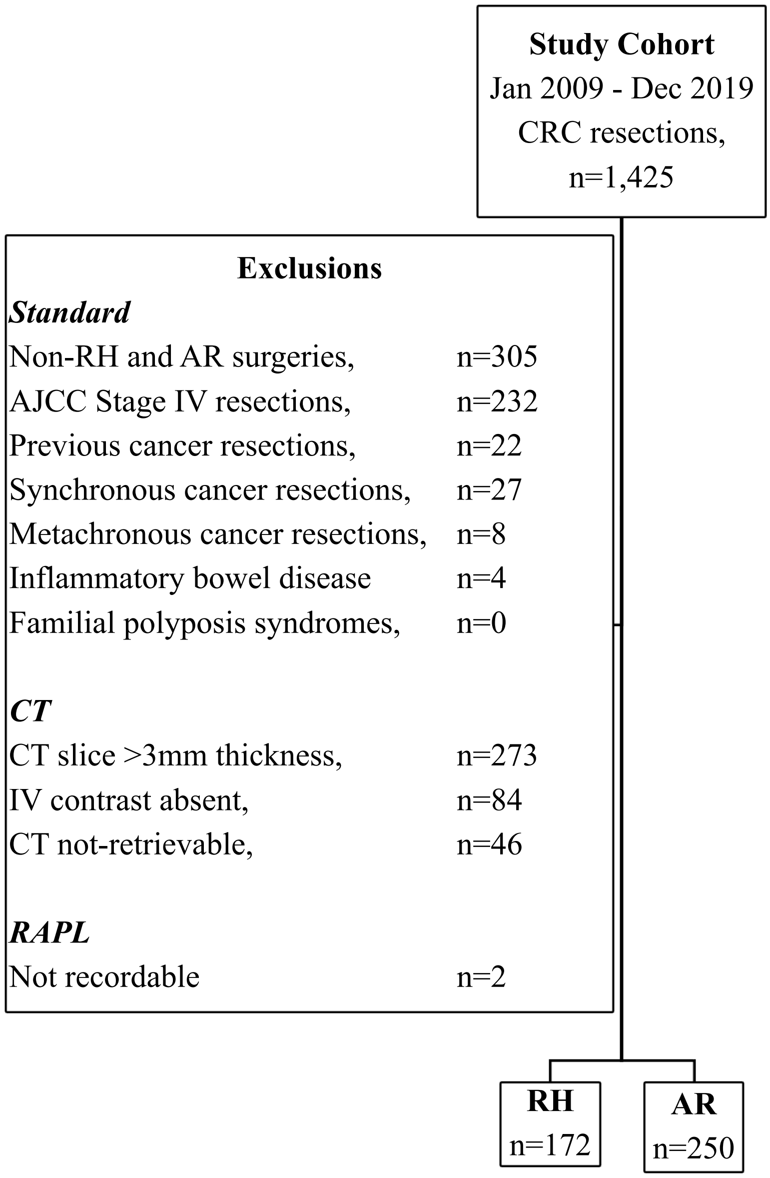


Figure 3: Flow diagram of cohort definition.

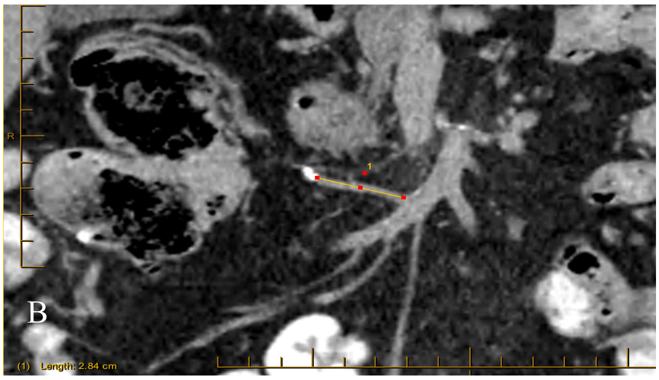
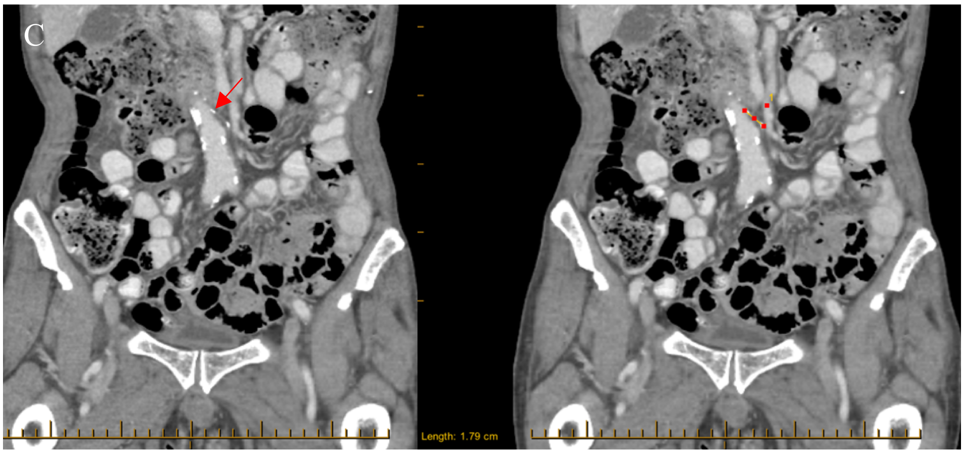


Figure 4: A - Coronal volume-rendered reformatted CT image of the IMA RAPL, defined by its termination at a surgical clip; B – Multi-planar reconstructed (MPR) CT image with ICA RAPL defined and measured in the coronal plane; C – MPR CT image with IMA RAPL defined (red arrow) and measured in the coronal plane. Surgical clip (B) and granuloma (C) denoted by hyper-density at maximal limits of measurement.



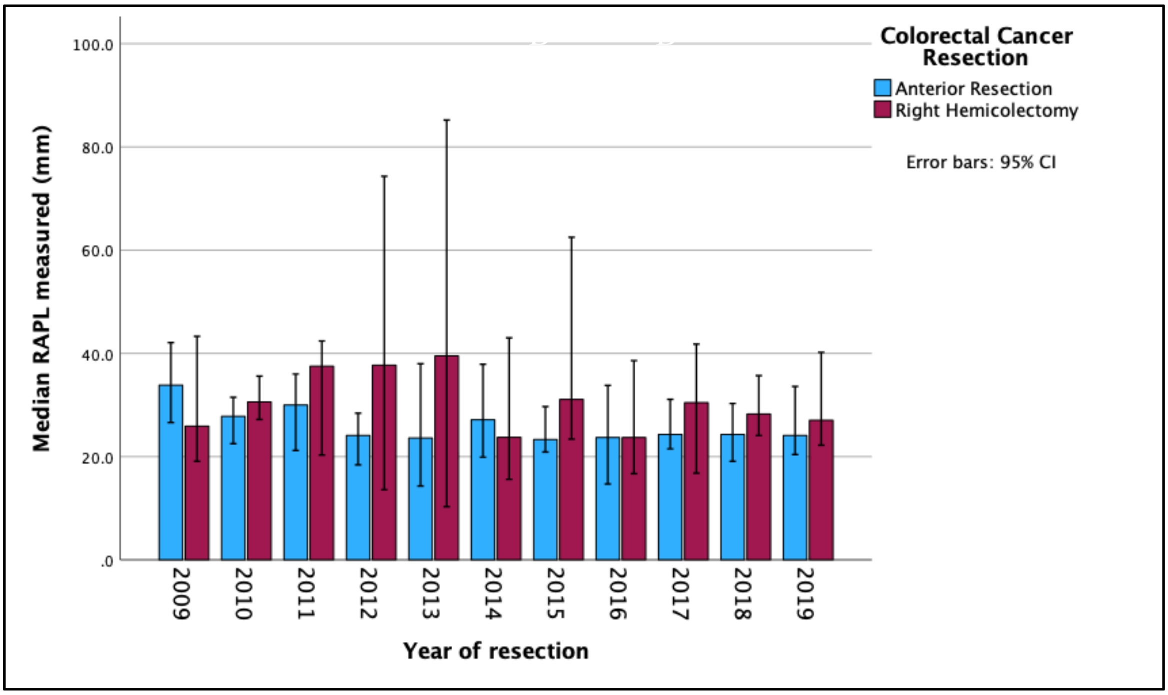
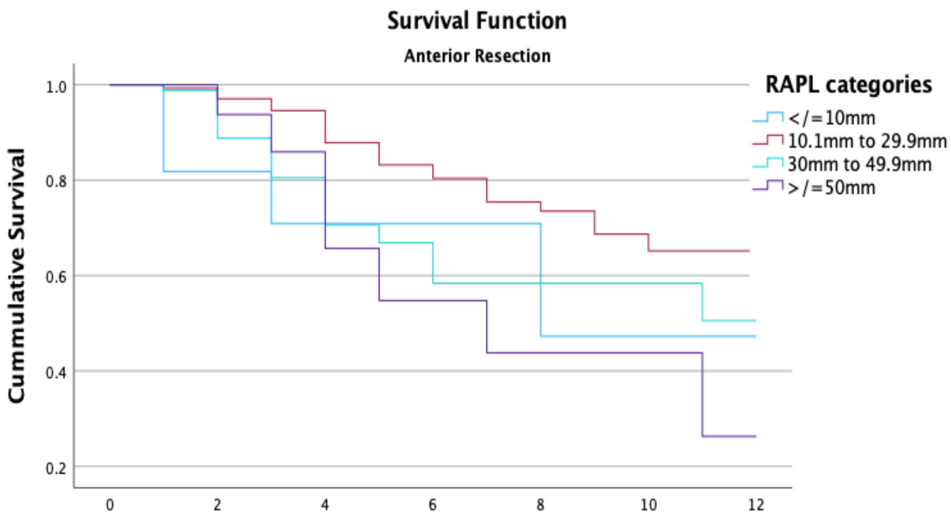


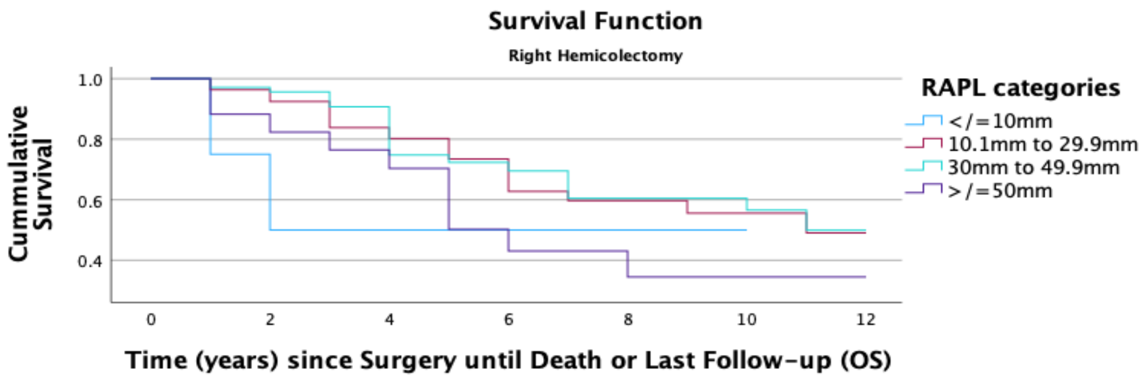
Figure 5: Trend of RAPLs over the 11-year study period in patients undergoing an AR or RH for CRC.



Number at Risk (Censored)

≤10mm	11(0)	8(1)	5(1)	3(0)	2(1)	1(0)	NR
10.1mm to 29.9mm	140(0)	128(20)	83(14)	52(7)	36(11)	15(2)	3(3)
30mm to 49.9mm	82(0)	70(12)	40(5)	26(4)	19(3)	9(3)	2(2)
≥ 50mm	17(0)	14(4)	6(0)	5(0)	4(1)	3(1)	1(1)

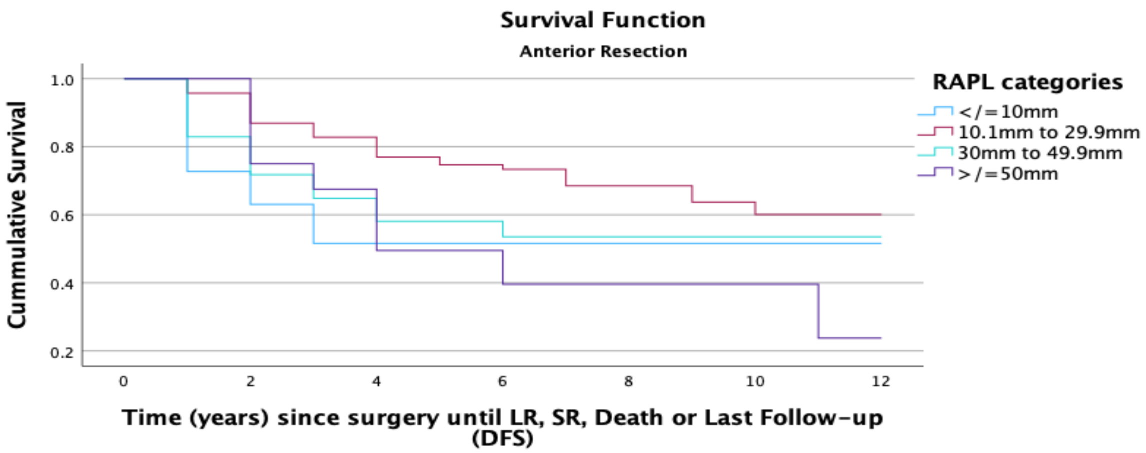
Figure 6A: AJCC Stage III Subgroup Overall Survival Analysis – AR



Number at Risk (Censored)

≤10mm	4(0)	2(0)	2(0)	1(0)	1(0)	NR	NR
10.1mm - 29.9mm	82(1)	69(10)	39(7)	22(3)	16(3)	10(3)	3(3)
30mm - 49.9mm	69(0)	64(11)	33(5)	24(2)	17(1)	14(11)	NR
≥ 50mm	17(0)	14(0)	11(1)	6(1)	4(1)	2(1)	NR

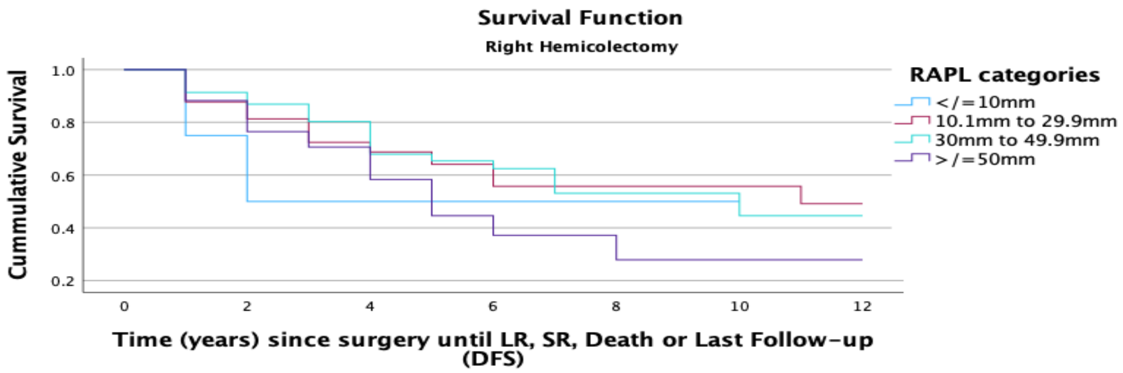
Figure 6B: AJCC Stage III Subgroup Overall Survival Analysis – RH



Number at Risk (Censored)

≤10mm	11(0)	6(1)	3(1)	1(0)	1(0)	1(0)	NR
10.1mm -29.9mm	140(0)	114(17)	73(10)	49(7)	34(11)	13(2)	3(3)
30mm - 49.9mm	82(0)	57(11)	32(5)	22(3)	17(3)	7(3)	2(2)
≥50mm	17(0)	11(2)	5(0)	6(0)	4(1)	3(1)	1(1)

Figure 6C: AJCC Stage III Subgroup Disease-Free Survival Analysis – AR



Number at Risk (Censored)

≤10mm	4(0)	2(0)	2(0)	1(0)	1(0)	NR	NR
10.1mm - 29.9mm	82(1)	60(10)	33(7)	19(3)	14(3)	10(3)	3(3)
30mm - 49.9mm	69(0)	58(11)	29(5)	21(2)	14(1)	10(8)	NR
≥ 50mm	17(0)	13(0)	9(1)	5(1)	3(0)	2(1)	NR

Figure 6D: AJCC Stage III Subgroup Disease-Free Survival Analysis – RH

TABLE 4. Comparison of Clinicopathological Factors Between Patients with AR and RH			
Variables	**Total (%) or Mean (SD) or Median (Range/IQR) (n=422)**	**Anterior Resection (n=250)**	**Right Hemicolectomy (n=172)**
RAPL (Median [IQR]), mm*	27.2(19.8-38.1)	26.4(19.2-34.3)	29.8(21.6-40.9)
CVL (RAPL < 10mm)			
No	407(96.4)	239(95.6)	168(97.7)
Yes	15(3.6)	11(4.4)	4(2.3)
Gender			
Male	228(54.0)	144(57.6)	84(48.8)
Female	194(46.0)	106(42.4)	88(51.2)
Age (Mean [SD]), years	69.0(12.3)	66.3(12.1)	72.8(11.4)
BMI (Mean [SD]), kg/m^2	27.5(5.8)	27.3(5.9)	27.9(5.6)
ASA Grade			
I	61(14.4)	44(17.6)	17(9.9)
II	229(54.3)	138(55.2)	91(52.9)
III/IV	132(31.3)	68(27.2)	64(37.2)
Duration of Operation (Median [IQR]), mins	221.0(171.5-274.5)	252.0(215.0-302.0)	169.0(143.75-201.0)
Emergency Operation			
No	396(93.8)	235(94.0)	161(93.6)
Yes	26(6.2)	15(6.0)	11(6.4)
Operation Modality			
Open	81(19.2)	57(22.8)	24(14.0)
Laparoscopy	341(80.8)	193(77.2)	148(86.0)
Procedure Conversion			
No	312(91.5)	173(89.6)	139(93.9)
Yes	29(8.5)	20(10.4)	9(6.1)
Blood Loss (mls)			
≤500	408(96.7)	239(95.6)	169(98.3)
>500	14(3.3)	11(4.4)	3(1.7)
Tumour Stage (TNM AJCC)			
Node Negative (Stage I and II)	184(43.6)	104(41.6)	80(46.5)
Node Positive (Stage III)	238(56.4)	146(58.4)	92(53.5)
Tumour Size (Median [IQR]), mm	4.0(3.0-5.0)	3.8(2.9-5.0)	4.5(3.1-6.4)
Tumour Perforation			
No	417(98.8)	247(98.8)	170(98.8)
Yes	5(1.2)	3(1.2)	2(1.2)
Histological Type			
Non-Mucinous or Signet ring	381(90.3)	237(94.8)	144(83.7)
Mucinous or Signet ring	41(9.7)	13(5.2)	28(16.3)
Histological Differentiation			
Well or Moderate	365(86.5)	235(94.0)	130(75.6)
Poor	57(13.5)	15(6.0)	42(24.4)
Histological Grade			
Low or Average	361(85.5)	234(93.6)	127(73.8)
High	61(14.5)	16(6.4)	45(26.2)
Lympho-Vascular Invasion			
No	291(69.0)	173(69.2)	118(68.6)
Yes	131(31.0)	77(30.8)	54(31.4)
Peri-Neural Invasion			
No	310(73.5)	170(68.0)	140(81.4)
Yes	112(26.5)	80(32.0)	32(18.6)
Number of Lymph Nodes Examined (Median [Range])	19(4-65)	19(4-65)	20(6-50)
Lymph Node Harvest (<12)			
No	388(91.9)	225(90.0)	163(94.8)
Yes	34(8.1)	25(10)	9(5.2)
Time to surveillance CT from index operation (Median [IQR), months	11.3 (7.3-16.7)	11.4(7.3-17.7)	11.0(7.1-14.7)
LOS (Median [Range]), days	7(2-77)	7(2-66)	6(3-77)

RAPL: **Residual Arterial Pedicle Length;** ***CVL:*** **Central Vascular Ligation;** ***ASA:*** **American Society of Anesthesiology;** ***TNM:*** **8th edition tumour, nodes, and metastasis staging system;** ***AJCC:*** **American Joint Committee on Cancer; and** ***LOS:*** **Length of Stay**

*** A longer RAPL was noted in those who underwent RH surgery (P=0.02)**

Table 4: Clinical characteristics and histopathological descriptions of AR and RH patients.

TABLE 5. Comparison of Oncological Outcomes Between Clinicopathological Features of AR and RH Patients								
	Overall Survival				Disease-Free Survival			
	Anterior Resection		Right Hemicolectomy		Anterior Resection		Right Hemicolectomy	
	HR (95%CI)	***P*-value**	**HR (95%CI)**	***P*-value**	**HR (95%CI)**	***P*-value**	**HR (95%CI)**	***P*-value**
RAPL (mm)	1.01(0.99-1.02)	0.14	1.01(0.99-1.03)	0.15	1.01(0.99-1.02)	0.26	1.01(0.99-1.03)	0.12
CVL								
No	ref	-	ref	-	ref	-	ref	-
Yes	1.70(0.62-4.67)	0.31	1.96(0.48-8.04)	0.35	1.87(0.76-4.61)	0.18	1.56(0.38-6.38)	0.54
Gender								
Male	ref	-	ref	-	ref	-	ref	-
Female	0.57(0.34-0.95)	0.03	0.66(0.39-1.12)	0.12	0.63(0.41-0.99)	0.045	0.77(0.48-1.24)	0.77
Age (years)	1.05(1.03-1.08)	<0.001	1.05(1.02-1.08)	0.001	1.03(1.01-1.05)	0.003	1.03(1.01-1.06)	0.01
BMI (kg/m^2)	1.01(0.97-1.05)	0.78	0.99(0.94-1.04)	0.61	1.01(0.98-1.04)	0.55	0.99(0.95-1.04)	0.81
ASA Grade								
I	ref	-	ref	-	ref	-	ref	-
II	2.15(0.90-5.15)	0.09	0.99(0.34-2.90)	0.98	1.99(0.94-4.25)	0.07	1.43(0.54-4.35)	0.43
III/IV	6.29(2.59-15.28)	<0.001	3.13(1.11-8.86)	0.03	4.94(2.27-10.73)	<0.001	3.14(1.11-8.86)	0.03
Emergency Operation								
No	ref	-	ref	-	ref	-	ref	-
Yes	1.31(0.47-3.60)	0.61	1.42(0.51-3.92)	0.50	1.06(0.43-2.62)	0.90	1.43(0.58-3.57)	0.44
Operation Modality								
Open	ref	-	ref	-	ref	-	ref	-
Laparoscopy	0.61(0.37-1.01)	0.05	0.39(0.22-0.71)	0.002	0.66(0.42-1.03)	0.07	0.27(0.16-0.46)	<0.001
Intraoperative Blood Loss (mls)								
≤500	ref	-	ref	-	ref	-	ref	-
>500	1.56(0.62-3.91)	0.34	9.77(2.23-42.85)	0.003	1.34(0.54-3.32)	0.53	4.19(1.00-18.48)	0.049
Tumour Stage (TNM)								
Stage I/II	ref	-	ref	-	ref	-	ref	-
Stage III	2.08(1.21-3.58)	0.008	1.57(0.91-2.68)	0.10	1.80(1.14-2.85)	0.01	1.89(1.15-3.10)	0.01
Tumour Size (cm)	1.13(0.98-1.30)	0.09	1.08(1.01-1.15)	0.02	1.16(1.03-1.30)	0.02	1.11(1.06-1.17)	<0.001
Histological Type								
Non-Mucinous/Signet Ring	ref	-	ref	-	ref	-	ref	-
Mucinous/Signet Ring	1.21 (0.44-3.33)	0.71	1.91 (1.02-3.56)	0.04	2.04(0.94-4.42)	0.07	1.83(1.03-3.25)	0.04
Histological Differentiation								
Well or Moderate	ref	-	ref	-	ref	-	ref	-
Poor	2.31(1.05-5.05)	0.04	1.21(0.66-2.22)	0.54	2.13(1.03-4.41)	0.04	1.37(0.80-2.35)	0.26
Histological Grade								
Low or Average	ref	-	ref	-	ref	-	ref	-
High	3.00(1.43-6.30)	0.004	1.18(0.65-2.13)	0.58	3.07(1.58-5.95)	<0.001	1.32(0.77-2.24)	0.31
Lympho-Vascular Invasion								
No	ref	-	ref	-	ref	-	ref	-
Yes	1.70(1.04-2.78)	0.03	2.18(1.27-3.74)	0.005	1.69(1.10-2.60)	0.02	2.91(1.78-4.75)	<0.001
Peri-Neural Invasion								

No Yes	ref 2.21(1.36-3.58)	- 0.001	ref 1.18(0.63-2.24)	- 0.61	ref 2.51(1.63-3.84)	- <0.001	ref 1.59(0.91-2.80)	- 0.10
Number of Lymph Nodes Examined	0.98(0.95-1.01)	0.15	0.97(0.94-1.00)	0.08	0.99(0.97-1.02)	0.57	0.98(0.95-1.01)	0.20
***HR:* Hazard Ratio*; RAPL:* Residual Arterial Pedicle Length; *CVL:* Central Vascular Ligation; *ASA:* American Society of Anesthesiology; *TNM: 8th* edition tumour, nodes, and metastasis staging system; and *AJCC:* American Joint Committee on Cancer**								

Table 5: Comparison of Survival Outcomes between Clinicopathological Characteristics in AR and RH patients.

TABLE 6A. Comparison of Correlations Between Clinicopathological Features and RAPLs – Univariate Analyses				
	Residual Arterial Pedicle Length (mm)			
	Anterior Resection (n=250)		**Right Hemicolectomy (n=172)**	
	β (95% CI)	***P*-value**	**β (95% CI)**	***P*-value**
Year of Operation (year)	-0.59(-1.13 to -0.06)	0.03	-0.54(-1.20 to 0.13)	0.11
Gender Male Female	-4.71(-8.46 to -0.96)	0.01	-6.87 (-11.29 to -2.45)	0.002
Age (years)	0.06 (-0.09 to 0.22)	0.43	0.27 (0.07 to 0.47)	0.008
BMI (kg/m^2)	0.34 (0.14 to 0.66)	0.04	0.30 (-0.12 to 0.71)	0.16
ASA Grade I II III/IV	3.66 (0.87 to 6.46)	0.01	5.26 (1.75 to 8.78)	0.004
Emergency Operation No Yes	3.43 (-4.46 to 11.32)	0.39	3.90 (-5.36 to 13.16)	0.41
Operation Modality Open Laparoscopy	-9.45 (-13.77 to -5.14)	<0.001	-7.75 (-14.20 to -1.31)	0.02
Intraoperative Blood Loss (mls) ≤500 >500	3.43 (-5.71 to 12.57)	0.46	10.95 (-6.31 to 28.21)	0.21
Tumour Stage (TNM AJCC) Stage I/II Stage III	-0.06 (-3.86 to 3.75)	0.98	-0.26(-4.81 to 4.29)	0.91
Tumour Size (cm)	0.29 (-0.72 to 1.31)	0.57	0.04 (-0.65 to 0.73)	0.91
Histological Type Mucinous or Signet Ring Non-Mucinous or Non-Signet Ring	2.40 (-6.08 to 10.81)	0.58	6.05 (-0.03 to 12.13)	0.05
Histological Differentiation Well or Moderate Poor	-3.02(-10.92 to 4.87)	0.45	0.35 (-4.93 to 5.63)	0.90
Histological Grade High Low or Average	-0.61(-8.27 to 7.06)	0.88	1.88(-3.28 to 7.04)	0.47
Lympho-Vascular Invasion No Yes	-0.55 (-4.62 to 3.51)	0.79	-2.21 (-7.09 to 2.67)	0.37
Peri-Neural Invasion No Yes	0.66 (-3.37 to 4.68)	0.75	-1.17 (-7.00 to 4.66)	0.69
Number of Lymph Nodes Examined	-0.07 (-0.28 to 0.15)	0.54	-0.20 (-0.48 to 0.08)	0.16
Time to Surveillance CT from Index Operation	0.05 (-016 to 0.25)	0.66	-0.18 (-046 to 0.09)	0.19
LOS (days)	0.11 (-0.14 to 0.35)	0.38	0.07 (-0.16 to 0.30)	0.56
***RAPL:* Residual Arterial Pedicle Length; *CVL:* Central Vascular Ligation; *ASA:* American Society of Anesthesiology; *TNM: 8th* edition tumour, nodes, and metastasis staging system; *AJCC:* American Joint Committee on Cancer; *CT:* Computed Tomography, and *LOS*: Length of Stay.**				

Table 6A: Uni-Variate Analyses of Clinicopathological Characteristics and RAPL Associations in AR and RH patients.

TABLE 6B. Comparison of Correlations Between Clinicopathological Features and RAPLs – Multi-Variate Analyses				
	Residual Arterial Pedicle Length (mm)			
	Anterior Resection (n=250)		**Right Hemicolectomy (n=172)**	
	aβ (95% CI)	***P*-value**	**aβ (95% CI)**	***P*-value**
Year of Operation (year)	-0.23(-0.82 to 0.35)	0.44		
Gender Male Female	-3.49(-7.23 to 0.24)	0.07	*-5.67 (-10.05 to -1.29)*	*0.01*
Age (years)			0.16 (-0.05 to 0.37)	0.14
BMI (kg/m^2)	0.22 (-0.10 to 0.54)	0.17		
ASA Grade I II III/IV	*3.08 (0.14 to 6.03)*	*0.04*	2.94 (-0.89 to 6.77)	0.13
Operation Modality Open Laparoscopy	*-8.70 (-13.51 to -3.89)*	*<0.001*	-4.91 (-11.30 to 1.48)	0.13
RAPL: **Residual Arterial Pedicle Length and** ***ASA:*** **American Society of Anesthesiology**				

Table 6B: Multi-Variate Analyses of Clinicopathological Characteristics and RAPL Associations in AR and RH patients.

TABLE 7. Oncological Associations Between AJCC Stage III Patients and RAPL (N=238)								
	Overall Survival				**Disease-Free Survival**			
	Anterior Resection		**Right Hemicolectomy**		**Anterior Resection**		**Right Hemicolectomy**	
	HR (95%CI)	***P***	**HR (95%CI)**	***P***	**HR (95%CI)**	***P***	**HR (95%CI)**	***P***
RAPL (mm)	1.03(1.00-1.05)	0.04	1.01(0.99-1.03)	0.27	1.02(0.99-1.04)	0.10	1.01(0.99-1.02)	0.64
HR: **Hazard Ratio** ***and RAPL:*** **Residual Arterial Pedicle Length**								

Table 7: AJCC Stage III Subgroup Analysis.

CHAPTER III: Tissue Morphometric Measurements Do Not Predict Survival Following Colorectal Cancer Surgery

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Preamble

In addition to the novel marker of surgical quality identified with the use of RAPL, some studies have investigated the impact of *ex vivo* TM measurements (e.g., area of mesentery excised) on clinico-pathological outcomes following CRC surgery, raising the possibility of this being a novel histopathological EoL metric by which quality of CRC surgery may be assessed[4,84,200]. However, successful application of these measurements for RC resections have been inconsistently reported[81,85]. Moreover, the prognostic significance of *ex vivo* TM measurements with respect to its influence on survival outcomes remains unknown, precluding its potential use as a reliable quality metric in CRC surgery[81,84,85]. These aspects will be explored further in **Chapter III**.

ABSTRACT

INTRODUCTION: *Ex vivo* TM measurements have been proposed as a quality marker for CRC surgery. However, their survival associations require clarification. This study aimed to evaluate the feasibility of capturing TM measurements based on *ex vivo* fresh specimen images and explore the association between these TM measurements and survival outcomes.

METHODS: A prospective cohort study at Concord Hospital, Sydney was conducted with Stage I to III CRC patients (2009-2019) who underwent an AR or RH. Using high-resolution digital photographs of fresh CRC specimens, *ex vivo* TM measurements - resected mesentery area (TM A), distances from HVT to tumour (TM B) and bowel wall (TM C), and bowel length (TM D) - were recorded using Image J. OS and DFS estimates and their associations to clinicopathological variables were investigated with Kaplan–Meier and Cox regression analyses. Linear regression models tested association between TM measurements and LNY.

RESULTS: Of the 1,425 patients who underwent CRC surgery, TM measurements were performed on 312 patients, with an average age of 69.4 years (SD 12.3), of whom 52.9% were male. The majority had an AR (57.8%). Among AR patients, a 5-year OS rate of 77.4% and a DFS rate of 70.1% were observed, with TM measurements bearing no relationship to survival outcomes. Similarly, RH patients exhibited a 5-year OS rate of 67.2% and a DFS rate of 63.1%, with TM measurements again showing no association with survival. Only TM D (P=0.02) measurements were associated with the number of LNs examined.

CONCLUSION: This study successfully demonstrates the feasibility of measuring TM measurements on photographs of *ex vivo* fresh specimens following CRC surgery. The lack of association with survival outcomes questions the utility of TM measurements as a quality metric of CRC surgery.

INTRODUCTION

CRC surgery is based on the principles of completely excising the primary tumour with clear margins, preserving the integrity of the lympho-vascular package along an avascular embryological (i.e., mesorectal or mesocolic) plane, and performing an adequate lymphadenectomy[12,13]. An array of metrics, described according to the structure, process and outcome framework, has been developed to assess the quality of CRC surgery holistically[4,11,18,200]. While substantial emphasis has been placed on surgical margins and plane of excision as process metrics, discussions regarding metrics that define the EoL have been less comprehensive[11,19]. Most studies have focused on assessment of LNY as a single marker of EoL[11].

More recently, studies have explored the impact of *ex vivo* TM measurements (e.g., area of mesentery excised) on clinico-pathological outcomes following CRC surgery, raising the possibility of this being a novel histopathological metric by which quality of CRC surgery may be assessed[4,84,200]. Our group has recently published the feasibility of performing *ex vivo* TM measurements using routine formalin fixed pathology specimen images post-CC surgery[84]. However, successful application of these measurements for RC resections have been inconsistently reported[81,85]. Moreover, the prognostic significance of *ex vivo* TM measurements with respect to its influence on survival outcomes remains unknown, precluding its potential use as a reliable quality metric in CRC surgery[81,84,85].

Therefore, the aim of this study was (i) to validate feasibility of *ex vivo* TM measurements using fresh pathology specimen images of CC and RC resections; and (ii) investigate the association between *ex vivo* TM measurements and survival outcomes. We hypothesised that TM measurements would be independently associated with patient survival.

MATERIAL and METHODS

A prospective observational cohort study was performed of patients who underwent a resection for a solitary primary CRC. Patients included were those who had any form of an AR (i.e., high or low) or a RH operation performed between January 2009 and December 2019 at CRGH, Sydney. These patients were identified from a prospectively maintained institutional database that has been in continual existence since 1971[227,228]. Patients with AJCC Stage IV cancer, synchronous or metachronous cancer, inflammatory bowel disease, or polyposis coli, were excluded (Figure 7). Ethical approval (2020/ETH03325 and CH62/62011-136-P Chapuis HREC/11/CRGH206) was granted by the Sydney Local Health District Ethics Committee, with included patients consenting for the use of their data and tumour specimens for research.

Surgical Procedures

The standard approach of our unit in performing an AR and RH operation has been previously described[226]. In our unit, routine exposure of the SMA or SMV and abdominal aorta is not performed.

Clinicopathological Variables of Interest – *ex vivo* TM measurements from fresh specimen images (Figure 8)

Four new variables were prospectively measured and recorded. These were based on routine photographs of fresh resected specimens, stored as high-resolution digital images. Each specimen was photographed from both anterior and posterior viewpoints, with the mesentery presented flat, without stretching. Each image was carefully calibrated with an included metric scale. As previously described, these calibrated images were then used to accurately determine several key measurements: the area of the resected mesentery (TM A, cm^2), the distance from the HVT to the tumour's centre (TM B, cm) and its nearest bowel wall (TM C, cm), and the length of the bowel segment removed (TM D, cm)[84]. If there was more than one artery supplying the tumour, the shortest was defined as TM B. These measurements were performed by KN using *Image J* (NIH, Maryland, USA)[84], blinded to patient outcomes. Importantly, patients whose specimens were photographed post formalin-fixation were excluded from analysis (Figure 7). *Image J* is a Java-based image processing program that can display, edit, and analyse a wide range of image types[243]. It features tools and supports simultaneous processing of multiple images for statistical analysis and measurements[243].

To understand our TM measurement data in the context of other published studies, we compared our measurements with those from resections performed at SJUH and UHE, which utilized an identical protocol for TM measurements[200].

Standard Clinicopathological Variables

The extraction of standard clinico-pathological data—including clinical information, operative details, tumour pathology, and follow-up data—as well as the details of pathology reporting and staging for adenocarcinomas (such as mucinous and signet ring variants), have been outlined in earlier publications[227,228]. These data included the LNY from each specimen; fat clearance techniques were not employed in node retrieval.

Surveillance and Follow-up

Patients underwent reviews at a minimum of every six months for the initial two years post-resection and were subsequently followed up on an annual basis either until their death or December 2021, barring instances of lost follow-up[232]. The surveillance protocol combined clinical examinations, laboratory tests, and advanced imaging, with periodic colonoscopies and multidisciplinary reviews for high-risk patients to evaluate adjuvant chemotherapy options[180].

The indications for post-operative adjuvant chemotherapy were routinely considered in a multidisciplinary setting for all patients, considering factors such as age, patient preferences, presence of comorbidities, adverse tumour pathological features, social circumstances, and best practice guidelines[180,232].

The follow-up period commenced from the date of resection. Follow-up times were censored at last contact for patients who did not experience the terminal event up to December 2021, who were lost to follow-up, or who remained alive. Death dates were primarily determined from the records of the patient's surgeon, family physician, or hospital. In a limited number of cases, this information was sourced from the national death registration system[233]. The primary cause of death was classified per the International Classification of Diseases-10. All clinical and surgical data were recorded by one of our team members (PC).

Outcome Measures

The primary outcome measures included:

(i) OS, defined as the time span from the date of resection to the date of death from any cause, with data censored at the last known contact for patients still alive[235]; and

(ii) DFS, which refers to the time period following CRC resection during which a patient remains alive and shows no signs of disease recurrence[174,234,235].

Statistical Analyses

The study population for this study was defined based on the period where photographs of fresh pathology specimens were routinely available. From January 2009 onwards, pathology specimens in our unit have been exclusively photographed in their fresh state. In the absence of pilot data, a sample size calculation was not performed. Continuous variables were reported as mean (SD) for normally distributed variables and as median (IQR or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages.

Survival estimates were modelled using the Kaplan-Meier function with log-rank tests performed to determine differences in survival distributions. Cox-regression modelling tested for associations between outcome measures and relevant clinicopathological variables, including *ex vivo* TM measurements. Linear regression models tested associations between TM measurements and LNY. The level for 2-tailed statistical significance was $P<0.05$ with confidence intervals at the 95% level. All the analyses were carried out using SPSS® v.29 (IBM, New York, USA).

AJCC Stage III Sub-group Analysis

It was anticipated that an increased EoL would provide the most significant survival advantage for patients undergoing CRC surgery for AJCC Stage III disease. A sub-group analysis was therefore conducted on this population, along with a separate Cox regression survival analysis.

RESULTS

Study Population

Some 1,425 patients underwent a resection for a CRC during the study period of which 1,113 patients were sequentially excluded (Figure 7) leaving 312 patients suitable for analysis. In these patients, 165 (52.9%) were male, the mean age was 69.4years (SD 12.3), the mean BMI was 27.6kg/m^2 (SD5.4), 179 patients had an ASA grade of II (57.4%), and the median hospital LOS was 7 days (range 2-66). An urgent operation was performed in nine patients (2.9%). Of those operated urgently, eight patients (88.9%) were obstructed. An open operation was performed in 47 patients (15.1%). Of the 265 patients (84.9%) managed with a laparoscopic operation, conversion to open surgery was required in 19 (7.2%).

Of the patients studied, 181 (57.8%) underwent an AR procedure and 131 (42.2%) had a RH operation. In those who had an AR procedure, the following mean TM measurements were recorded: TM A – 164.4cm^2 (SD 69.1), TM B – 14.1cm (SD 4.9), TM C – 9.9cm (SD 3.9), and TM D – 27.7cm (SD 9.2). Comparatively, in patients who had RH surgery the mean TM measurements were: TM A – 108.4cm^2 (SD 49.0), TM B – 10.7cm (SD 3.0), TM C – 7.3cm (SD 2.4), and TM D – 24.7cm (SD 9.4). The comparisons of these measurements with those previously documented at SJUH and UHE are presented in Table 8. Detailed clinical characteristics, histopathological descriptions, and *ex vivo* TM measurements for both the AR and RH cohorts are presented in Table 9.

Comparison of Survival Outcomes between Clinico-pathological Characteristics in AR and RH patients (Table 10)

Table 10 summarises the associations between clinico-pathological characteristics, including *ex vivo* TM measurements, and survival outcomes in patients who underwent either an AR or RH resection.

Anterior Resection

Amongst those who underwent an AR, death occurred in 42 patients (23.2%). The 5-year OS and DFS rates were 77.4% (95%CI 73.7-81.1) and 70.1% (95%CI 66.3-73.9) respectively. A LR was diagnosed in nine patients (5.0%), while SR was identified in 34 patients (18.8%). The median time to LR and SR was 1.3 years (95%CI 1.2-1.4) and 1.5 years (95%CI 1.2-1.8), respectively.

None of the *ex vivo* TM measurements were associated with OS or DFS. With respect to other clinico-pathological characteristics, poorer OS was associated with increasing age (P=0.004), ASA grade greater than II (P=0.001), AJCC Stage III tumours (P=0.03) and those with PNI (P<0.001). Similarly, poor prognosis characterised by increased DFS hazards mirrored all the factors above.

Right Hemicolectomy

Amongst the RH patients studied, death occurred in 47 patients (35.9%). The median OS and DFS was 7.7 years (95% CI 4.0-11.3) and 6.8 years (95% CI 3.2-10.4), respectively. The 5-year OS and DFS rates were 67.2% (95%CI 62.3-72.1) and 63.1% (95%CI 58.3-67.9) respectively. A LR was diagnosed in three patients (2.3%), while SR was seen in 24 patients (18.3%). The median time to LR and SR was 2.8 years (95%CI 0.3-5.2) and 1.7 years (95%CI 0.6-2.7), respectively.

Similar to the AR cohort, none of the *ex vivo* TM measurements for RH surgery patients were associated with OS or DFS. Clinically, poorer OS was associated with an increasing age (P=0.002) and patients with ASA grade greater than II (P=0.02). Pathologically, poorer OS was noted in tumours that were AJCC Stage III (P=0.02), harbouring a mucinous or signet-ring pathology (P=0.03), had LVI (P<0.001) or PNI (P=0.004). Conversely, a longer OS was associated with patients who underwent laparoscopic surgery (P<0.001).

Regarding DFS, factors associated with a poorer prognosis included an increasing age (P=0.006), an ASA grade greater than II (P=0.02), AJCC Stage III tumours (P=0.02), the presence of mucinous or signet-ring pathology (P=0.03), and tumours harbouring LVI (P<0.001) or PNI (P<0.001). Meanwhile, patients who underwent laparoscopic surgery were associated with a reduced DFS hazard (P<0.001).

AJCC Stage III Sub-group Analysis (Table 11)

There were 133 AJCC Stage III patients who had TM measurements recorded. Of these, 80 had an AR and 53 had a RH. In those who had an AR procedure, the following mean TM measurements were recorded: TM A – 163.5cm^2 (SD 67.3), TM B – 13.8cm (SD 5.1), TM C – 9.8cm (SD 4.0), and TM D – 27.5cm (SD 9.3). Comparatively, in patients who had RH surgery the mean TM measurements were: TM A – 106.4cm^2 (SD 55.8), TM B – 10.6cm (SD 3.1), TM C – 6.9cm (SD 2.5), and TM D – 25.9cm (SD 10.7).

The survival associations of the sub-cohort are summarised in Table 11. In both AR and RH sub-cohorts, none of the *ex vivo* TM measurements were associated with OS or DFS.

Associations between *ex vivo* TM measurements and Lymph Node Yield following AR and RH operations (Table 12)

Table 12 summarises the associations between *ex vivo* TM measurements and the LNY of all study patients.

With each additional unit of bowel resection length, there was a corresponding increase in the number of LNs examined, as indicated by the TM D (length of resected bowel) index (β 0.11 [95% CI 0.02-0.20; P=0.02]). No significant associations were observed between LNY and other TM indices.

DISCUSSION

In a previous study, we demonstrated the feasibility of performing *ex vivo* TM measurements using routine photographs of fixed CRC specimens, and explored surgical, patient, and disease factors associated with these measurements. Our present prospective study builds on that work by investigating the association between such TM measurements and survival outcomes, applied to a cohort of CC and RC resections spanning an eleven-year period. By determining their prognostic significance, this study sought to confirm or deny the utility of *ex vivo* TM measurements as a quality metric of 'good CRC surgery'.

Practically, assessment of the quality of CRC surgery encompasses two key considerations: (i) the plane of excision, and (ii) the EoL. While both elements are presumed to have equal significance for patient outcomes, much of the initial research in assessing surgical quality concentrated on the correct plane of excision to ensure intactness of the enveloping mesorectal or mesocolic fascia and avoidance of tumour transection. For instance, the MRC CR07 trial revealed that 3-year LR rates were estimated at 4%, 7%, and 13% for mesorectal, intramesorectal, and muscularis propria dissections, respectively[19]. Also, when in the mesocolic plane, a 15% survival advantage at 5 years is conferred, compared to operating in the muscularis propria plane, with the benefit peaking to 27% in patients diagnosed with Stage III disease[4].

The twin consideration for surgical quality - EoL - has received relatively less attention. The evaluation of the EoL in CRC surgery is traditionally based on LN harvest, with a nodal count of at least twelve generally considered adequate for staging purposes[21,237]. However, relying solely on LNY as a marker of EoL has its limitations. Firstly, the assessment of LNY depends on the thoroughness of the pathologist[200]. Furthermore, the increasing use of neoadjuvant therapy may lead to a reduction in the number of nodes excised[25,26]. Despite these challenges, the growing focus on CVL signifies a shift in assessing the EoL aspect of CRC surgery quality given the emphasis on high tie of the parent pedicle[12,81,84]. Therefore, when *ex vivo* analysis of CVL specimens are performed, TM measurements of resected specimens have revealed greater area of mesentery resected, longer amounts of bowel divided and longer distances from the tumour to the ligation of tumour-supplying vessels or closest bowel wall than non-CVL cases[200,218,244]. A natural inference of this is to expect a significant association between *ex vivo* TM measurements and survival outcomes in CRC surgery, which if proven, would provide argument for its role as a quality metric of CRC (in particular, CVL) surgery.

However, contrary to our initial hypothesis, we found no association between *ex vivo* TM measurements and either OS or DFS in the AR or RH surgery cohorts. This lack of association was also observed specifically in the Stage III group where it could be expected that an increased EoL would provide the most significant survival advantage. The absence of survival association was consistent for both: (i) 'oncologically relevant' TM measurements (TM A, TM B, and TM C), which would be expected to reflect mesenteric excision of LNs along the central tumour-draining pedicle, and (ii) the arguably 'less oncologically relevant' TM measurement (TM D), which would explain excision of longitudinal peri-colic LNs not necessarily draining the primary tumour. Consequently, while feasible and reproducible, the utility of *ex vivo* TM measurements as quality metric for CRC surgery was not demonstrated.

Notably, few studies have previously investigated a survival relationship with *ex vivo* TM measurements, and of these, focus has been on populations predominantly undergoing non-CVL surgery for colonic malignancies[4,8,207]. Specifically, West *et al.*[4] confined their investigation of area and lengths to *cross-sectional measurements* and conducted these measurements on fixed tissue specimens. Storli *et al.*[8] solely focussed on the length of the resected bowel measured from fixed CC specimens, while Galizia *et al.*[207] documented the length from the vascular tie to the bowel wall in addition to the length of the bowel resected in those who had a right sided CC resection but applied median cut-offs when examining survival association. In keeping with our findings, none of these studies observed significant differences in patient survival according to TM measurement(s).

Identifying the specific reasons for the lack of association between *ex vivo* TM measurements and survival outcomes is challenging. It is plausible that any increase in TM measurements was attributed to excision of mesenteric fat without concomitant increase in LN harvest. This is supported by our finding that TM measurements were not associated with LNY (except for a positive association observed with length of bowel resected [i.e., TM D], owing to an increased harvest of longitudinal peri-colic LNs with increased TM D). Clearly, increased TM measurements without increase in 'oncologically relevant' LN harvest, would not be expected to confer survival benefit. It is also noteworthy that *ex vivo* TM measurements record parameters of the excised specimen but give no understanding of the residual *in vivo* vascular pedicle measurement, which in many ways is more oncologically relevant as the residual pedicle contains draining LNs left *in situ*. Measurements of *in vivo* residual pedicles post resection have previously been described[81], and may be a more sensitive predictor of survival post CRC surgery.

This study validates the feasibility of measuring *ex vivo* TM parameters using high-definition images of non-formalin fixed specimens. To ensure the standardised measurements on these fresh specimens, we excluded 269 fixed specimen records, mitigating the risk of shrinkage artefact. Moreover, the variation in *ex vivo* TM measurements between the AR and RH groups underscores our rationale for analysing AR and RH patients separately. Our standardised approach to TM measurements also allows comparison of data with other groups which have employed similar methodology. The *ex vivo* TM measurements in our study (at CRGH) exhibit comparability to those recorded at SJUH in Leeds (Table 8). However, when compared to the measurements from UHE, both CRGH and SJUH demonstrate smaller measurements globally[200]. Notably, the standard practice at CRGH and SJUH involves non-routine CVL surgery, in contrast to UHE where CVL surgery is routinely performed[200]. This discrepancy in surgical practices is presumed to contribute to the observed differences, with routine CVL surgery at UHE likely being a contributing factor to the larger TM A and the extended lengths in TM B, TM C and TM D[218,244].

The comparison between the resected specimens from a Japanese D3 resection and our non-routine CVL surgery reveals both distinct contrasts and similarities[5,245,246]. Specifically, the excised mesentery area (i.e., TM A) and the length of the resected bowel (i.e., TM D) were observed to be smaller and shorter in the cohort undergoing D3 dissection[5]. The observed discrepancies in TM measurements between the Japanese cohort and our study could reflect divergent surgical techniques, particularly the adherence of Japanese surgeons to the stringent '10cm rule' and their routine practice of CVL[245]. Furthermore, phenotypic variations in body structure and large bowel anatomy between the populations of the two regions might also have influenced the differences in TM measurements[5,247]. Despite these variances, the oncological outcomes after D3 resection is similar to that reported by us[248,249]. Additionally, central radicality, as indicated by the lengths of TM B and TM C, remained comparable across the Japanese literature and our data[5,245].

The association (or the apparent lack thereof) between survival outcomes and either LN positivity or LNY merits discussion. Expectedly, Stage III disease was associated with poorer survival. Interestingly though, the removal of additional LNs seemed to have no impact on OS and DFS. These observations suggest that once LN metastatic disease is established, survival may be influenced by other clinicopathological factors, including the progression to systemic disease. Therefore, although our practice is to perform a comprehensive lymphadenectomy that incorporates central nodes from the vessel root into the specimen's resection margin, the advantage of removing additional LNs (i.e., more than twelve nodes) in Stage III CRC patients is unclear based on our study's data. It is important to note that this conclusion might not apply to LR rate, which was low in our series.

This prospective study has several limitations. Notably, the absence of height records for some patients in the database prevented us from adjusting *ex vivo* TM measurements based on the patients' body mass indices. Furthermore, we could not account for the plane of mesocolic excision due to missing data for some patients. Given the lack of association between TM measurements and survival on univariate analysis, though, it is unlikely that any significant association would have been confounded by plane of excision status. Finally, this study may be underpowered as there was an absence of pilot data to guide a robust sample size calculation. However, our use of fresh specimens over an eleven-year period to record TM measurements addresses concerns about specimen shrinkage artefact. Moreover, incorporating both urgent operations and a range of surgical approaches—from minimally invasive to open— improves the generalizability of our findings.

This study focused on evaluating the feasibility of using *ex vivo* fresh specimen images for recording TM measurements, with the expectation that it could be useful as a quality metric of 'good CRC surgery'. However, the absence of significant association between TM measurements and survival outcomes does not support its use as a quality metric. This finding serves to further highlight the inherent challenges in assessing quality of CRC surgery but also underscores the need to further explore aspects of EoL, particularly as *ex vivo* TM measurements do not offer insight.

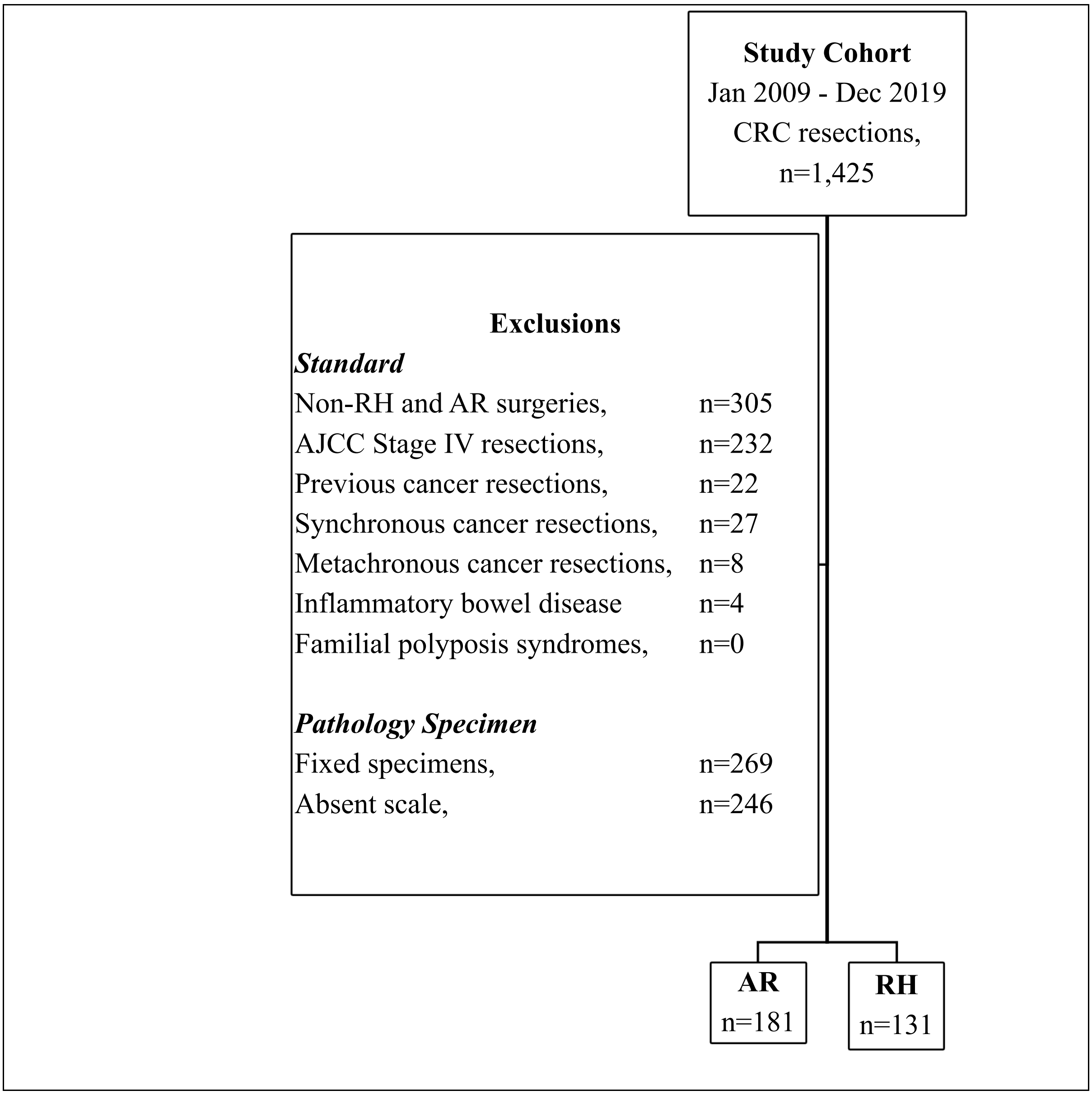

Figure 7: Flow diagram of cohort definition.

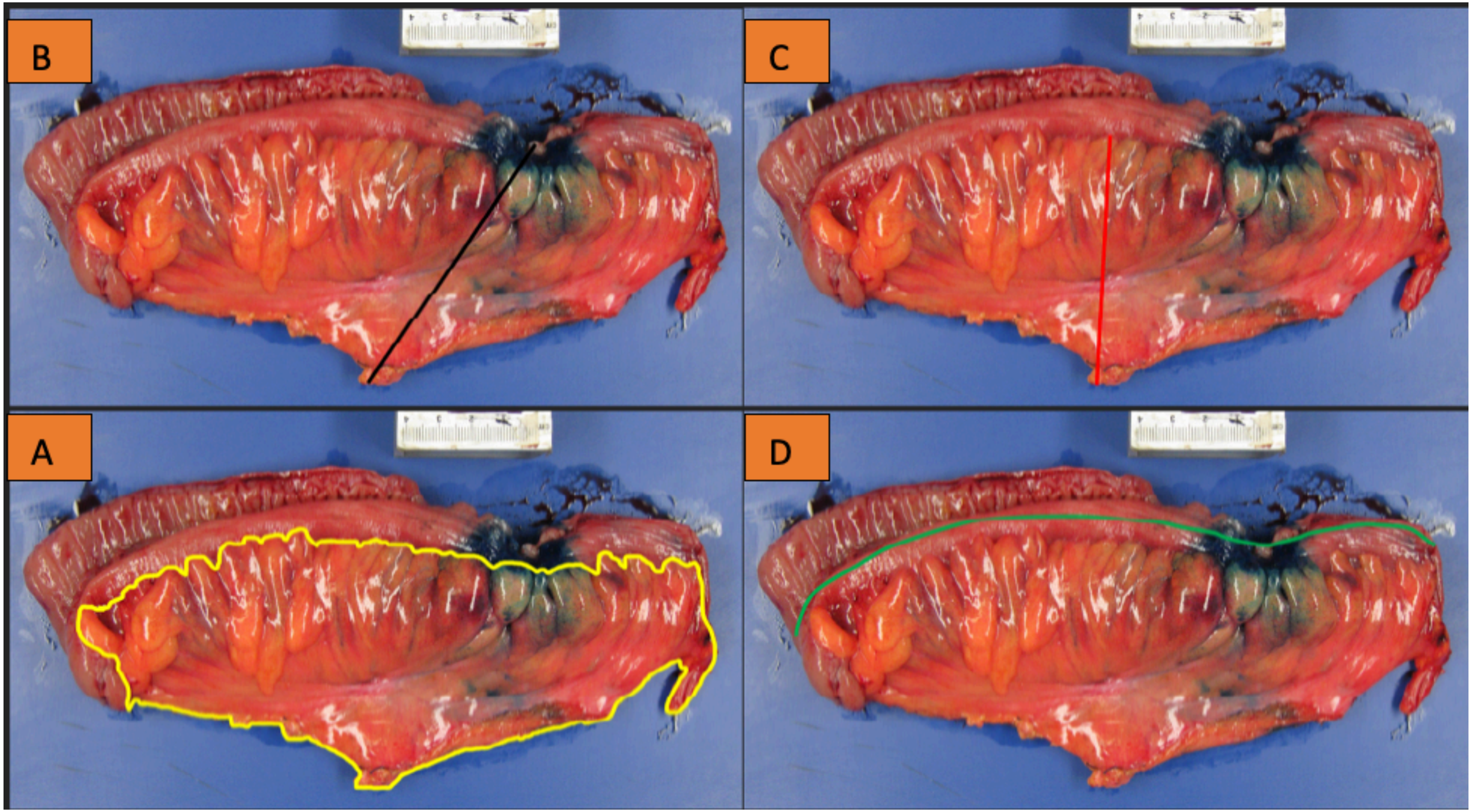

Figure 8: TM measurements (A-D) of a fresh high AR specimen includes - (A) the area of resected mesentery, (B) distance from the vascular tie to the tumour or (C) closest bowel wall and (D) length of bowel resected. The tumour is inked blue.

TABLE 8. Median tissue morphometry (TM) measurements based upon fresh resection specimens, comparing Concord Hospital (n=312), St. James's University Hospital (Leeds [n=40])[200], and University Hospital of Erlangen (Erlangen [n=49])[200]						
	Concord Hospital		St. James's University Hospital^		University Hospital of Erlangen^	
	Anterior resection	Right hemicolectomy	Left sided resection	Right sided resection†	Left sided resection	Right sided resection†
TM A, cm^2	149.6	99.8	131.7	88.8	241.3	167.7
TM B, cm	13.6	10.4	9.7	8.1	14.5	12.9
TM C, cm	9.2	7.2	8.5	7.2	10.8	10.2
TM D, cm	26.4	23.0	26.0	24.3†	39.2	34.8†

TM A: **Area of mesentery resected*****; TM B:*** **Distance from high vascular tie to tumour*****; TM C:*** **Distance from high vascular tie to nearest bowel wall*****; and TM D:*** **Length of bowel resected.**
^Interquartile ranges were not reported
†Right sided resections at Leeds and Erlangen recorded small bowel and large bowel lengths separately. These were combined to reflect the total bowel resected

Table 8: Comparison of TM measurements between Concord Hospital St. James's University Hospital in Leeds and University Hospital of Erlangen.

TABLE 9. Comparison of clinicopathological factors between AR and RH surgery patients			
	Total (%) or Mean (SD) or Median (Range/IQR) (n=312)	**Anterior Resection (n=181)**	**Right Hemicolectomy (n=131)**
TM A (Mean [SD]), cm^2	140.9(67.3)	164.4(69.1)	108.4(49.0)
TM B (Mean [SD]), cm	12.6(4.5)	14.1(4.9)	10.7(3.0)
TM C (Mean [SD]), cm	8.8(3.6)	9.9(3.9)	7.3(2.4)
TM D (Mean [SD]), cm	26.5(9.4)	27.7(9.2)	24.7(9.4)
Gender			
Male	165(52.9)	107(59.1)	58(44.3)
Female	147(47.1)	74(40.9)	73(55.7)
Age (Mean [SD]), years	69.4(12.3)	66.8(12.3)	72.9(11.5)
BMI (Mean [SD]), kg/m^2	27.6(5.4)	27.2(5.2)	28.1(5.7)
ASA Grade			
I	41(13.1)	29(16.1)	12(9.2)
II	179(57.4)	110(60.8)	69(52.7)
III/IV	92(29.5)	42(23.2)	50(38.2)
Emergency Operation			
No	303(97.1)	175(96.7)	128(97.7)
Yes	9(2.9)	6(3.3)	3(2.3)
Emergency Operation (Reason)			
Non-emergency	303(97.1)	175(96.7)	128(97.7)
Obstruction	8(2.6)	5(2.8)	3(2.3)
Perforation	1(0.3)	1(0.6)	-
Operation Modality			
Laparoscopy	265(84.9)	149(82.3)	116(88.5)
Open	47(15.1)	32(17.7)	15(11.5)
Procedure Conversion			
No	246(92.8)	137(92.6)	109(94.0)
Yes	19(7.2)	12(8.1)	7(6.0)
Blood Loss (mls)			
≤500	302(96.8)	172(95.0)	130(99.2)
>500	10(3.2)	9(5.0)	1(0.8)
Tumour Stage (TNM AJCC)			
Stage I	68(21.8)	44(24.4)	24(18.3)
Stage II	111(35.6)	57(31.5)	54(41.2)
Stage III	133(42.6)	80(44.4)	53(40.5)
Tumour Size (cm) (Median [Range])	4.0(0.8-34.0)	3.5(0.8-9.0)	4.5(0.8-34.0)

Tumour Perforation			
No	308(98.7)	179(98.9)	129(98.5)
Yes	4(1.3)	2(1.1)	2(1.5)
Histological Type			
Non-Mucinous or Signet ring	282(90.4)	175(96.7)	107(81.7)
Mucinous or Signet ring	30(9.6)	6(3.3)	24(18.3)
Histological Differentiation			
Well or Moderate	272(87.2)	171(94.5)	101(77.1)
Poor	40(12.8)	10(5.5)	30(22.9)
Histological Grade			
Low or Average	270(86.5)	170(93.9)	100(76.3)
High	42(13.5)	11(6.1)	31(23.7)
Lympho-Vascular Invasion			
No	226(72.4)	127(70.2)	99(75.6)
Yes	86(27.6)	54(29.8)	32(24.4)
Peri-Neural Invasion			
No	247(79.2)	136(75.1)	111(84.7)
Yes	65(20.8)	45(25.0)	20(15.3)
Number of Lymph Nodes Examined (Median [Range])	19(4-47)	19(4-45)	20(6-47)
Lymph Node Harvest (<12)			
No	286(91.7)	161(89.0)	125(95.4)
Yes	26(8.3)	20(11.0)	6(4.6)
LOS (Median [Range]), days	7(2-66)	7(2-66)	6(3-30)
TM: **Tissue Morphometry A-D*; BMI:* Body Mass Index; *ASA:* American Society of Anesthesiology; *TNM: 8th* edition tumour, nodes, and metastasis staging system; and *AJCC:* American Joint Committee on Cancer**			

Table 9: Clinical characteristics and histopathological descriptions of AR and RH patients.

TABLE 10. Clinicopathological Features and Oncological Associations (N=312)								
	Overall Survival				Disease-Free Survival			
	AR		RH		AR		RH	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
TM A (cm^2)	1.00(1.00-1.01)	0.38	1.00(1.00-1.00)	0.30	1.00(1.00-1.01)	0.40	1.00(0.99-1.00)	0.57
TM B (cm)	0.95(0.86-1.06)	0.78	0.95(0.86-1.06)	0.36	1.01(0.96-1.07)	0.60	0.97(0.88-1.06)	0.50
TM C (cm)	0.99(0.92-1.04)	0.79	0.92(0.82-1.04)	0.17	0.98(0.92-1.05)	0.56	0.93(0.83-1.04)	0.19
TM D (cm)	1.02(0.99-1.04)	0.19	1.00(0.97-1.03)	0.83	1.01(0.99-1.04)	0.35	1.01(0.99-1.04)	0.36
Gender								
Male	ref	-	ref	-	ref	-	ref	-
Female	0.53(0.27-1.04)	0.07	0.63(0.36-1.12)	0.12	0.64(0.37-1.13)	0.12	0.85(0.49-1.46)	0.55
Age (years)	1.05(1.02-1.08)	0.003	1.05(1.02-1.08)	0.002	1.02(1.00-1.05)	0.049	1.04(1.01-1.07)	0.006
BMI (kg/m^2)	1.02(0.96-1.07)	0.55	0.99(0.94-1.05)	0.84	1.02(0.98-1.07)	0.35	1.01(0.96-1.06)	0.68
ASA Grade								
I	ref	-	ref	-	ref	-	ref	-
II	2.66(0.79-8.86)	0.11	1.47(0.34-6.42)	0.61	1.91(0.74-4.92)	0.18	1.85(0.43-7.94)	0.41
III/IV	8.08(2.34-27.9)	<0.001	5.36(1.27-22.54)	0.02	5.57(2.07-14.97)	<0.001	5.85(1.39-24.53)	0.02
Emergency Operation								
No	ref	-	ref	-	ref	-	ref	-
Yes	0.05(0.00-513.03)	0.52	2.02(0.28-14.83)	0.49	0.63(0.09-4.58)	0.65	1.49(0.20-10.82)	0.70
Operation Modality								
Open	ref	-	ref	-	ref	-	ref	-
Laparoscopy	0.69(0.36-1.1.35)	0.29	0.33(0.17-0.63)	<0.001	0.70(0.39-1.28)	0.25	0.28(0.15-0.53)	<0.001
Intraoperative Blood Loss (mls)								
≤500	ref	-	ref	-	ref	-	ref	-
>500	1.60(0.57-4.52)	0.38	NA	0.84	1.33(0.48-3.71)	0.59	NA	0.77
Tumour Stage (TNM)								
Stage I	ref	-	ref	-	ref	-	ref	-
Stage II	1.53(0.56-4.13)	0.41	1.44(0.52-3.98)	0.48	1.91(0.83-4.40)	0.13	1.23(0.48-3.14)	0.70
Stage III	2.66(1.09-6.48)	0.03	3.00(1.15-7.80)	0.02	2.51(1.15-5.49)	0.02	2.96(1.23-7.12)	0.02
Histological Type								
Non-Mucinous or Non-Signet Ring	ref	-	ref	-	ref	-	ref	-
Mucinous or Signet Ring	0.05(0.00-52.51)	0.39	2.10(1.08-4.09)	0.03	0.53(0.07-3.84)	0.53	2.05(1.09-3.86)	0.03
Lympho-Vascular Invasion								
No	ref	-	ref	-	ref	-	ref	-
Yes	1.82(0.98-3.38)	0.06	3.57(1.87-6.83)	<0.001	1.64(0.95-2.82)	0.07	3.74(2.03-6.91)	<0.001
Peri-Neural Invasion								
No	ref	-	ref	-	ref	-	ref	-

Yes	3.08(1.67-5.68)	<0.001	2.62(1.35-5.08)	0.004	3.07(1.80-5.25)	<0.001	2.92(1.55-5.50)	<0.001
Number of Lymph Nodes Examined	0.97(0.93-1.02)	0.20	0.98(0.94-1.02)	0.23	0.99(0.96-1.03)	0.62	0.98(0.94-1.02)	0.23
HR: **Hazard Ratio*; TM:*** **Tissue Morphometry A-D; *BMI:*** **Body Mass Index*; ASA:*** **American Society of Anesthesiology and *TNM: 8th*** **edition tumour, nodes, and metastasis staging system**								

Table 10: Comparison of Survival Outcomes between Clinicopathological Characteristics in AR and RH patients.

TABLE 11. Tissue Morphometric Measurements and Oncological Associations in AJCC Stage III patients (N=133)								
Variable	**Overall Survival**				**Disease-Free Survival**			
	AR		**RH**		**AR**		**RH**	
	HR (95%CI)	***P*-value**	**HR (95%CI)**	***P*-value**	**HR (95%CI)**	***P*-value**	**HR (95%CI)**	***P*-value**
TM A (cm^2)	1.00(1.00-1.01)	0.46	1.00(1.00-1.01)	0.63	1.00(1.00-1.01)	0.30	1.00(1.00-1.01)	0.46
TM B (cm)	1.00(0.93-1.08)	0.96	0.96(0.84-1.10)	0.54	1.01(0.94-1.08)	0.87	1.00(0.89-1.14)	0.95
TM C (cm)	0.99(0.89-1.09)	0.82	0.92(0.80-1.07)	0.29	0.97(0.89-1.07)	0.56	0.94(0.82-1.08)	0.36
TM D (cm)	1.01(0.98-1.05)	0.50	1.00(0.97-1.04)	0.86	1.01(0.98-1.04)	0.66	1.01(0.98-1.04)	0.55
HR: **Hazard Ratio*; TM:*** **Tissue Morphometry A-D**								

Table 11: Comparison of Survival Outcomes between TM measurements in AJCC Stage III AR and RH patients.

TABLE 12. Differences in the association of TM measurements and LNY in all CRC patients (n=312)		
	Number of LN examined	
	β (95% CI)	***P*-value**
TM A (cm^2)	0.01(-0.004 to 0.02)	0.20
TM B (cm)	-0.07(-0.26 to 0.12)	0.47
TM C (cm)	0.07(-0.18 to 0.31)	0.59
TM D (cm)	*0.11(0.02 to 0.20)*	*0.02*
TM: **Tissue Morphometry A-D, *LNY:*** **Lymph Node Yield and *LN:*** **Lymph node(s)**		

Table 12: TM measurements and LNY associations in 312 RC and CC resections.

CHAPTER IV: Lymph Node Ratio Prognosticates Overall Survival in Patients with Stage IV Colorectal Cancer

This chapter has been prepared, submitted and published in *Techniques in Coloproctology* as **"Naidu K, Chapuis PH, Connell L, Chan C, Rickard MJFX, Ng KS. Lymph Node Ratio Prognosticates Overall Survival in Patients with Stage IV Colorectal Cancer. Tech Coloproctol. 2024 Aug 23;28(1):115. doi: 10.1007/s10151-024-02984-6. PMID: 39177674; PMCID: PMC11343919".** The published manuscript is included as **APPENDIX III** in **Chapter IX**.

Preamble

LNR is a metric derived from LNY and thus an important consideration for EoL. LNR is described as the ratio of metastatic LNs to the total LNs examined. It has been identified as a prognostic indicator in several non-CRCs, such as upper gastrointestinal, breast, thyroid, non-small cell lung, and oropharyngeal cancers[250-254]. In CRC, although pathological nodal staging remains a key predictor of patient outcomes, numerous studies have investigated LNR's potential as an additional prognostic marker, some of which are summarised in Table 13.

Studies have demonstrated that LNR is a valuable independent predictor of survival in CRC, particularly in patients with AJCC Stage III disease. Early research by Berger *et al*. identified LNR as the most significant predictor of DFS and OS in AJCC Stage II and III CC patients with more than ten LNs retrieved (pooled data from the Intergroup 0089 trial)[44]. This study highlighted dramatic changes in recurrence rates within nodal classifications based on LNR values. Notably, all patients in the study received adjuvant chemotherapy, making it unlikely that the benefits of improved nodal staging were due to intensified treatment. Instead, the better prognosis associated with a lower LNR may reflect factors such as the quality of surgery performed, with a *higher* LNY necessarily yielding a *lower* LNR. Similarly, in 2008, Wang *et al.* analysed 24,477 AJCC Stage III CC patients from the SEER registry and found that LNR provided greater prognostic accuracy than TNM staging for specific subgroups[255].

Evidence for LNR's relevance also extends to RC[256-259]. For example, Junginger *et al.* showed that LNR offers valuable prognostic information, helping to offset the effects of inadequate LN dissection in AJCC Stage III RC patients who did not undergo neoadjuvant therapy[259]. Furthermore, early systematic reviews on this topic concluded that LNR offers superior prognostic stratification for OS, DFS, and CSS[258,260].

The potential of LNR as a prognostic marker has even inspired attempts to integrate it into staging systems. For example, Pei *et al.*[261] proposed the use of total LNR classification, based on 62,294 early-stage CC patients from the SEER registry and an additional 3,327 patients from an external validation cohort. This classification system showed promise, outperforming the AJCC 8th TNM classification in predicting OS and DFS[261].

Considerations for EoL in unique patient populations, particularly those with AJCC Stage IV CRC, requires investigation. In patients with mCRC undergoing primary tumour resection, LNY is often suboptimal compared to non-metastatic cases, likely due to the perception that LN evaluation holds little value once distant metastasis has occurred[262-266]. However, LN status may still be relevant in mCRC, as it enables a more nuanced distinction of patients by categorising them based on their observed routes of spread – an aspect LNY alone is unable to achieve. Specifically, mCRC patients with an LNR=0 may represent those with tumours that have spread exclusively via haematogenous routes and/or transcoelemic spread, but not by lymphatic spread. In contrast, those with an LNR>0 may potentially demonstrate all three routes of dissemination. Thus, the absence of LN spread alongside hematogenous or transcoelemic spread, as seen in LNR=0 patients, may point to tumours with potentially favourable biological behaviour and a lower tumour burden.

While LNR has been evaluated as a prognostic marker in mCRC[265,267,268], its significance, particularly in patients with LNR=0, remains unclear. Further investigation into the survival implications of LNR dichotomization (LNR=0 vs. LNR>0) could enhance understanding of these patients and underscore the value of adequate lymphadenectomy in mCRC cases undergoing primary tumour resection[269]. Therefore, **Chapter IV** aims to explore the impact of LNR on survival in patients with mCRC specifically exploring the prognostic implications of dividing patients into LNR=0 and LNR>0 categories.

TABLE 13. Overview of Publications on LNR and LODDS and Their Associations with Survival (up to 2023)												
Study	**Sample size (n)**	**Study Type**	**Tumour location**	**LNY**	**Positive LNs**	**LNR**	**LNR cut offs**	**Modality**	**Urgency**	**AJCC Stage**	**Follow-up (months)**	**Oncological Outcomes**
Ahmad *et al*.[268] 2017 USA	53	RSCS	Colon Rectum	Median = 17 (Range 2–39)	NR	Median = 0.25 (Range, 0–0.94)	Low LNR (L-LNR, ≤0.25). High LNR (H-LNR, >0.25)	NR	NR	IV	Median = 16 (Range 0–117)	**Each increase of LNR value by 0.1 was associated with a decrease in OS by 3.1 months (P=0.009).** **3-year OS (P=0.027)** H-LNR 9% L-LNR 34% **Multivariate analysis** H-LNR (HR2.63; 95% CI, 1.13–6.14; P=0.025)
Berger *et al*.[44] 2005 USA	3,411	RSCS; Based on INT-0089 trial	Colon	Median – 11 LNY 1= <10 LNY 2=10-15 LNY 3= >15	N1 + N2 = 2,763; 81%	NR	LNR 1: <0.05 LNR 2: 0.05 to 0.19 LNR 3: 0.2 to 0.39 LNR 4: 0.4 to 1.0	NR	NR	II to III	Median=79; (range 1-131)	**5 yr OS [ALL PATIENTS]** (P<0.0001): LNR1 – 79%; LNR2 – 73%; LNR 3 – 63%; LNR 4 – 52% **5 yr DFS [ALL PATIENTS]** (P<0.0001): LNR1 – 77%; LNR2 – 70%; LNR 3 – 62%; LNR 4 – 50% **5 yr CSS [ALL PATIENTS]** (P<0.0001): LNR1 – 84%; LNR2 – 79%; LNR 3 – 69%; LNR 4 – 57% **5 yr OS [N1 DISEASE]** (P<0.0001): LNR1 – 79%; LNR2 – 73%; LNR 3 – 67%; LNR 4 – 60% **5 yr DFS [N1 DISEASE]** (P<0.0001): LNR1 – 84%; LNR2 – 70%; LNR 3 – 65%; LNR 4 – 59% **5 yr CSS [N1 DISEASE]** (P<0.0001): LNR1 – 85%; LNR2 – 79%; LNR 3 – 72%; LNR 4 – 67% **5 yr OS [N2 DISEASE]** (P<0.0001): LNR2 – 73%; LNR 3 – 55%; LNR 4 – 45% **5 yr DFS [N2 DISEASE]** (P<0.0001): LNR2 – 66%; LNR 3 – 54%; LNR 4 – 43% **5 yr CSS [N2 DISEASE]** (P<0.0001): LNR2 – 74%; LNR 3 – 60%; LNR 4 – 50%

												LNY 2 and LNR - OS – HR 3.9 (P<0.001) - OS – HR 5.0 (P<0.001) **LNY 3 and LNR** - OS – HR 12.4 (P<0.001) - OS – HR 13.2 (P<0.001)
Chang *et al.*[90] 2012 Taiwan	9,644	RSCS	Colon	Mean= 19.3 (SD 12.9)	Mean= 3.9 (SD 4.2)	LNR- Mean= 0.3 (SD 0.2) LODDS - Mean= -0.6 (SD 0.5) -	Continuous only	NR	NR	I to III	Mean=49.4 (SD18.4)	**MR:** 27.7% **LNR (AUC)** -5-year OS 0.70 (95%CI 0.68–0.73) -5-year DFS 0.70 (95%CI 0.68–0.72) -5-year DDS 0.71 (95%CI 0. 0.69–0.73 **LODDS (AUC)** -5-year OS 0.70 (95%CI 0.68–0.72) -5-year DFS 0.70 (95%CI 0.68–0.72) -5-year DDS 0.71 (95%CI 0. 0.69–0.73
Chen *et al.*[92] 2011 USA	36,712	RSCS	Colon	<12 nodes: Mean=7.2 (SD 2.7) >12 nodes: Mean=19.8 (SD 8.9)	<12 nodes: Mean=2.6 (SD 2) >12 nodes: Mean=4.2 (SD 4.4)	NR	0.10,0.25, 0.50, 0.99, and 1.0 Reported as % positive nodes: <10%, 25%-49%, 50%-99%, 100%	NR	NR	I to III	NR	**LNY** <12 nodes vs ≥12 nodes (median survival of 53 months versus 66 months; P<0.001) **LNR (Multivariate)** < 12 nodes - <10% positive: ref - 10%–24% positive: HR 1.2 (1.1–1.4); P<0.001 - 25%–49%positive: HR 1.5 (1.4–1.6)); P<0.001 - 50%–99% positive: HR 1.8 (1.6–2.0);P<0.001 - 100% positive: HR 2.3 (2.0–2.5); P<0.001 ≥ 12 nodes - <10% positive: ref - 10%–24% positive: HR 1.3 (1.2–1.4); P<0.001 - 25%–49%positive: HR 1.6 (1.5–1.8); P<0.001

												- 50%–99% positive: HR 2.9 (2.6–3.2);P<0.001 - 100% positive: HR 5.1 (4.3–6.1); P<0.001
Chin *et al*.[270] 2009 Taiwan	624	RSCS	Colon	LNR 1 Mean = 25.3 (range 12–147) LNR 2 Mean = 19.5 (range 12–53) LNR 3 Mean = 16.8 (range 14–21)	LNR 1 Mean = 2.9 (range 1–18) LNR 2 Mean = 10.5 (range 5–32) LNR 3 Mean = 14.0 (range 11-19)	Mean = 0.20 (SD 0.19)	LNR 1: ≤0.4 LNR 2: >0.4 to ≤0.7 LNR 3: > 0.7	NR	NR	III	NR	**5-year DFS (P<0.0001)** LNR 1 = 66.7% LNR 2 = 35.1% LNR 3 = 0%
Deng *et al*.[267] 2018 China	154	RSCS	Colon Rectum	Median = 12 (range 1–44)	Median = 1 (range 0–19)	Median = 0.33	LNR≤0.33 LNR>0.33	NR	NR	IV	Median = 34 (range 2–126	**3-year RFS (P=0.001)** Low LNR = 47.2% High LNR = 16.7% **3-year OS (P=0.003)** Low LNR = 72.8% High LNR = 45.3% **Multivariate analysis – LNR independent factor** 3-year RFS (HR=2.12; 95% CI 1.34–3.37; P=0.001) 3-year OS (HR=2.29; 95% CI 1.28–4.08; P=0.005)
Derwinger *et al*.[262] 2008 Sweden	265	RSCS	Colon	Median -11 (Range 4-160)	Median - 2 (Range 1-20)	NR	LNR 1: <0.125 LNR 2: 0.126 to 0.266 LNR 3: 0.267 to 0.450 LNR 4: 0.451 to 1	NR	Emergency =24%	III	NR	**LNR** 3-year DFS (P<0.001) - LNR 1: 80% - LNR 2 : 60% - LNR 3: 57% - LNR 4: 29%

Dolan *et al.*[71] 2018 UK	896	RSCS	Colon	Median - 17 (Range 1–71)	NR	LNR in node-positive disease - 0.16 (Range 0.03–1)	LNR <0.25 vs ≥0.25	Lap = 166 (18.5%)	Emerg 118 patients	I to IV	NR	**LNY** < 12 nodes vs ≥12 nodes - CSS: 77% vs 78% (P=0.18) - OS: 64% vs 67% (P=0.30) **LNR ≥0.25** - CSS 50% (P=.002) - OS 44% (P=.048)
Edler *et al.*[38] 2007 Sweden Denmark	1,025	Prospective RCT	Colon – 727 Rectum 298	Median - 5 (Range 0-32)	NR	NR	LNR 1: <0.2. LNR 2: 0.2 to 0.49 LNR 3: 0.5 to 0.69. LNR 4: 0.7 to 1.0	NR	NR	I to III	Median = 60	**Stage III 5 yr OS** (P<0.0001): LNR1 – 77%; LNR2 – 56%; LNR 3 – 51%; LNR 4 – 44%
Huh *et al.*[271] 2010 Korea	514	RSCS	Colon Rectum	Median = 14 (Range, 2–67)	Median = 2 (Range, 1–31)	NR	LNR 1: <0.09 LNR 2: 0.09 to 0.18 LNR 3: >0.18 to <0.34 LNR 4: ≥0.34	NR	NR	III	Median = 48.5	**5 yr OS**(P<0.001): LNR 1= 79% LNR 2 = 72% LNR 3 = 62% LNR 4 = 55% **5 yr DFS** (P<0.001): LNR 1= 73% LNR 2 = 67% LNR 3 = 54% LNR 4 = 42%, [MV ANALYSIS] LNR was an independent prognostic factor for OS (P=0.012) and DFS (P=0.009)
Jakob *et al.*[272] 2018 Switzerland	166	RSCS	Colon	Median = 23 (Range 8–54)	Median = 3 (Range 1–19)	Median = 0.13	Low = LNR<0.13 High = LNR>0.13	Open	NR	I to III	Median = 34.3 (95% CI 24.4–41.1)	**5 yr OS(P= 0.034):** LNR High = 19.9% LNR Low = 32.1% **5 yr DFS(P=0.032):** LNR High = 15% LNR Low = 26.9%
Junginger *et al*[259] 2014 Germany	237	RSCS	Rectal	Median = 15	Median = 3	Median = 0.2	LNR ≤ 0.1 LNR ≤ 0.2 LNR ≤ 0.4 LNR ≥ 0.4	NR	NR	III	Median = 55	**MULTIVARIATE** **OS** LNR 1 (P=0.001) LNR 2: HR 2.35 (P=0.015) LNR 3: HR 2.72 (P=0.014)

												LNR 4: HR 5.25 (P<0.001) **DFS** **LNR 1 (P=0.003)** LNR 2: HR 2.34 (P=0.011) LNR 3: HR 2.56 (P=0.014) LNR 4: HR 4.52 (P<0.001)
Kobayashi *et al.*[273] 2011 Japan	820	RSCS	Colon	Mean = 20 (SD 12)	Median = 3 (Range 1–23)	NR	LNR 1: >0 to < 0.07 LNR 2: ≥0.07 to <0.15 LNR 3: ≥0.15 to <0.30 LNR 4: ≥0.30	NR	NR	I to III	Recurrence: Median = 49.2	Recurrence: P=0.0099 There was no difference in OS between patients with LNR< 0.15 and those with Stage II cancer (P=0.45)
La Torre *et al.*[79] 2013 Italy	508	RSCS	Rectum	Neoadjuvant group: Mean = 15.2 (SD3.1) Primary surgery group: Mean= 19.7 (SD4.5)	Neoadjuvant group: Mean = 2.7 (SD 2.4) Primary surgery group: Mean= 4.1 (SD3.*5)*	NR	LNR<0.2 LNR≥0.2	NR	Elective = 100%	I to III	Median = 50.4 (Range 9–120)	Analysis of the LNR in node-positive patients showed that a value of > 0.2 was related to a poorer DFS and OS compared with patients in whom it was < 0.2 (P=0.001)
Lee *et al.*[274] 2007 South Korea	201	RSCS	Colon	Median = 17 (Range, 7–58)	Median =3; (Range, 1–28)	Median=0.16 (Range, .01–.92).	LNR1: 0.01 to 0.11 LNR 2: 0.12 to 0.16 LNR 3: 0.17 to 0.24 LNR 4: 0.25 to 0.92	NR	NR	III	Median= 52 (Range, 13–96)	**5 yr DFS (P<0.0001)** LNR 1 = 83.6% LNR 2 and LNR 3= 61.1% LNR 4 = 20% LNR 1: (Ref) LNR 2: HR 2.24 (95%CI 0.19–0.98; P=0 .04) LNR 3: HR 1.38 (95%CI 0.37–1.42; P=0.35) LNR 4: HR 2.66 (95%CI: 0.21–0.60; P=0.0001)
Leonard *et al.*[275] 2015 Belgium	1,470	RSCS	Rectal	Mean=12.3 (SD 7.9).	NR	Mean= 0.08 (SD 0.17)	Continuous LNR	Open - 999 (68%)	NR	I to III	NR	**5 yr OS: only P value reported (<0.0001)**

								Lap - 467 (32%)				- MV analysis: HR 1.012 (95%CI 1.002–1.021; P=0.02) **5 yr Distant Metastasis: only P value reported (<0.0001)** - MV analysis: HR 1.014 (1.002–1.025; P= 0.02)
Leonard *et al.*[276] 2016 Belgium	357	RSCS	Rectal	Mean= 12.8 (SD 8.8)	Mean= 0.8 (SD 1.97)	Mean=0.07 (SD 0.16)	LNR 1: <0.2 LNR 2: ≥0.2	Open – 243 (68.1%) Lap – 103 (28.9%)	Elective 100%	I to III	NR	**5 yr OS:** LNR 1 vs LNR 2: HR 2.85 (95%CI 1.73–4.70;P<0.001) vs HR 5.04 (95%CI 1.78–14.27; P= 0.002) **5 yr RFS:** LNR 1 vs LNR 2: HR 2.77 (95%CI 1.79–4.29; P<0.001) vs HR 4.55 (95%CI 2.00–10.36; P<0.001)
Li Destri *et al.*[120] 2017 Italy	432	RSCS	Colon Rectum	Median = 13 (Range 1 – 77)	NR	ROC curve analysis identified the LNR value of 0.194 as the best cut-off to differentiate patients who are likely to relapse (AUC:0.626 ;95%CI:0.547–0.706;p= 0.003) with a sensitivity of 65% and a specificity of 61%.	LNR 1 = 0.013 to 0.096 LNR 2 = 0.097 to 0.185 LNR 3 = 0.186 to 0.360 LNR 4 = 0.361 to 1.000	NR	NR	II to III	NR	**5 yr DFS of patients with LNR below the cut- off value was 71%, whereas that of patients with LNR above the cut-off value was 45% (P< 0.001).** **In particular, patients with an LNR below 0.194 were at a lower risk of disease relapse than patients with an LNR above 0.194 (OR: 0.482; 95%CI: 0.245–0.950).**
Mammaen *et al.*[123] 2007 USA	5,823	RSCS	Colon	Mean= Stage I:9.6 Stage II: 12.9	Mean= Stage III: 3.3	Quartile 1: 1 Quartile 2: 2 Quartile 3: 3-4 Quartile 4:>4	LNR 1: ≤0.1 LNR 2: 0.1 to 0.21 LNR 3: 0.22 to 0.43 LNR 4: >0.43	NR	NR	I to III	NR	**5yr OS (Stage III)** LNR 1: 44% LNR 2: 49% LNR 3: 30% LNR 4: 27%

				Stage III: 13.6								[Reported as significant but P value not reported]
Madbouly *et al*.[256] 2014 Egypt	115	Prospective	Rectal	Mean = 12.1 (Range 5 to 25)	Mean = 3.5 (Range 1 to 19).	Mean = 0.37 (Range 0 to 1.00).	NR	NR	NR	III	Mean = 37 (Range, 24 to 63)	**MV analysis - Recurrence** LNR: HR 3.7 (95%CI 1.5–9.3; P=0.003)
Mohan *et al*.[277] 2017 Ireland	402	RSCS	Colon	NR	NR	NR	LNR 1 = <0.27 LNR 2 = ≥ 0.27	NR	NR	III	Mean = 34.9	**3 yr OS** Univariate - LNR: HR 2.22 (95% CI 1.69–2.93;P< 0.001) Multivariate – LNR: HR 1.97 (95%CI 1.49–2.6; P< 0.001)
Molnar *et al*.[278] 2019 Romania	72	RSCS	Rectum	NR	NR	NR	LNR 1: <0.2 LNR 2: >0.2	NR	NR	I to IV	Median=62 (Range 52-80)	**5 yr OS**: LNR 1 (>80%) vs LNR 2 (<40%) (P=0.001)
Moug *et al*.[279] 2009 UK	206	RSCS	Colon Rectum	Median = 11·5 (Range 2–39	NR	NR	LNR 1: <0·05 LNR 2: 0·05 to 0·19 LNR 3: 0·20 to 0·39 LNR 4: 0·40 to1·00	NR	NR	I to III	Median=44.4 (Range 1.2–81.6)	**5 yr OS**: HR 1·51 (95% CI 1·24-1·84); P<0·001) - **SUB GROUP ANALYSIS** LNR 3&4 VS LNR 1: HR4·10 (95%CI 2·24-7·53; P<0·001) VS HR 2·79 (95%CI 1·22-6·39; P<0·001) LNR 1 vs LNR 2: NS
Onitilo *et al*.[134] 2013 USA	1,397	RSCS	Colon Rectum	NR	NR	NR	Low LNR =0 High LNR >0	NR	Elective: 1,091 Emerg: 143	I to III	Median = 63	**Unadjusted** Group I (Low LNR and adequate LNY) REF Group 2 (Low LNR and inadequate LNY) – HR 1.15 (95%CI 0.92–1.44; P=0.22) Group III (High LNR and Adequate LNY) - HR1.76 (95%CI 1.47–2.11; P<0.001) Group IV (High LNR and Inadequate LNY) - HR 2.36 (95%CI 1.77–3.16; P <0.001) **Adjusted** Group I (Low LNR and adequate LNY) REF

												Group 2 (Low LNR and inadequate LNY) – HR 1.32 (95%CI 1.02–1.72; P=0.04) Group III (High LNR and Adequate LNY) – HR3.11 (95%CI 1.55–6.26; P<0.001) Group IV (High LNR and Inadequate LNY) - HR 4.14 (95%CI 2.04–8.41; P <0.001)
Osterman *et al*.[280] 2020 Sweden	416	RSCS	Colon	NR	NR	Cutoff to stratify recurrences by ROC analysis = 0.13 44% (83/190) of Stage III patients were LNR-high	NR	NR	NR	I to III	Median = 66	LNR correlated strongly with recurrence unadjusted HR 37.2 (95% CI 15.6-88.6; P<0.001). Lost significance on multivariate analysis. Increased LNR, correlated with increased hazard of mortality when adjusting for baseline variables.
Park *et al*.[281] 2009 South Korea	318	RSCS	Colon	Mean = 24 (SD 15)	N1 = 229 (72%) N2 = 89 (28%)	Mean= 0.2 (Range, 0.01–1)	<0.06; 0.06 to 0.23; >0.23	NR	NR	III	Median = 37 (Range1–122)	**3 yr DFS** (P=0.002) LNR<0.06 = 83.6%, LNR 0.06 to 0.23 = 71.1% LNR>0.23 = 55% **MV DFS analysis** **Odds Ratio:** OR 4.74 (95%CI 2.0–11.22; P <0.001**)**
Pen *et al*.[282] 2008 China	318	RSCS	Rectal	Median = 12 (Range 2–38)	Median = 3.8 (Range 1–27)	Mean = 0.34 (Range 0.03–1)	<0.14; 0.14 to 0.49; 0.5–1	NR	NR	III	Median = 41	**5 yr OS** (P=0.002) <0.14 = 72.2% 0.14–0.49 = 61.9% 0.5–1 = 38.5% **5yr DFS** (P=0.0001) <0.14 = 72.6% 0.14–0.49 = 58.5% 0.5–1 = 34.8%

												5 yr LR: LNR 0.14 to 1 vs LNR<0.14(15.6% vs 3.6%, P=0.019)
Persiani *et al.*[283] 2012 Italy	258	RSCS	Colon	NR	NR	NR	**LNR -** ≤0.05; 0.05 to ≤0.20; >0.20 **LODDS -** ≤ -1.36; -1.36 to ≤ -0.53; > -0.53	NR	NR	I to IV Methods states not including IV but tables include I to IV	Median = 26 (range 2-76)	**LNR 3 yr OS** (P=0.001): LNR</=0.05 – 87.8; LNR 0.05 to </=0.20 – 62.7; LNR>0.20 – 34.6 [HR1.03(95%CI 1.02-1.04; P<0.001)] [UNIVARIATE] **LODDS 3 yr OS** (P=0.001): LODDS</=-1.36 – 91.1; LODDS -1.36 to </=-0.53- 75.2; LODDS >-0.53 – 33.1 [HR LODDS 0 vs 1 – 3.69 (95%CI 1.55-8.79; P=0.003); HR LODDS 0 vs 2 – 9.44 (95%CI 3.92-22.76;P<0.001)] [MULTIVARIATE]
Peschaud *et al.*[284] 2008 France	307	RSCS	Rectal	Mean= 22 (SD 12)	NR	Mean LNR= 0.10 to 0.18 in the 175 who received no preoperative treatment; LNR=0.13 to 0.25 in the 50 who received preoperative SCRT. LNR 0.11 0.20 in the 82 who received preoperative LCRT	0; 0.01 to 0.0; >0.07 to 0.2; >0.2.	NR	NR	I to III	Mean = 38 (SD 20)	LNR was a significant prognostic factor for both DFS (P=0.006) and OS (P= 0.0003).
Qiu *et al.*[285] 2011 China	626	RSCS	Colon = 406 Rectal = 220	Median =10 (Range 1–33)	Median = 2 (Range 1-23)	NR	LNR 1: 0 to ≤0.1 LNR 2: 0.1 to ≤0.25 LNR 3: (0.25 to ≤ 0.5 LNR 4: >0.5	NR	NR	III	Median= 42.2 (SD	**MR:** 43.8% **5-year OS**: LNR1: 73% LNR2: 64% LNR3: 44% LNR4 : 22%

												LR: LNR </=0.1 = 3.2% LNR >0.1 and </=1 =14.6% In the multivariate analysis, the LNR was an independent prognostic factor for survival (P<0.001).
Rosenberg *et al.*[286] 2008 Germany	3,026	RSCS	Colon= 1,763 Rectal= 1, 263	Mean= 18.3 (SD 10.5)	Mean= 2.6 (SD 5.3)	Mean= 0.14 (SD 0.22)	LNR 0; 0.01 to 0.17; 0.18 to 0.41; 0.42 to 0.69; ≥0.70	NR	NR	I to IV	Median=79 (Range 12-275)	5-year OS (P<0.001): LNR 0.01 to 0.17 : 60.6%, LNR 0.18 to 0.4:34.4% LNR 0.42 to 0.69: 17.6% LNR ≥0.70: 5.3% LNR 0 to 0.17: HR 1.92 (95%CI 1.58-2.34) LNR 0.18 to 0.41: HR 2.92 (95%CI 2.38-3.58) LNR 0.42 to 0.69: HR 3.62 (95%CI 2.86-4.57) LNR >0.70: HR 4.31 (95%CI 3.28-5.66)
Rosenberg *et al.*[287] 2010 Germany	17,309	RSCS	Colon= 17,134 (61.6%) Rectal= 10,669 (38.4%)	Mean= 16.8 (SD 8.4)	Mean= 2.2 (SD 4.3)	NR	LNR 0 LNR 0.01 to 0.17 LNR 0.18 to 0.4 LNR 0.42 to 0.69 LNR ≥0.70	NR	NR	I to IV	Mean=70.8 (SD 49.2)	LNR 0.01–0.17 HR 1.32 LNR 0.18–0.41 HR 1.70 LNR 0.42–0.69 HR 2.09 LNR >/=0.70 HR 2.87 **5 yr OS and RFS decreased with increasing LNR (P<0.001).**
Sabbagh *et al.*[288] 2014 France	178	RSCS	Colon	Median = 15 6 (Range: 3–36)	Mean= 2 (SD 3)	NR	LNR 1= <0.07 LNR 2= 0.07 to 0.25 LNR 3= >0.25 to 0.5 LNR 4= >0.5	Laparoscopy (11 patients;6 %)	Elective(127 patients;71%)	III	NR	**Overall OS (P=0.002)** **3 yr OS (P=0.06)** LNR 1 = 88% LNR 2 = 82.5% LNR 3 = 64.5% LNR 4 = 72% **3 yr DFS (P=0.03)** LNR 1 = 88%

												LNR 2 = 67.5% LNR 3 = 61% LNR 4 = 64% **ROC curve analysis and determination of an LNR cutoff.** An LNR of 10% is associated with the greater sensitivity and specificity for predicting DFS (sensitivity: 80%; specificity: 53%; positive predictive value: 21%; negative predictive value: 89%, area under the curve: 0.71) **Survival data as a function of the LNR cutoff of 10%.** Mean OS (P<0.01) **1 yr OS** - LNR<10% = 97%; LNR ≥10%= 87%; P <0.009 **3 yr OS** - LNR<10% = 82%; LNR ≥10%= 63%; P <0.02 **Mean DFS** (log rank P<0.01) **1 yr DFS** - LNR<10% = 92%; LNR ≥10%= 77%; P <0.009 **3 yr DFS** - LNR<10% = 82%; LNR ≥10%= 63%; P <0.02
Schumacher *et al.*[289] 2007 USA	232	RSCS	Colon	Median = 17	NR	Median = 0.18	<0.18 ≥0.18	NR	NR	I to III	NR	**Stage III 5 yr OS (NS)** <0.18 = 50% >/= 0.18 = <40% **Stage III 5 yr DFS** (P<0.0005) <0.18 = >80% >/= 0.18 = 50%
Shimomura *et al.*[290] 2011 Japan	266	RSCS	Colon Rectum	Median = 17 (Range 1-76)	Median = 2 (Range 1-27)	Median = 0.16 (Range 0.01 to 1)	Low = 0.01 to 0.20 High = 0.21 to 1.0	NR	NR	III	Median= 42.4 (Range 0.59–183.7)	**5 yr DFS (**P <0.0001) Low LNR=70.2% High LNR= 46.4%

												MV Analysis LNR (high): HR=2.4247 (95% CI 1.50–3.92; P=0.0003) **No. of LNs examined and survival (P=0.6319)** ≤11 = 63% ≥12 = 60%
Sjo *et al.*[46] 2012 Norway	950	PSOC	Colon	1993 to 1999: Median =7 2005-2009: Median =15	NR	NR	0 to 0.10; 0.11 to 0.18; 0.19 to 0.40; 0.41 to 1	NR	Elective – 805 (85%) Emergency – 145 (15%)	I to III	NR	**Stage III 5 yr OS** (P=0.001): LNR<0.11 – 70%; LNR>/= 0.11 – 46% (P=0.002). **Stage III 5 yr TTR (MV analysis)** (P=NR): LNR 2 HR 2.5 (95% CI, 1.2–5.3); LNR 3 HR 2.7 (95% CI, 1.3–5.7); LNR 4 HR 3.4 (95% CI, 1.6–7.2)
Vaccaro *et al.*[291] 2009 Argentina	362	RSCS	Colon	Median = 20(95% CI 19-21)	Median =2 (95%CI 2-2)	Median = 0.11 (95%CI 0.09-0.14)	<0.06; 0.06 to 0.12; >0.12 to 0.24; ≥0.25 Eventually grouped into <0.25 and >0.25	NR	NR	III	Median = 42 (95%CI 35-54)	**5 yr OS** (P=0.001): LNR<0.25 – 64.9 (95% CI, 58.1–71.9); LNR>0.25 – 38.3 (95% CI, 25.5–51.1) **5 yr DFS** (P=0.001): LNR<0.25 – 74.5 (95% CI, 67.9–80.9); LNR>0.25 – 40.1 (95% CI, 27.1–53.1). **5 yr CSS** (P=0.001): LNR<0.25 – 68.3 (95% CI, 61.5–75.2); LNR>0.25 – 31.5 (95% CI, 19.4–43.5). **MV analysis**: LNR but not N categorization has independent prognostic value
Vather *et al.*[43] 2009 New Zealand	4,309	RSCS	Colon	Median: 11(Range 1–99)	NR	NR	0.0 to 0.1; 0.1 to 0.2; 0.2 to 0.3; 0.3 to 0.4; 0.4 to 0.5; 0.5 to 0.6; 0.6 to 0.7; 0.7 to 0.8; 0.8 to 0.9; 0.9 to 1.0;	NR	NR	II to III	NR	**5yr MR:** 31.0% **5 yr OS** - Stage II: 1-4 nodes - <65% 5-8 nodes - >65% 9-12 nodes – 70% 13-16 nodes - >70% >17 nodes - >70% - Stage III: 1-4 nodes - 30% 5-8 nodes - >35%

												9-12 nodes – <40% 13-16 nodes - <50% >17 nodes - <50% - LNR: 55% to 60% in the lowest ratio; 10% to 20% in the higher ratio groups.
Wang *et al.*[255] 2008 USA	24,477	RSCS	Colon	<10: 9,610 patients (39.3%) 10–15: 7,398 patients (30.2%) >15: 7,469 patients (30.5%)	N1: 16,819 (68.7%) N2: 7,658 (31.3%)	LNR1: 2860 (11.7% LNR 2: 9729 (39.7%) LNR 3: 6058 (24.7%) LNR 4: 5830 (23.8%).	LNR 1: <0.07; LNR 2: 0.07 to <0.25; LNR 3: 0.25 to <0.50; LNR 4: 0.5 to1.0	NR	NR	III	NR	**5 yr OS** (P=0.001) - LNR 1: 64.8%; LNR 2: 56.2%; LNR 3: 45.1% and LNR 4: 29.6%.

RSCS: **Retrospective Cohort Study;** ***PSOC:*** **Prospective Observational Cohort Study;** ***RCT:*** **Randomised Controlled Trial;** ***MR:*** **Mortality Rate;** ***CCS:*** **Cancer-Specific Survival;** ***TTR:*** **Time to Recurrence;** ***DFS:*** **Disease-Free Survival;** ***RFS:*** **Recurrence-Free Survival;** ***OS:*** **Overall Survival;** ***HR:*** **Hazard Ratio;** ***MV:*** **Multi-Variate;** ***NR:*** **Not Reported;** ***ROC:*** **Receiver Operating Curve;** ***AUC:*** **Area Under the Curve;** ***SCRT:*** **Short Course Chemoradiotherapy;** ***LCRT:*** **Long Course Chemoradiotherapy;** ***and AJCC:*** **American Joint Committee on Cancer**

Table 13: Publications on LNR or LODDS and Survival Associations.

ABSTRACT

INTRODUCTION: LNR is suggested to address the shortcomings of using only LNY or status in CRC prognosis. This study explores how LNR affects survival in mCRC patients, seeking to provide clearer insights into its application.

METHODS: This observational cohort study investigated Stage IV CRC patients (1995-2021) who underwent an upfront resection of their primary tumour at Concord Hospital, Sydney. Clinico-pathological data were extracted from a prospective database, and LNR was calculated both continuously and dichotomously (LNR=0 and LNR>0). The primary endpoint was OS. The associations between LNR and various clinico-pathological variables were tested using regression analyses. Kaplan–Meier and Cox regression analyses estimated OS in univariate and multivariate survival models.

RESULTS: 464 patients who underwent a primary CRC resection with clear margins (mean age 68.1 years [SD 13.4]; 58.0% M; CC [n=339,73.1%]) had AJCC Stage IV disease. The median LNR was 0.18 (IQR:0.05-0.42) for CC resections, and 0.21 (IQR:0.09-0.47) for RC resections. 84 patients had an LNR=0 (CC=66 patients; RC=18 patients). The 5-year OS for the CC cohort was 10.5% (95%CI 8.7-12.3) and 11.5% (95%CI 8.4-14.6) for RC. Increasing LNR demonstrated a decline in OS in both CC (P<0.001) and RC (P<0.001). In patients with non-lymphatic dissemination only (LNR=0 or N0 status), there was better survival compared to those with lymphatic spread (CC: aHR1.50 [95%CI 1.08-2.07;P=0.02], RC: aHR 2.21 [95%CI 1.16-4.24;P=0.02]).

CONCLUSION: LNR is worthy of consideration in patients with mCRC. LNR=0 patients have a better prognosis, underscoring the need for adequate lymphadenectomy to facilitate precise mCRC staging.

INTRODUCTION

In patients with mCRC who undergo a primary tumour resection with clear margins, a distinct observation is noted in that the LNY from resected specimens is frequently *substandard* (i.e., fewer than twelve LNs are retrieved), when compared to their non-metastatic counterparts[262-265]. This observation may be embedded in the perception that once distant metastatic disease has occurred, addressing LN evaluation is futile[266].

While it is true that the presence of distant metastases represents the most advanced stage of the disease, it may not negate the importance of LN evaluation in mCRC management. Understanding the extent of regional LN involvement may still be relevant, as LN status (even in the presence of distant metastases) may potentially reflect differences in disease behaviour, tumour aggressiveness, and OS. Similarly, precise LN evaluation might help inform decisions on the use of post-operative therapy, targeted treatments, and the frequency of follow-up care[264].

LNR, calculated as the number of metastatic LNs divided by the total number of examined LNs[44], may be a useful parameter that incorporates information relating to both LN status and LNY. The prognostic significance of LNR in Stage III CRC has been well-documented, with previous studies demonstrating that those with higher LNR values tend to have poorer survival[44,260,292]. Notably, LNR offers a more distinct prognostic differentiation when compared to the sole reliance on a simple count of positive nodes[260,292]. However, the impact of LNR on mCRC patients' survival remains unclear. In particular, mCRC patients' survival with LNR=0 (i.e. node negative, or N0 status) have not been sufficiently explored in the literature.

In mCRC patients with LNR=0, the absence of metastatic LNs coexists with metastatic spread of disease through other routes such as haematogenous or transcoelomic spread. Intuitively, these patients are presumed to have a better prognosis due to a potentially decreased tumour burden. Investigating the survival implications of dichotomizing LNR (LNR=0 and LNR>0) may refine our understanding of LNR=0 patients. Additionally, such a dichotomization may provide insight into the need to perform an adequate lymphadenectomy in mCRC patients undergoing primary tumour resection (i.e., upfront surgery).

This study aims to examine if LNR is an independent predictor of OS in mCRC patients following resection of the primary tumour. We analysed LNR both as a continuous variable and dichotomously (LNR=0 and LNR>0), hypothesising that the rising LNR correlates with poorer survival, while LNR=0 would be associated with better outcome.

MATERIAL and METHODS

A cohort study of consecutive patients who had an upfront resection for a newly diagnosed AJCC Stage IV colorectal adenocarcinoma at Concord Hospital, Sydney, between January 1995 and December 2021 was performed. The study population was drawn from a prospectively maintained institutional database, which has been in continual existence since January 1971. Patients with *in-situ* neoplasia, AJCC Stage I to III CRC, polyposis coli, inflammatory bowel disease and/or synchronous or metachronous CRC were excluded (Figure 9). The study received ethical approval from the Sydney Local Health District Ethics Committee (2019/ETH07841).

Pre-operative Assessment

For patients planned for an elective operation, pre-operative tumour status was assessed in a multidisciplinary setting based on clinical examination, colonoscopy, CT, ultrasonography, MRI, and, more recently, selective positron emission tomography.

Neo-adjuvant Therapy

In more recent study periods, systemic nCT was introduced for mCRCs patients. These patients were excluded from this study for several reasons. Firstly, nCT has been shown to artefactually influence LNY[26], potentially confounding LNR's impact on outcomes. Moreover, an upfront resection followed by post-operative treatment of synchronous metastases formed the mainstay of management during much of the study period and today remains a recognised strategy in the treatment of mCRC[109,263,265,293-295].

Neo-adjuvant Therapy in RC

For non-urgent RCs located in the lower two-thirds of the rectum, a patient's suitability for neo-adjuvant RT (short vs. long-course with or without chemotherapy) was decided by multidisciplinary consensus based on: the patient's individual needs, tumour location, clinical and radiological features, biopsy findings, metastasis extent, history of previous pelvic irradiation, and the patient's fitness for operation.

Surgical Management

Primary CRC:

During the majority of the study period, the predominant approach to managing mCRC entailed upfront surgical resection of the primary CRC followed by post-operative chemotherapy. This strategy continues to be a recognized method in mCRC treatment[109,263,265,293-297]. For patients who underwent emergency primary CRC resection, the surgical indications were typically large bowel obstruction, perforation, or bleeding.

A resection for a primary CRC was classified into three categories: standard, extended, or a segmental operation. This classification was based on the extent of the vascular ligation of the tumour which consequently determined the extent of lymphadenectomy performed[166]. Standard resections included RH, LH, transverse colectomy, sigmoid colectomy, a Hartmann's procedure, AR, and APE, wherein the operation involved the ligation of strategic vessels, such as the ICA, RCA (if present), MCA, ascending LCA, and IMA. An extended resection comprised either an ERH or a SC, involving the ligation of a combination of the aforementioned vessels. For instance, in the case of an ERH, the ligation includes the ICA, RCA (if present), and MCA. Lastly, SgRs entailed the removal of the primary tumour without a dedicated vascular pedicle ligation, resulting in varying LNY[166]. Tumours between (and including) the caecum and rectosigmoid junction were defined as colonic. RCs were defined as those whose inferior edge was within 15cm from the anal verge, measured by rigid sigmoidoscopy[233].

Metastasectomy:

For patients who underwent a metastasectomy, this was performed during the index operation or as a separate staged operation. The decision and timing hinged on factors including the clinical presentation of the primary cancer, the extent of metastatic spread, the patient's fitness for surgery, and response to post-operative chemotherapy.

In selected cases where metastasectomy was deemed unsuitable but the patient was symptomatic and had a good performance status, a palliative resection of the primary tumour and regional lymphadenectomy was performed followed by palliative chemotherapy, if appropriate.

Pathology Reporting and Staging

Specialist pathologists examined resected specimens using a standard synoptic protocol, with pathology data coded by CC[166]. Adenocarcinomas including mucinous and signet ring types were analysed. Tumour staging followed the AJCC pTNM system[22].

Standard Clinicopathological Variables

Clinical information, operative details, tumour pathology, treatment information and follow-up data were extracted from the database for analysis, as previously described[166]. Groupings for year of resection were also performed to acknowledge the potential changes in treatments approaches over time.

Clinicopathological Variable of Interest - LNR

The LNR was calculated and documented as an additional variable. It was also dichotomised as LNR=0 and LNR>0. This dichotomisation aimed to identify mCRC patients with metastatic spread exclusively through non-lymphatic pathways (i.e., LNR=0).

Surveillance

In patients with mCRC who underwent a synchronous metastasectomy and primary tumour resection, their follow-up mostly resembled that of individuals with non-metastatic disease[166,232]. Generally, this involved clinical examinations and liver function tests every three months, along with serial carcinoembryonic antigen measurements for the first two years post-operation, followed by these evaluations every six months for an additional three years, if clinically appropriate. Imaging typically included annual CT scans for the first two years, with subsequent imaging guided by clinical and biochemical findings. More recently, selective positron emission tomography imaging has also been utilized. Patients were monitored annually until death or December 2021, with less than 5% of patients being lost to follow-up. Colonoscopy was generally conducted at one-year post-resection.

For patients with unresectable metastatic disease following the resection of the primary tumour, were referred to medical oncology and palliative care services for consideration of palliative chemotherapy. In these patients, a personalised and less demanding follow-up protocol was often employed.

Post-operative chemotherapy

A multidisciplinary team routinely considered post-operative chemotherapy for all mCRC patients who underwent primary tumour resection. Factors such as age, patient preferences, comorbidities, adverse pathological features, social circumstances, and best practice guidelines were considered.

The chemotherapy regimens utilised varied but were in accordance with best practice at the time and for the most part were: bolus injections of 5-FU and leucovorin administered daily in five-day blocks and repeated every month for six months, as per the Mayo Clinic regimen[298]; 5-FU and leucovorin repeated weekly for six doses with a 2-week rest between, as per the Roswell Park regimen[299]; semi-monthly 22-hour 5-FU infusion with leucovorin[300] or modified oxaliplatin, leucovorin and 5-FU (FOLFOX) every 2 weeks[301]. In some, oral capecitabine and IV oxaliplatin (CAPOX or XELOX) was considered for six cycles. In some palliative settings, duplet (i.e., leucovorin, 5-FU with either oxaliplatin[FOLFOX] or irinotecan [FOLFIRI]) or triplet (i.e., leucovorin, 5-FU, oxaliplatin and irinotecan [FOLFOXIRI]) chemotherapy was considered.

Outcome Measures

The primary outcome measure was OS. This was considered the most pragmatic measure of survival in this patient cohort.

Patient follow-up commenced from the date of resection. Follow-up times were censored at the last contact for patients who did not experience a terminal event up to December 2021 or were lost to follow-up or remained alive. The date of death was primarily obtained from the patient's surgeon, family physician, or hospital records, with occasional use of the national death registration system[166]. The cause of death was coded by PC based on the International Classification of Diseases-10.

Statistical Analyses

Continuous variables were reported as mean (SD) for normally distributed variables and as median (IQR or range) for non-normal distributions. Categorical variables were reported as frequencies and percentages. Linear regression tested association between LNR (as a continuous variable) and other clinico-pathological variables. Logistic regression tested association between dichotomised LNRs (LNR=0 or LNR>0) and other clinico-pathological variables. These regression models were performed for CC and RC separately, due to perceived differences in their outcomes, biological behaviour, and overall surgical treatment approach. Survival estimates were modelled using the Kaplan-Meier function with log-rank test performed to determine difference in survival distributions.

The association between LNR and OS was assessed by Cox regression, performed for CC and RC separately. To adjust for confounding, all clinico-pathological variables that showed associations ($P<0.05$) with LNR on their respective linear (for continuous LNR) and logistic (for dichotomised LNR) regression analyses were also included in multivariable models. To avoid collinearity, LNR considered as continuous and dichotomised variables, were entered into two separate models. $P<0.05$ was considered significant. All analyses were performed using SPSS® v.29 (IBM, New York, USA).

RESULTS

Study Population

A total of 3,859 patients underwent primary CRC resection, including 464 with AJCC Stage IV disease. Among these, 269 (58.0%) were male, and the mean age was 68.1 years (SD 13.4). Of the total, 339 patients (73.1%) had CC. A *standard* operation was performed in 306 CC patients and 125 RC patients. Specifically, among the CC patients, there were 122 (39.9%) cases of RH, four (1.3%) cases of transverse colectomy, 13 (4.2%) cases of LH, eleven (3.6%) cases of sigmoid colectomy, 50 (16.3%) cases of Hartmann's procedure, and 106 (34.6%) cases of AR. In the RC group, there were 28 (22.4%) cases of Hartmann's procedure, 70 (56.0%) cases of AR, and 27 (21.6%) cases of APE. The study population's characteristics are summarized in Table 14.

Of the 464 Stage IV patients, 286 (61.6%) had isolated liver metastases, while 96 (20.7%) had metastases to other organs (lung and/or brain) exclusively. In 75 patients (16.3%), metastatic disease affected both the liver and other organs. A metastasectomy was performed in 83 patients (17.9%). Sixty-five patients (78.3%) had a staged metastasectomy; of these, 50 (76.9%) were performed for synchronous metastatic disease, and 15 (23.1%) for metachronous disease.

LNR

Both CC and RC resection specimens showed a median LN count of 17 nodes (CC range: 2-87, RC range: 3-49). Most resections yielded twelve or more nodes (see Table 15). In CC resections, a median of three positive nodes (range: 0-28) were found, while RC resections had a median of four involved nodes (range: 0-44). This resulted in a median LNR of 0.18 (IQR:

0.05-0.42) for CC resections and 0.21 (IQR: 0.09-0.47) for RC resections. Notably, 66 (19.5%) CC patients and 18 (14.4%) RC patients had an LNR=0.

Clinico-pathological Characteristics and LNR Associations in mCRC Patients (Table 16, Table 17 and Table 18)

The associations between standard clinico-pathological factors and LNR are summarised in Table 16 (LNR as a continuous variable), Table 17 and 18 (LNR as a dichotomous variable).

Colon Cancer

(i) LNR (Continuous)

On linear regression, LNR was significantly associated with the year of resection (β -0.02 [95%CI -0.04, -0.003]; P=0.02), left-sided CCs (β -0.07 [95%CI -0.12, -0.01]; P=0.02), number of metastatic sites (β 0.33 [95%CI 0.24, 0.42]; P<0.001), distant metastasectomy (β -0.09 [95%CI -0.16, -0.02]; P=0.02) and depth of tumour invasion (β 0.16 [95%CI 0.07, 0.17]; P<0.001)

(ii) LNR (Dichotomised)

On logistic regression, LNR>0 was associated with age (OR 0.96 [95%CI 0.94, 0.99]; P=0.001), number of metastatic sites (OR 2.63 [95%CI 1.002, 6.89]; P=0.049), tumour size (OR 0.87 [95%CI 0.78, 0.98]; P=0.02) and depth of tumour invasion (OR 2.24 [95%CI 1.33, 3.77]; P=0.002).

Rectal Cancer

(i) LNR (Continuous)

On linear regression, LNR was significantly associated with number of metastatic sites (β 0.41 [95%CI 0.26, 0.57]; P<0.001), distant metastasectomy (β -0.13 [95%CI -0.25, -0.02]; P=0.03) and histology type (β -0.26 [95%CI -0.45, -0.07]; P=0.008).

(ii) LNR (Dichotomised)

On logistic regression, LNR>0 was associated with decreasing rectal tumour height (OR 0.45 [95%CI 0.23, 0.87]; P=0.02), neo-adjuvant (chemo)RT (OR 0.23 [95%CI 0.07, 0.73]; P=0.01), and depth of tumour invasion (OR 6.55 [95%CI 1.86, 23.09]; P=0.003).

Comparison of Survival Outcomes between Clinico-pathological Characteristics including LNR in mCRC Patients (Table 19 and Table 20)

The associations between LNR and OS for both CC and RC patients, are presented in Table 19 and 20, respectively. Additionally, Figure 10 and Figure 11 present the Kaplan-Meier plots illustrating the OS for these patients.

Colon Cancer

Death occurred in 289 CC patients (85.3%), primarily due to CRC, with only 34 patients (11.8%) succumbing to non-cancer causes. The 5-year OS was 10.5% (95%CI 8.7-12.3).

i. LNR (Continuous)

On multivariate analysis with LNR as a continuous variable, increasing LNR (P=0.02) and T stage (P=0.006) correlated with poorer survival. Conversely, improved OS was associated with later years of the study (P<0.001), left-sided tumours (P=0.002) and distant metastasectomy (P<0.001).

ii. LNR (Dichotomised)

When analysed as a categorical variable in a multivariate model, LNR>0 was associated with poorer survival (P=0.02), as was increasing age (P<0.001), number of metastatic sites (P=0.03), and increasing depth of tumour invasion (P=0.003).

Rectal Cancer

In RC patients, 109 individuals (87.2%) died, with 103 (94.5%) deaths attributed to RC and six to other causes. The 5-year OS rate was 11.5% (95%CI 8.4-14.6).

i. <u>LNR (Continuous)</u>

In a multivariate model, poorer survival was noted with increasing LNR (P<0.001), while improved survival was observed in those undergoing a distant metastasectomy (P<0.001).

ii. <u>LNR (Dichotomised)</u>

On multivariate analysis, only LNR>0 was associated with poorer survival (P=0.02).

DISCUSSION

This extensive 27-year cohort study aimed to investigate the prognostic significance of LNR in patients with Stage IV CRC who underwent upfront resection of their primary tumour. The relationship between LNR and OS was tested (adjusting for confounders of LNR), treating LNR as both a continuous and dichotomous variable. In both CC and RC patients, an increasing LNR was found to worsen OS. Moreover, patients with mCRC who exclusively had non-lymphatic routes of dissemination (i.e., LNR=0, or N0 status) displayed a more favourable prognosis than those with lymphatic spread (i.e., LNR>0).

The significance of LNR as a prognostic factor in mCRC has been previously investigated, but with varying outcome measures and study populations[262,263,265,302-308]. Most studies, like ours, have confirmed an association between increasing LNR and poorer prognosis[262,263,265,302-310]. Some, however, included Stage I/II CRC patients in their study cohorts, wherein defining an LNR would seem inappropriate (given these patients all have an LNR=0, by definition). This approach may skew the nodal metastasis and survival outcome data[306,308]. Additionally, a few studies investigated only CC patients[263,305], focussed solely on either palliative resection[262] or curative resection[265,307], and violated principles of collinearity[302,310]. Notwithstanding this, our study adds to a growing body of literature that promotes the prognostic importance of LNR and its valuable contribution to patient survival following resection of the primary tumour, even in patients with Stage IV CRC.

This study uniquely dichotomised patients into those with an LNR=0, and those with LNR>0. To date, limited studies have explored survival differences based on this LNR dichotomization[264,286]. In the study by Zhang *et al.*[264], LNR was stratified into five groups, including LNR=0, to validate the discriminatory performance of previously published LNR cut-offs by Rosenberg *et al.*[286]. However, in both studies, the large number of LNR=0 patients were confounded by the inclusion of early-stage CRC patients. Dichotomisation of patients into those with an LNR=0 and LNR>0 is more meaningful, as it groups tumours based on their observed routes of spread. Specifically, patients with an LNR=0 represent those with tumours that have spread exclusively via haematogenous routes and/or transcoelomic spread, but not by lymphatic spread. By contrast, tumours with an LNR>0 are those demonstrating potentially all three routes of dissemination. Intuitively, the *absence* of LN spread in the *presence* of haematogenous or transcoelomic spread, as observed in LNR=0 patients, may identify patients whose tumours have potentially favourable biological behaviour and decreased tumour burden. Indeed, in our study, LNR=0 served as an independent predictor of improved survival in mCRC patients.

Notably, the majority of patients in our cohort had at least twelve LNs harvested during surgery. This probably reflects a standardised surgical approach to performing a CRC resection in our unit, irrespective of the presence of distant metastases. This approach is not universally embraced, though, as some surgeons argue that the lymphadenectomy holds little prognostic value for a mCRC resection as the presence of distant metastases overrides prognostication in these circumstances[266,311]. Nevertheless, a separate study by Jiao *et al.* demonstrated improved survival in mCRC patients with at least twelve LNs harvested, highlighting the role of an oncologically adequate lymphadenectomy as an element of good quality mCRC surgery[109]. Building on this, the demonstrable prognostic importance of LNR (specifically LNR=0, or N0 status) as demonstrated in our study can only be reliably ascertained in the presence of an adequate lymphadenectomy (i.e., LNY of at least twelve nodes).

The finding of a relatively small proportion of patients (16.6%) in our series who underwent an urgent operation warrants discussion. Published literature suggests that patients requiring urgent surgery represent a population with poorer outcomes compared to those with non-urgently resected CRCs[166]. This disparity in outcomes might stem from complex clinical presentations, such as perforation, obstruction or bleeding, and potentially insufficient LNY, which could lead to imprecise staging[166]. However, contrary to the perception that emergency surgery may be responsible for a *substandard* oncological resection, this study revealed no significant difference between urgent surgery and LNR, irrespective of its assessment as a continuous or dichotomous variable. In our study, the majority of operations were elective. This approach of elective upfront primary tumour surgery for mCRC remains widely recognised and practiced today, underlining the generalisability of our findings[109,263-265,295,296,303].

The optimal extent of resection and lymphadenectomy for patients with mCRC remains controversial. While SgR with limited lymphadenectomy may be considered in some cases, our study does not support its routine adoption. As already mentioned, the significant difference in survival observed between those with exclusively haematogenous or transcoelemic spread (i.e., LNR=0, or N0 status) and LNR>0 (where lymphatic routes are also implicated), emphasizes the importance of conducting an adequate lymphadenectomy to accurately ascertain the nodal status of mCRCs. Achieving this may be more feasible with a standard or extended resection compared with SgRs. While our study was not primarily focused on evaluating the extent of resection in mCRC patients, it highlights the benefits of performing a standardised approach to CRC resection whenever possible in all patients, regardless of whether the operation is executed laparoscopically or by an open approach. Furthermore, one could argue that standardising surgical techniques across all stages of CRC, regardless of metastatic status, contributes to a safer surgical approach by eliminating the risk of taking "short-cuts".

This study has limitations. The retrospective design introduces inherent biases. The limited rate of distant metastasectomy and the omission of patients who underwent nCT reduces our study population and narrows the generalizability of our findings. Likewise, the use of neo-adjuvant (chemo) RT in RC patients may have influenced LNY and nodal positivity rates. Whilst only 13% of patients received neo-adjuvant (chemo) RT, selection was primarily based on a patient's individual needs in accordance with contemporary practice guidelines at the time. Moreover, while our study primarily focused on mCRC patients who received upfront primary tumour resection—a recognized treatment strategy[295,296]—the uptake of nCT and *liver-first surgery* in managing mCRC may limit this study's generalizability to other treatment paradigms[312]. Finally, the limited availability of tumour biomarkers (e.g., BRAF, NRAS) restricts our analysis and comprehensive understanding of the biological behaviour of the tumour in mCRC patients.

In conclusion, this study enhances our understanding of mCRC management by investigating the prognostic value of the LNR in patients who underwent upfront surgery for the primary tumour. Notably, this study stands out as the only cohort study to date that has specifically examined the survival outcomes using dichotomization of LNR into LNR=0 and LNR>0. Our results highlight that this dichotomization holds useful prognostic information, with LNR=0 (i.e., N0 status) patients identified as a unique subgroup with a better prognosis. Thus, rather than dismissing it as futile, we emphasize the importance of conducting an adequate lymphadenectomy (i.e., harvesting at least twelve LNs) to accurately stage mCRC patients. Furthermore, identifying LNR=0 patients as a distinct subgroup calls for further research into their molecular and genetic characteristics, paving the way for refined, individualized treatment approaches, and targeted therapies in the management of mCRC.

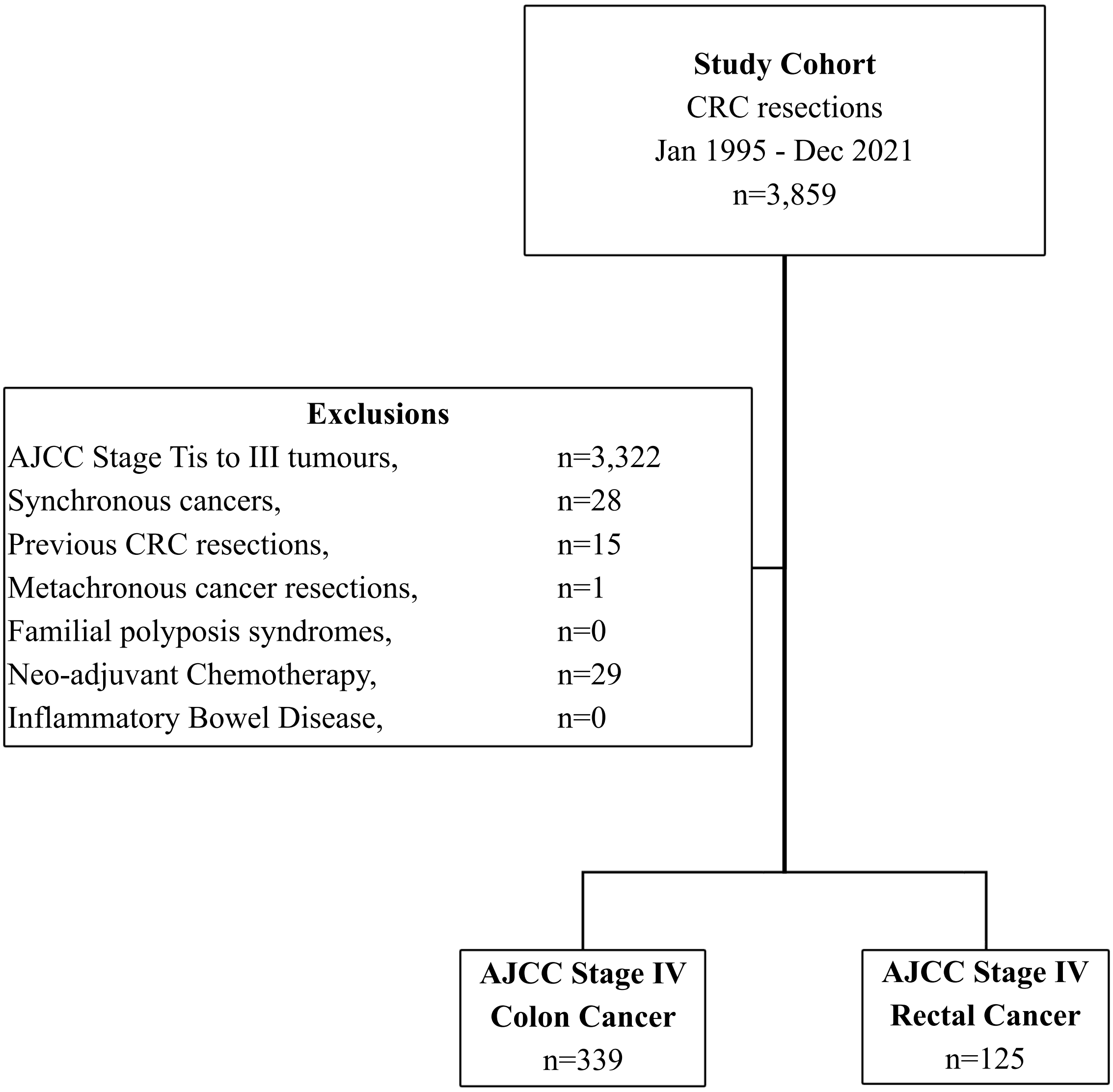

Figure 9: Flow diagram of cohort definition.

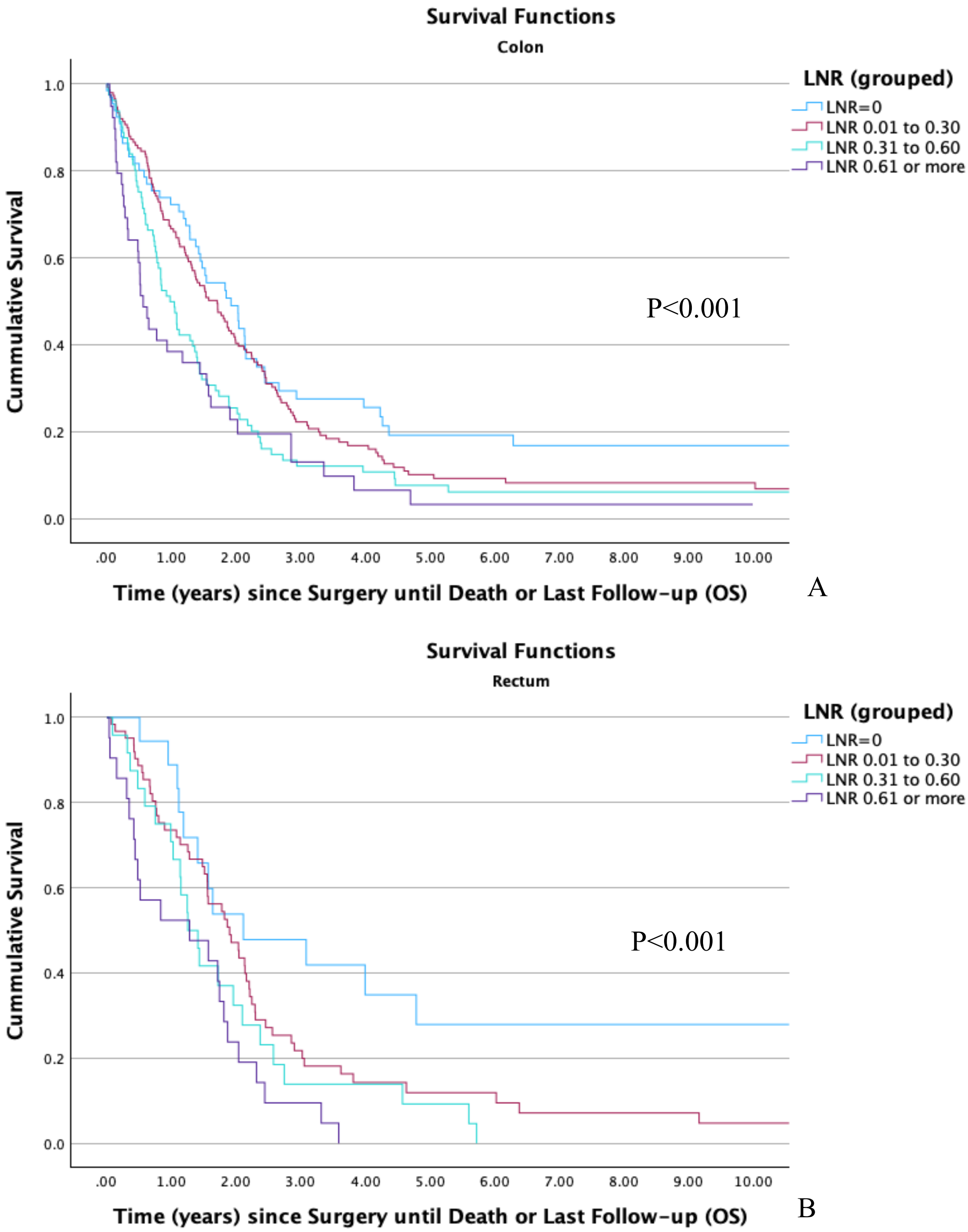

Figure 10: Kaplan-Meier plots of OS of Stage IV CC (A) and RC (B) patients stratified by LNRs.

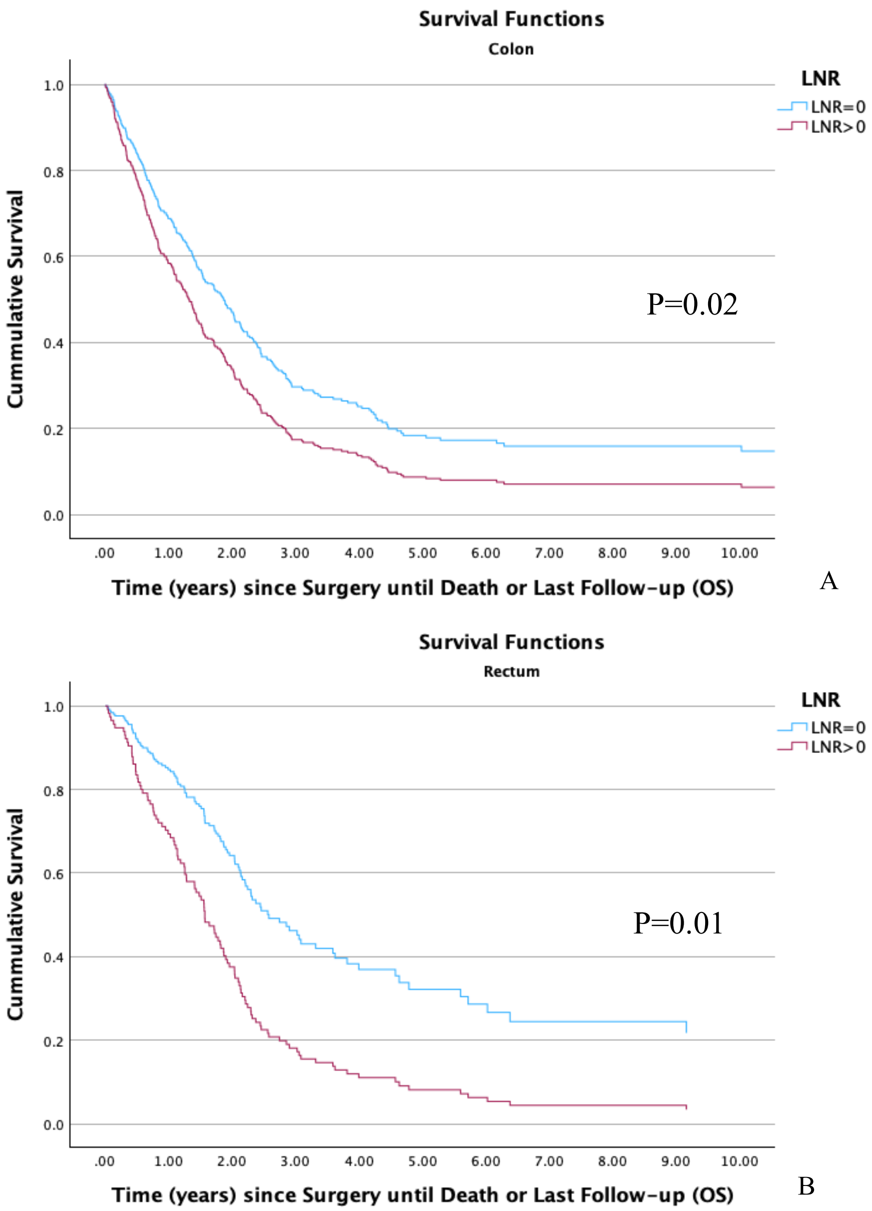

Figure 11: Kaplan-Meier plots of OS of Stage IV CC (A) and RC (B) patients stratified by LNR=0 and LNR>0.

TABLE 14. Comparison of Clinicopathological Factors Between Patients with CC and RC (n=464)			
	N (%) or Mean (SD) or Median (Range/ IQR)	**Colon Cancer (n [%], Total=339)**	**Rectal Cancer (n [%], Total=125)**
Gender			
Male	269(58.0)	191(56.3)	78(62.4)
Female	195(42.0)	148(43.7)	47(37.6)
Age (Mean [SD]), years	68.1(13.4)	69.1(13.6)	65.4(12.6)
BMI (Mean [SD]), kg/m^2	27.1(5.5)	27.1(5.7)	27.0(5.0)
ASA Grade			
I	68(14.7)	40(11.8)	28(22.4)
II	232(50.0)	164(48.4)	68(54.4)
III/IV	164(35.3)	135(39.8)	29(23.2)
Year of Resection			
1995 to 1999	95(20.5)	61(18.0)	34(27.2)
2000 to 2004	95(20.5)	59(17.4)	36(28.8)
2005 to 2009	94(20.3)	68(20.1)	26(20.8)
2010 to 2014	82(17.7)	71(20.9)	11(8.8)
2015 to 2019	74(15.9)	64(18.9)	10(8.0)
2020 onwards	24(5.2)	16(4.7)	8(6.4)
Emergency Operation			
No	387(83.4)	265(78.2)	122(97.6)
Yes	77(16.6)	74(21.8)	3(2.4)
Type of Operation			
Segmental Resection	3(0.6)	3(0.9)	-
Standard Resection	431(92.9)	306(90.3)	125(100.0)
Extended Resection	30(6.5)	30(8.8)	-
Operation Modality			
Open	321(69.2)	229(67.6)	92(73.6)
Laparoscopy	143(30.8)	110(32.4)	33(26.4)
Blood Loss (millilitres)			
≤ 500	428(92.2)	317(93.5)	111(88.8)
>500	36(7.8)	22(6.5)	14(11.2)
Tumour location (Colon)			
Right	145(42.8)	145(42.8)	
Left	194(57.2)	194(57.2)	
Tumour location (Rectum)			
Upper	47(37.6)		47(37.6)
Mid	43(34.4)		43(34.4)
Lower	35(28.0)		35(28.0)

Neoadjuvant RT (Rectal)			
No	108(86.4)	NA	108(86.4)
Yes	17(13.6)	NA	17(13.6)
Distant Metastatic Sites			
Liver or Other(s)	352(82.3)	279(82.3)	103(82.4)
Liver and Other(s)	75(16.3)	54(15.9)	21(16.8)
Distant Metastasectomy			
No	374(80.6)	275(81.1)	99(79.2)
Yes	83(17.9)	58(17.1)	25(20.0)
Tumour Size (Median [Range]), cm	5.0(0.6-15.0)	5.0(0.6-15.0)	5.0(0.6-10.0)
Tumour Perforation			
No	413(89.0)	307(90.6)	106(84.8)
Yes	51(11.0)	32(9.4)	19(15.2)
Obstructing Tumour			
No	402(86.6)	280(82.6)	122(97.6)
Yes	62(13.4)	59(17.4)	3(2.4)
T Stage			
T1 and T2	13(2.8)	6(1.8)	7(5.6)
T3	262(56.5)	173(51.0)	89(71.2)
T4	189(40.7)	160(47.2)	29(23.2)
Histological Type			
Mucinous/Signet ring	47(10.1)	39(11.5)	8(6.4)
Non-Mucinous/Signet ring	417(89.9)	300(88.5)	117(93.6)
Histological Differentiation			
Well or Moderate	297(64.0)	212(62.5)	85(68.0)
Poor	167(36.0)	127(37.5)	40(32.0)
Histological Grade			
Low or Average	251(54.1)	184(54.3)	67(53.6)
High	213(45.9)	155(45.7)	58(46.4)
Lympho-Vascular Invasion			
No	160(34.5)	120(35.4)	40(32.0)
Yes	304(65.5)	219(64.6)	85(68.0)
Peri-Neural Invasion			
No	254(54.7)	188(55.5)	66(52.8)
Yes	210(45.3)	151(44.5)	59(47.2)
Lymph Nodes – Examined (Median [Range])	17(2-87)	17(2-87)	17(3-49)
Lymph Node Yield			
< 12	103(22.2)	72(21.2)	31(24.8)
≥ 12	361(77.8)	267(78.8)	94(75.2)

Lymph Node Ratio - Positive nodes/ total nodes examined (Median [IQR])	0.20(0.06-0.43)	0.18(0.05-0.42)	0.21(0.09-0.47)
Lymph Node Ratio (LNR [Dichotomised])			
LNR=0	84(18.1)	66(19.5)	18(14.4)
LNR>0	380(81.9)	273(80.5)	107(85.6)
BMI: **Body Mass Index;** ***ASA:*** **American Society of Anesthesiology;** ***RT:*** **Radiotherapy;** ***NA:*** **Not Applicable;** ***and T stage:*** **Tumour Stage (part of AJCC TNM system)**			

Table 14: Clinical characteristics and histopathological descriptions of Stage IV CC and RC patients.

TABLE 15. Comparison of LNY between CC and RC Patients with LNR=0 and LNR>0 (n=464)					
	N (%) or Mean (SD) or Median (Range/ IQR)	**Colon Cancer (Total=339)**		**Rectal Cancer (Total=125)**	
		LNR=0 (n [%], Total=66)	**LNR>0 (n [%], Total=273)**	**LNR=0 (n [%], Total=18)**	**LNR>0 (n [%], Total=107)**
Lymph Node Yield					
< 12	103(22.2)	16(24.2)	56(20.5)	9(50.0)	22(20.6)
≥ 12	361(77.8)	50(75.8)	217(79.5)	9(50.0)	85(79.4)
LNR: **Lymph Node Ratio**					

Table 15: Comparison of dichotomised LNR by lymph node yield in Stage IV CC.

TABLE 16. Comparison of clinicopathological factors and LNR (Stage IV)				
	Lymph Node Ratio			
	Colon (n=339)		Rectum (n=125)	
	β (95% CI)	P-value	β (95% CI)	P-value
Gender Male Female	 Ref 0.004(-0.05 to 0.06)	 - 0.89	 Ref -0.004(-0.10 to 0.09)	 - 0.93
Age (Mean [SD]), years	-0.001(-0.003 to 0.001)	0.39	0.0001(-0.004 to 0.003)	0.87
BMI (Mean [SD]), kg/m^2	0.0001(-0.01 to 0.01)	0.88	0.004(-0.01 to 0.02)	0.61
ASA Grade I II III/IV	-0.03(-0.07 to 0.01)	0.15	-0.02(-0.09 to 0.05)	0.61
Year of Surgery 1995 to 1999 2000 to 2004 2005 to 2009 2010 to 2014 2015 to 2019 2020 onwards	-0.02(-0.04 to -0.003)	0.02*	-0.02(-0.05 to 0.01)	0.19
Emergency Operation No Yes	 Ref -0.03(-0.10 to 0.03)	 - 0.32	 Ref 0.07(-0.25 to 0.38)	 - 0.68
Tumour Perforation No Yes	 Ref -0.05(-0.14 to 0.05)	 - 0.30	 Ref 0.11(-0.02 to 0.24)	 - 0.09
Obstructive Presentation No Yes	 Ref -0.04(-0.11 to 0.04)	 - 0.35	 Ref 0.07(-0.25 to 0.38)	 - 0.68
Operation Modality Open Laparoscopy	 Ref -0.03(-0.09 to 0.03)	 - 0.27	 Ref -0.09(-0.19 to 0.02)	 - 0.12
Operation Type Segmental Standard Extended	-0.09(-0.18 to 0.002)	0.05	†	
Colon Cancer Location Right Left	 Ref -0.07(-0.12 to -0.01)	 - 0.02*		
Rectal Cancer Location Upper Mid Lower			-0.04(-0.10 to 0.02)	0.17
Neoadjuvant RT (Rectal) No Yes			 Ref -0.06(-0.19 to 0.08)	 - 0.44
Distant Metastatic Sites Liver or Other Liver and Other(s)	 Ref 0.33(0.24 to 0.42)	 - <0.001*	 Ref 0.41(0.26 to 0.57)	 - <0.001*
Distant Mets Resected No Yes	 Ref -0.09(-0.16 to -0.02)	 - 0.02*	 Ref 0.13(-0.25 to -0.02)	 - 0.03*
Tumour Size (Median [IQR]), cm	0.003(-0.01 to 0.02)	0.67	0.01(-0.02 to 0.04)	0.40
T Stage T1 and T2 T3 T4	0.16(0.07 to 0.17)	<0.001*	0.09(-0.01 to 0.18)	0.07
Histological Type Mucinous/Signet ring	 Ref	 -	 Ref	 -

Non-Mucinous/Signet ring	-0.05(-0.14 to 0.03)	0.22	*-0.26(-0.45 to -0.07)*	*0.008**
BMI: Body Mass Index; ASA: American Society of Anesthesiology; RT: Radiotherapy*; and T stage:* Tumour Stage (part of AJCC TNM system); † All Stage IV rectal cancers were managed with a standard resection*;* * P<0.05				

Table 16: Association of clinicopathological factors with LNR (continuous) in Stage IV CC and RC patients.

TABLE 17. Comparison of clinicopathological factors between patients with LNR=0 and LNR>0 in Stage IV Colon Cancer (n=339)					
	N (%) or Mean (SD) or Median (Range/ IQR)	**LNR=0 n (%) (Total=66)**	**LNR>0 n (%) (Total=273)**	***P*-value**	**Odds Ratio (95% CI)**
Gender					
Male	191(56.3)	41(62.1)	150(54.9)	-	ref
Female	148(43.7)	25(37.9)	123(45.1)	0.29	1.35(0.78-2.33)
Age (Mean [SD]), years	69.1(13.6)	74.0(11.1)	67.9(13.9)	*0.001**	*0.96(0.94-0.99)*
BMI (Mean [SD]), kg/m^2	27.1(5.7)	26.5(4.9)	27.3(5.8)	0.50	1.03(0.95-1.11)
ASA Grade				0.12	0.72(0.47-1.09)
I	40(11.8)	7(10.6)	33(12.1)		
II	164(48.4)	26(39.4)	138(50.5)		
III/IV	135(39.8)	33(50.0)	102(37.4)		
Year of Surgery				0.47	1.07(0.89-1.28)
1995 to 1999	61(18.0)	14(21.2)	47(17.2)		
2000 to 2004	59(17.4)	9(13.6)	50(`8.3)		
2005 to 2009	68(20.1)	19(28.8)	49(17.9)		
2010 to 2014	71(20.9)	10(15.2)	61(22.3)		
2015 to 2019	64(18.9)	10(15.2)	54(19.8)		
2020 onwards	16(4.7)	4(6.1)	12(4.4)		
Emergency Operation					
No	265(78.2)	46(69.7)	219(80.2)	-	Ref
Yes	74(21.8)	20(30.3)	54(19.8)	0.07	0.57(0.31-1.04)
Tumour Perforation					
No	307(90.6)	58(87.9)	249(91.2)	-	Ref
Yes	31(9.1)	8(12.1)	23(8.8)	0.41	0.70(0.30-1.63)
Obstructive Presentation					
No	280(82.6)	52(78.8)	228(83.5)	-	Ref
Yes	59(17.4)	14(21.2)	45(16.5)	0.37	0.73(0.38-1.43)
Operation Modality					
Open	229(67.6)	47(71.2)	182(66.7)	-	Ref
Laparoscopy	110(32.4)	19(28.8)	91(33.3)	0.48	1.24(0.69-2.23)
Operation Type				0.31	1.68(0.62-4.55)
Segmental	3(0.9)	1(1.5)	2(0.7)		
Standard	306(90.3)	61(92.4)	245(89.7)		
Extended	30(8.8)	4(6.1)	26(9.5)		
Colon Cancer Location					
Right	145(42.8)	22(33.3)	123(45.1)	-	Ref

Left	194(57.2)	44(66.7)	150(54.9)	0.09	0.61(0.35-1.07)
Distant Metastatic Sites Liver or Other Liver and Other(s)	 279(82.3) 54(15.9)	 59(89.4) 5(7.6)	 220(80.5) 49(17.9)	 - *0.049**	 *Ref* *2.63(1.002-6.89)*
Distant Mets Resected No Yes	 275(81.1) 58(17.1)	 57(86.4) 7(10.6)	 218(79.9) 51(18.7)	 - 0.13	 Ref 1.91(0.82-4.42)
Tumour Size (Median [IQR]), cm	5.0(4.0-6.0)	5.5(4.1-7.3)	5.0(4.0-6.0)	*0.02**	*0.87(0.78-0.98)*
T Stage T1 and T2 T3 T4	 6(1.8) 173(51.0) 160(47.2)	 1(1.5) 46(69.7) 19(28.8)	 5(1.8) 127(46.5) 141(51.6)	*0.02**	*2.24(1.33-3.77)*
Histological Type Mucinous/Signet ring Non-Mucinous/Signet ring	 39(11.5) 300(88.5)	 8(12.1) 58(87.9)	 31(11.4) 242(88.6)	0.86	1.08(0.47-2.47)
***BMI: Body Mass Index*; *ASA:* American Society of Anesthesiology*; and T stage:* Tumour Stage (part of AJCC TNM system)*;* * P<0.05**					

Table 17: Comparison of clinicopathological factors between LNR=0 and LNR>0 in Stage IV colon cancer patients.

TABLE 18. Comparison of clinicopathological factors between patients with LNR=0 and LNR>0 in Stage IV RC (n=125)					
	N (%) or Mean (SD) or Median (Range/ IQR)	**LNR=0 n (%) (Total=18)**	**LNR>0 n (%) (Total=107)**	***P*-value**	**Odds Ratio (95% CI)**
Gender					
Male	78(62.4)	9(50.0)	69(64.5)	-	Ref
Female	47(37.6)	9(50.0)	38(35.5)	0.89	0.92(0.30-2.82)
Age (Mean [SD]), years	65.4(12.6)	66.8(12.8)	65.1(12.6)	0.61	0.99(0.95-1.03)
BMI (Mean [SD]), kg/m^2	27.0(5.0)	28.0(6.8)	26.9(4.7)	0.61	0.96(0.81-1.14)
ASA Grade				0.49	0.77(0.36-1.62)
I	28(22.4)	2(11.1)	26(24.3)		
II	68(54.4)	12(66.7)	56(52.3)		
III/IV	29(23.2)	4(22.2)	25(23.4)		
Year of Surgery				0.50	1.13(0.79-1.62)
1995 to 1999	37(29.6)	8(44.4)	26(24.3)		
2000 to 2004	36(28.8)	4(22.2)	32(29.9)		
2005 to 2009	26(20.8)	1(5.6)	25(23.4)		
2010 to 2014	11(8.8)	2(11.1)	9(8.4)		
2015 to 2019	10(8.0)	2(11.1)	8(7.5)		
2020 onwards	8(6.4)	1(5.6)	7(6.5)		
Emergency Operation					
No	122(97.6)	18(100.0)	104(97.2)		
Yes	3(2.4)	-	3(2.8)		
Tumour Perforation					
No	106(84.8)	17(94.4)	89(83.2)	-	Ref
Yes	19(5.2)	1(5.6)	18(16.8)	0.24	3.44(0.43-27.51)
Obstructive Presentation					
No	122(97.6)	18(100.0)	104(97.2)	-	Ref
Yes	3(2.4)	-	3(2.8)	1.00	NA
Operation Modality					
Open	92(73.6)	13(72.2)	79(73.8)	-	Ref
Laparoscopy	33(26.4)	5(27.8)	28(26.2)	0.89	0.92(0.30-2.82)
Operation Type					
Segmental	-	-	-		
Standard	125(100.0)	18(100.0)	107(100.0)		
Extended	-	-	-		
Rectal cancer location				*0.02***	*0.45(0.23-0.87)*
Upper	47(37.6)	3(16.7)	44(41.1)		

Mid Lower	43(34.4) 35(28.0)	6(33.3) 9(50.0)	37(34.6) 26(24.3)		
Neoadjuvant RT (Rectal) No Yes	 108(86.4) 17(3.6)	 12(66.7) 6(33.3)	 96(89.7) 11(10.3)	 - *0.01**	 *Ref* *0.23(0.07-0.73)*
Distant Metastatic Sites Liver or Other Liver and Other(s)	 103(82.4) 21(16.8)	 18(100.0) -	 85(79.4) 21(19.6)	 - NA	 Ref NA
Distant Mets Resected No Yes	 99(79.2) 25(20.0)	 12(66.7) 6(33.3)	 87(81.3) 19(17.8)	 - 0.14	 Ref 0.44(0.15-1.31)
Tumour Size (Median [IQR]), cm	5.0(4.0-6.0)	5.3(4.5-7.1)	5.0(4.0-6.0)	0.35	0.88(0.66-1.16)
T Stage T1 and T2 T3 T4	 7(5.6) 89(71.2) 29(23.2)	 3(16.7) 15(83.3) -	 4(3.7) 74(69.2) 29(27.1)	*0.03**	*6.55(1.86-23.09)*
Histological Type Mucinous/Signet ring Non-Mucinous/Signet ring	 8(6.4) 117(93.6)	 - 18(100.0)	 8(7.5) 99(92.5)	 - NA	 Ref NA
***BMI: Body Mass Index; ASA:* American Society of Anesthesiology; *RT: Radiotherapy; and T stage:* Tumour Stage (part of AJCC TNM system); * P<0.05**					

Table 18: Comparison of clinicopathological factors between LNR=0 and LNR>0 in Stage IV RC patients.

TABLE 19. Clinicopathological Features and Oncological Associations in Stage IV Patients(n=464) – LNR CONTINUOUS								
	Overall Survival							
	Colon				**Rectum**			
	HR (95%CI)	***P*-value**	**aHR (95%CI)**	***P*-value**	**HR (95%CI)**	***P*-value**	**aHR (95%CI)**	***P*-value**
Year of Surgery	*0.84(0.77-0.91)*	*<0.001**	*0.84(0.77-0.92)*	*<0.001**				
1995 to 1999								
2000 to 2004								
2005 to 2009								
2010 to 2014								
2015 to 2019								
2020 onwards								
Colon Cancer Location								
Right	*ref*	-	*ref*	-				
Left	*0.68(0.54-0.86)*	*0.001**	*0.68(0.54-0.87)*	*0.002**				
Distant Metastatic Sites								
Liver or Other	*ref*	-	ref	-	ref	-	ref	-
Liver and Other(s)	*1.52(1.12-2.06)*	*0.007*	1.24(0.90-1.71)	0.18	1.34(0.81-2.22)	0.26	0.82(0.48-1.40)	0.46
Distant Mets Resected								
No	*ref*	-	*ref*	-	*ref*	-	*ref*	-
Yes	*0.36(0.25-0.50)*	*<0.001**	*0.43(0.30-0.62)*	*<0.001**	*0.36(0.22-0.60)*	*<0.001**	*0.39(0.23-0.65)*	*<0.001**
T Stage	*1.42(1.14-1.76)*	*0.02**	*1.41(1.11-2.68)*	*0.006**				
T1 and T2								
T3								
T4								
Histological Type								
Mucinous/Signet ring					*ref*	-	*ref*	-
Non-Mucinous/Signet ring					0.80(0.39-1.64)	0.54	0.95(0.46-1.98)	0.90
LNR (Continuous)	*2.72(1.78-4.16)*	*<0.001**	*1.72(1.11-2.68)*	*0.02**	*3.83(1.98-7.40)*	*<0.001**	*3.48(1.76-6.88)*	*<0.001**
HR: **Hazard Ratio;** ***aHR:*** **Adjusted Hazard Ratio;** ***LNR:*** **Lymph Node Ratio; and** ***NA*****: Not Applicable*;* * P<0.05**								

Table 19:Uni-variate and Multi-variate Analyses of Survival Outcomes between Clinicopathological Characteristic Including LNR (Continuous) in mCRC patients.

TABLE 20. Clinicopathological Features and Oncological Associations in Stage IV Patients (n=464) - LNR CATEGORISED								
	Overall Survival							
	Colon				**Rectum**			
	HR (95%CI)	**P-value**	**aHR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**	**aHR (95%CI)**	**P-value**
Age, years	*1.02(1.01-1.03)*	*<0.001**	*1.02(1.02-1.03)*	*<0.001**				
Rectal Cancer Location Upper Mid Lower					1.00(0.79-1.26)	0.99	1.09(0.84-1.43)	0.52
Neoadjuvant RT (Rectal) No Yes					 *Ref* 0.64(0.34-1.20)	 - 0.17	 *Ref* 0.77(0.40-1.49)	 - 0.43
Distant Metastatic Sites Liver or Other Liver and Other(s)	 *Ref* *1.52(1.12-2.06)*	 - *0.007**	 *Ref* *1.42(1.04-1.94)*	 - *0.03*				
Tumour Size, cm	1.01(0.96-1.07)	0.62	1.00(0.95-1.06)	0.91				
T Stage T1 and T2 T3 T4	*1.42(1.14-1.76)*	*0.002**	*1.41(1.12-1.77)*	*0.003**	1.08(0.73-1.58)	0.71	0.94(0.60-1.48)	0.80
LNR (Categorised) LNR=0 LNR>0	 *Ref* *1.44(1.06-1.96)*	 - *0.02**	 *Ref* *1.50(1.08-2.07)*	 - *0.02**	 *Ref* *2.21(1.21-4.06)*	 - *0.01**	 *Ref* *2.21(1.16-4.24)*	 - *0.02**
***HR*: Hazard Ratio; *aHR:* Adjusted Hazard Ratio; *RT*: Radiotherapy and *LNR*: Lymph Node Ratio; *P<0.05**								

Table 20: Uni-variate and Multi-variate Analyses of Survival Outcomes between Clinicopathological Characteristic Including LNR (Dichotomised) in mCRC patients.

PART II:"APPROPRIATENESS" OR "CORRECTNESS" OF LYMPHADENECTOMY

CHAPTER V: Survival Following Resection of Splenic Flexure Cancer: A 25-year Experience and Implications for Complete Mesocolic Excision (CME) and Central Vascular Ligation (CVL) Surgery

This chapter has been prepared, submitted and published in *Australia and New Zealand Journal of Surgery* as **"Naidu K, Chapuis PH, Brown K, Chan C, Rickard MJFX, Ng KS. Splenic Flexure Cancer Survival: A 25-year Experience and Implications for Complete Mesocolic Excision (CME) and Central Vascular Ligation (CVL). ANZ J Surg. 2023 Jul-Aug;93(7-8):1861-1869. doi: 10.1111/ans.18434. Epub 2023 Mar 28. PMID: 36978261".** The published manuscript is included as **APPENDIX IV** in **Chapter IX**.

Preamble

Having addressed the EoL as a measure of quality in CRC surgery, Part II of this thesis shifts focus to a complementary aspect of understanding quality of CRC surgery: whether an "appropriate" lymphadenectomy has been performed.

As described in the Introduction (**Chapter I**) of this thesis, consideration regarding "appropriateness" of a lymphadenectomy is pertinent for cancers of watershed areas of the colorectum. These watershed areas, such as at the SF and mid-distal rectum, are characterised by variable (and often, unpredictable) lymphatic drainage. Such variability of lymphatic drainage makes a complete lymphadenectomy challenging, as radical excision of all potential draining LN packages adds unnecessary morbidity, and/or results in excessive (and unwarranted) colonic devascularisation without oncological advantage. **Chapters V** and **VI** will focus specifically on the SF, guiding the reader through unique considerations regarding the "appropriateness" or "correctness" of lymphadenectomy practices when managing patients with SFC. It is beyond the scope of this thesis to look at the watershed area of the rectum.

Given the potential for SFCs to drain to either MCA or IMA nodal basins, it would seem intuitive to suppose that the outcomes of SFC patients to be inferior to their non-SFC counterparts. This is because radical excision of both MCA and IMA pedicles is rarely performed (due to extensive devascularisation incurred to the left colon), raising the question of whether surgery for SFCs necessarily incurs a sub-standard, or incomplete, lymphadenectomy. Moreover, as many SFCs are diagnosed following an emergency presentation with obstruction or perforation, it could be argued that outcomes of SFCs are further worsened by the nature of their emergency presentation.

Nevertheless, as described in the Introduction of this thesis, evidence surrounding long-term survival in SFC patients is conflicting. **Chapter V** seeks to add to existing literature and provide context regarding outcomes of contemporary SFC management. Through the *Concord experience*, this Chapter aims to define an SFC phenotype and elucidate long-term oncological outcomes in relation to key clinicopathological factors.

ABSTRACT

INTRODUCTION: The management of SFCs in the era of CME and CVL is challenging because of its variable lymphatic drainage. This study aimed to compare survival outcomes for SFCs and non-SFCs and better understand the clinicopathological characteristics which may define a distinct SFC phenotype.

METHODS: An observational cohort study at Concord Hospital, Sydney was conducted with patients who underwent resection for colon adenocarcinoma (1995-2019). Clinicopathological data were extracted from a prospective database. OS and DFS estimates and their associations to clinicopathological variables were investigated with Kaplan–Meier and Cox regression analyses.

RESULTS: Of 2,149 patients with CC, 129 (6%) had an SFC. The overall 5-year OS and DFS rates were 63.6% (95%CI 62.5-64.7) and 59.4% (95%CI 58.3-60.5), respectively. SFCs were not associated with OS (P=0.6) or DFS (P=0.5). SFCs were more likely to present urgently (P<0.001) with obstruction (P<0.001) or perforation (P=0.03), and more likely to require an open operation (P<0.001). These characteristics were associated with poorer survival outcomes. No differences were noted between SFCs and non-SFCs with respect to tumour stage (P=0.3).

CONCLUSION: SFCs have a distinct phenotype, the individual characteristics of which are associated with poorer survival. However, the survivals of SFCs and non-SFCs are similar, possibly because the most important determinant of outcome, tumour stage, is no different between the groups. This may have implications for the surgical approach to SFCs with respect to standardisation of CME and CVL surgery for these cancers.

INTRODUCTION

In the era of CME and CVL, dissection along bloodless embryonic tissue planes with a HVT theoretically ensures removal of a pre-defined lympho-neuro-vascular package enveloped between intact parietal peritoneum. This approach has been demonstrated to result in superior specimen quality[5,200] and convey a survival advantage in several cohort studies[7,9,12,200,218]. However, it is difficult to apply the principles of CME to areas of watershed blood supply such as the SF owing to the lack of clarity as to whether SF lymphatic drainage preferences the MCA or the IMA nodal basins, or both[156,157,174].

From early cadaveric studies to more recent *in vivo* non-physiological experimentation, metastatic routes stretching from the paracolic nodes to the SMA, the MCA, the accessory MCA, the IMA and LCA nodal basins have been described[153,156,157,191,313-315]. These random anatomical incongruences, together with the lack of dedicated CME and CVL guidelines for SFC[12] have invariably led to different surgical approaches, and crucially, a failure to target a dominant lymphovascular drainage pedicle[12]. Intuitively, it could be argued that SFCs, unlike other CC subsites are at risk of a substandard and potentially non-curative resection.

We hypothesize that SFCs are associated with poorer survival outcomes compared to other CC subsites. Therefore, the primary aim of this study was to analyse a single centre's 25-year experience with colectomy for CC, comparing long-term survival outcomes of SFCs and non-SFCs. A subsidiary aim was to explore whether clinicopathological characteristics and macroscopic features might indicate a unique phenotype of SFCs which would account for any differences in their long-term survival outcomes compared to other subsites.

MATERIAL and METHODS

Consecutive patients who underwent a potentially curative colectomy for a solitary CC between January 1995 and December 2019 at CRGH, Sydney, Australia, were included in this observational retrospective cohort study. Patients were identified from a prospectively maintained institutional database, where clinical, operative details, tumour pathology, and follow-up data were extracted for analysis, comparing SFC and non-SFC resections[227]. Patients excluded were those with a RC, in-situ neoplasia, polyposis coli, a previous CC, and those who developed a metachronous cancer (Figure 12).

Ethical approval was granted by the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206).

Pathology Reporting and Staging

The precise tumour location was marked by the surgeon on a diagram at the completion of the operation, with SFCs defined as tumours located between the distal 10cm of the transverse colon to the proximal 10cm of the descending colon[174]. All resected specimens were examined by pathologists with a special interest in CRC using a standard synoptic protocol, and all pathology data were coded by one of us (ChC)[229]. Only adenocarcinomas (including mucinous and signet ring types) were included in the analyses. Tumour morphology was defined as pedunculated, where a tumour was attached to the colonic mucosa by a stalk or pedicle, regardless of base size; sessile, where a tumour was flat and lacked a pedicle; ulcerated, where a tumour had a crater-like appearance; and stenosing, where a tumour had an occluding and narrowed lumen. These morphological characteristics were not mutually exclusive. Tumours were staged according to the AJCC pTNM system[22]. All pathology variables included in the data set were recorded for every specimen and their presence or absence noted explicitly.

Surgical Management of SFCs

At our institution, SFC patients were managed with either an ERH, a LH, or a SgR. An ERH was defined as resection of the distal terminal ileum through to the proximal descending colon with ligation of the ICA, RCA (if present), MCA and LCA. In a LH, the distal third of the transverse colon, SF and proximal descending colon is resected with ligation of the left branch of the MCA and LCA. In the SgR technique, the cancer is resected with 5cm of colon either side of it, with no dedicated pedicle ligation. All resections were performed by colorectal surgeons with CVL not routinely practised[316]. An urgent operation was defined as an unscheduled operation.

Surveillance and Follow-up

Patients were reviewed at least six-monthly for the first two years post-resection and followed up annually thereafter until death or December 2021 (unless lost to follow-up)[232]. Surveillance included clinical examination, liver function tests, serial carcinoembryonic antigen measurements and, more recently, selective CT and positron emission tomography imaging. Colonoscopy was generally performed one, three- and five-years post-resection. Patients with LN positive tumours and associated poor prognostic features such as age, comorbidities, post-operative complications, and adverse prognostic features (e.g., PNI or LVI) were routinely discussed at a multi-disciplinary meeting, generally with a view for offering adjuvant chemotherapy.

The date of resection was the starting point for follow-up times. Follow-up times were censored at last contact for patients who did not experience the terminal event up to December 2021, who were lost to follow-up, or who remained alive. The underlying cause of death was coded according to the International Classification of Diseases-10. All clinical and operative data were recorded by one of us (PC) in addition to information provided by the GP, operating surgeon, and close family members.

Outcome Measures

The primary outcome measures were OS and DFS[174,235]. Secondary outcome measures included LR and SR, where recurrence was defined as clinically or radiologically suspected or biopsy-proven tumour in the peritoneal cavity or newly diagnosed distant metastasis[235].

Statistical Analyses

Continuous variables were reported as mean (SD) for normally distributed variables and median (IQR or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages. Logistic regression analyses were performed to determine any differences in continuous or categorical data between the SFC and non-SFC cohorts. Survival and recurrence estimates were modelled using the Kaplan-Meier function with log-rank test performed to determine difference in survival distributions. Cox-regression modelling tested associations between outcome measures and relevant clinicopathological variables. The level for 2-tailed statistical significance was $P<0.05$ with confidence intervals at the 95% level. All analyses were completed using SPSS v.28 (IBM, New York, USA).

RESULTS

Study Population

A total of 3,657 patients underwent a resection for a primary CRC during the study period of which 1,508 patients were sequentially excluded (Figure 12), leaving 2,149 suitable patients for analysis. Of these, 129 patients (6%) had a SFC, with 45 (34.9%), 64 (49.6%), 20 (15.5%) patients operated on in the first decade, second decade and last five years of the study period, respectively. The features of all these patients are summarised in Table 21.

Amongst the SFC patients, 67 (51.9%) were male; their mean age was 69.4 years (SD 14.7) and the median hospital length of stay (LOS) was 10 days (range 8-15). An ERH was performed in 59 patients (45.7%) and this was regardless of the mode of presentation. However, in 101 SFC patients operated electively, 45 (53.6%) had a LH performed, 35 (41.7%) had an ERH, and four (4.7%) had a SgR. When grouped according to exact tumour location, 66 patients (51.2%) had a cancer located at the "true" SF (i.e., corresponding to the colonic angulation relating to the spleno-colic ligament), 41 (31.8%) had a cancer at the distal transverse colon and 22 (17.0%) had a cancer situated at the proximal descending colon. Of the 66 patients with a "true" SFC, 28 (42.4%) underwent an ERH whilst 26 (39.4%) had a LH performed. Of the 41 patients with a distal transverse CC, 27 (65.9%) underwent an ERH whilst 9 (22.0%) underwent a LH. Of the 22 patients with a proximal descending CC, 4 (18.2%) underwent an ERH whilst 11 (50.0%) had a LH. An urgent operation was performed in 28 SFC patients (21.7%). Of those operated urgently, 23 patients (82.1%) were obstructed. An open operation was performed in 99 patients (76.7%).

Detailed clinical characteristics, the predominant macroscopic tumour morphology, and histopathological descriptions of SFCs and non-SFCs and comparisons of clinicopathological factors between SFC and other subsites are shown in Table 21. The comparison of survival outcomes between clinicopathological characteristics of all cases are detailed in Table 22.

Follow-up and Survival Outcomes

For the entire study population (SFCs and non-SFCs), death occurred in 1,195 patients (55.6%). Of the 954 surviving patients, the median follow-up time was 10 years. The median OS and DFS was 9.4 years (95% CI 8.6-10.3) and 8.4 years (95% CI 7.4-9.3), respectively. The 5-year OS and DFS rates were 63.6% (95%CI 62.5-64.7) and 59.4% (95%CI 58.3-60.5), respectively. A LR was diagnosed in 69 patients (3.2%) whilst a SR was diagnosed in 361 patients (16.8%). The median time to LR and SR of the study population was 1.2 years (95% CI 0.9-1.5) and 1.5 years (95% CI 1.2-1.7), respectively.

Comparison of Survival Outcomes between SFCs and non-SFCs (Table 22)

The median OS and DFS of patients with SFC were 9.9 years (95% CI 6.1-13.7) and 9.5 years (95% CI 5.5-13.5), respectively. The 5-year OS and DFS rates of patients with SFC were 65.1% (95%CI 60.8-69.4) and 61.4% (95%CI 57.1-65.7), respectively. SFCs were not associated with OS (P=0.6), DFS (P=0.5), LR (P=0.9) or SR (P=0.5). These findings persisted with adjustment for age at surgery, with no association seen with OS [aHR=1.0 (95%CI 0.8-1.3), P=1.0] or DFS [aHR=1.0 (95%CI 0.8-1.2), P=0.8]. Further, on a subgroup analysis of patients undergoing elective surgery, SFCs were not associated with OS (P=0.2) or DFS (P=0.2).

With respect to clinicopathological characteristics within the entire cohort, a poorer OS was associated with being male (P=0.01), increasing age (P<0.001), ASA >1 (P<0.001), having an urgent resection (P<0.001) performed for either obstruction (P<0.001) or perforation (P<0.001), having an open surgery (P<0.001) and an intraoperative blood loss of more than

500mls (P=0.01). For pathological features, a poorer OS was associated with AJCC Stage III (P<0.001) and IV (P<0.001) tumours, and those that were predominantly stenosed (P<0.001) or ulcerated (P<0.001). A lymphadenectomy with a LN harvest of less than twelve LNs (P=0.002) and an increased LOS were also associated with poorer OS (P<0.001). OS was longer in the presence of a pedunculated lesion (P<0.001). Poor prognosis characterised by increased DFS hazards mirrored all the factors associated with OS above and are displayed in Table 22.

Clinicopathological Variables and SFC Associations (Table 21)

Table 21 summarises the associations between SFCs and other clinicopathological variables. When compared with non-SFCs, SFCs were associated with an urgent operation (P<0.001), and an obstructed (P<0.001) or a perforated (P=0.03) presentation. SFCs were more likely to be managed by an open procedure (P <0.001) and, when begun laparoscopically, showed no difference in rates of requiring an open conversion (P=0.2). Surgery for SFCs were more likely to be associated with a splenic injury (P<0.001) or requiring a splenectomy (P<0.001).

No differences were noted between SFCs and non-SFCs with regards to AJCC tumour stage (Stage III [P=0.3] and Stage IV [P=0.9]) or tumour size (P=1.0). Macroscopically, SFC patients were more likely to harbour a stenosing tumour (P<0.001). SFC tumours also favoured a sessile morphology (P=0.004). The median LN harvest for SFC resections was 18 nodes (range 3-79; P<0.001). The LN harvest (median [range]) for each specific tumour subsite are as follows: “true” SF – 17 (3-79); distal transverse colon – 20 (6-79); proximal descending colon – 14 (6-35). The median length of hospitalization for SFC patients was longer than for non-SFC patients (10 days [8-15] vs. 9 days [7-12]; P=0.003). There was no difference between SFCs and non-SFCs with regards to gender, age, ASA grade, anastomotic leak rates, tumour perforation status, histological type, degree of tumour differentiation, overall tumour grade and the presence of LVI or PNI.

DISCUSSION

This large cohort study of SFC resections, over a long historical period (25 years), highlights key findings that may re-define the approach to SFC management. It appears that SFCs may behave differently and have distinct macroscopic characteristics, some of which are individually associated with poorer survival outcomes when applied to the entire study population. Despite description of such a distinct phenotype, survival outcomes between SFCs and non-SFCs are similar, and the tumour stage remains a critical determinant of the oncological outcome. The question of which surgery should be performed for SFCs is difficult to answer from the results of this study alone, but the similar propensity of SFCs to metastasise when compared with non-SFCs (reflected by their similar tumour stages at presentation) suggests standardised oncological principles with respect to adequate lymphadenectomy (as is recognised for non-SFC resections) should still apply, despite challenges in performing an appropriate lymphadenectomy for this watershed colonic region.

To the best of our knowledge, this study is the first to characterise a distinct phenotype of SFCs using a constellation of macroscopic tumour characteristics and clinicopathological features. In our study, SFCs are more likely to present urgently with obstruction (due to their stenosing nature) or perforation, and thus more likely to require an open operation. These factors are associated with poorer survival outcomes. SFCs were also less likely to have a pedunculated morphology which was associated with better survival outcomes. Despite this over-representation of adverse prognostic features amongst SFCs, no differences in survival outcomes were observed between SFCs and non-SFCs. This is likely because the most important determinant of oncological outcomes *viz.* their propensity to metastasise (reflected by tumour stage) were similar between the two groups; correspondingly, no difference in their OS and DFS were noted.

Studies principally comparing clinicopathological factors between SFCs and non-SFCs with a view to understanding their survival association are limited. As such, no studies have previously reported macroscopic features of SFCs and their survival associations[161-163,165,167-169,174,184,186,191,196,317,318]. Furthermore, interpretation of previous studies investigating SFC outcomes are confounded by inconsistencies in the definition of the SF[162,163,167,169,317], reporting only short-term non-oncological outcomes[196], failing to distinguish elective and emergency SFC surgeries[163,165] and comparing the survival outcomes of combined colonic flexure cancers to other colonic sub-sites[161]. Additionally, some studies considered patients who underwent a conversion from minimally-invasive to an open operation to be classified as part of the minimally-invasive cohort[318] while other studies excluded AJCC Stage IV cancers[184,186,191,318]. Indeed, the overwhelming majority of studies on SFCs have focussed on describing feasibility of specific surgical approaches[184,186,190,191,196] and a comparison of the operation modality (i.e., minimally-invasive vs. open)[318] rather than understanding the relationship between their tumour characteristics and survival outcomes when compared with non-SFCs.

Our finding of similar survival outcomes between SFCs and non-SFCs is supported by several studies in the literature[162,165,167,168]. However, reported 5-year OS rates range widely between 28% to 84%[151,162]. Two studies have reported poorer survival outcomes between SFCs and non-SFCs[163,164]. Specifically, Aldridge *et al*. noted a higher rate of LR (4%) and lower 5-year OS rate (50%)[163], which may be a consequence of the increased rates of obstruction (49%) and perforation (10%) reported in that study compared with ours. Lykke *et al.* reported that SFCs (5-year OS rate of 56.6%) were associated with poorer prognosis compared to sigmoid cancers but did not define the limits of the SF and limited their analysis to AJCC Stage I to III CC OS (i.e., recurrence data had not been registered)[164]. They speculated that a poorer prognosis was likely due to operational difficulties with central ligation of the branches of the MCA. That said, heterogeneity of SF blood supply with shared lymphatic drainage patterns between IMA and MCA pedicles suggest that central ligation of the IMA should receive an equivalent focus. Indeed, a recent study using lymphatic scintigraphy mapping in patients with non-pathological SFs demonstrated preferential lymphatic drainage to LCA LNs in the vast majority of cases[157]. Thus, failure to recognise these disparate drainage routes may contribute to performing a non-radical potentially curative resection.

Despite these inconsistencies, tumour stage of a CC at presentation consistently remains the most important indicator of survival outcome following surgery[22,174,266]. This is reaffirmed in our study with poorer OS and DFS noted in those with AJCC Stage III and IV disease. Importantly, there was no difference between SFCs and non-SFCs in AJCC stage at presentation. Only one other study could support this but failed to surmise the reason[168]. Nonetheless, our observation that tumour stage at time of diagnosis was similar between SFCs and non-SFCs is relevant to the explanation of equivalent oncological outcomes between the two groups.

Our study was not intended to establish the appropriate operation for SFCs (i.e., ERH, SgR, or LH). While it would be intuitive that the operative approach could be guided by the precise location of the cancer (i.e., distal transverse, "true" SF, or proximal descending colon), our available data was insufficient to suitably answer this. This is because separate survival analyses for each operative approach sub-analysed according to tumour location would need to be performed and the numbers for these subgroup analyses would be too small for any meaningful interpretation. Further, it is difficult to predict the oncological outcome of any of these operations as none automatically translate to having a HVT. Nevertheless, even when limiting consideration to elective SFC resections, we confirmed variation in practice within one surgical unit, where the choice of procedure was likely contingent on the surgeon's experience and preference. That said, given their similar propensity to metastasise (reflected by the similar tumour stages at diagnosis), any push towards a limited resection and lymphadenectomy for SFCs[178,190,196,198,199] was not supported by the results of this study, especially considering the increasing move towards CME and CVL as standard practice for other CCs. Further, in recognition of technical challenges to applying CME and CVL principles to the watershed area of the SF, one key to better understanding the surgical approach and in turn standardising a SFC resection may lie in *in vivo* techniques designed to map SF lymphatics to better anatomically characterise a DLP[153,157]. Such a tailored approach, applied to individual patients (in recognition of the heterogenous and disparate drainage patterns between different patients), may facilitate a targeted lymphadenectomy that conforms to CME and CVL principles without incurring excessive colonic devascularisation that may ultimately prove oncologically unnecessary.

This study was limited by the retrospective nature of analysis and the impact of bias thereof. The comparison of SFCs and non-SFCs is confounded by the relatively small number of patients in the SFC group, but this is a recognised biological phenomenon. Any desire to perform a randomised-controlled trial comparing CME and CVL for SFC would be challenging owing to the low incidence of this disease. However, strengths of this study include the large cohort size, long study duration, application of standardized surgery by specialist colorectal surgeons following anatomical planes, detailed pathology reporting and a minimum loss of patient follow-up. Also, this study emphasizes the importance of a standardised SF definition as inconsistencies in the previous literature with regards to its distal limit, together with the description of SFC location, has led to controversy[160,163,165].

In conclusion, this study is the first to report a distinct phenotype of SFCs, the individual characteristics of which are associated with poorer outcomes. While the over-representation of these adverse clinicopathological features would intuitively suggest poorer survival amongst SFC patients, this was not observed in our study. The similar survival outcomes between SFCs and non-SFCs is likely because the most critical determinant of oncological outcome *viz.* tumour stage was similar between the two groups. Recognising that the propensity of SFCs to metastasise is no different to non-SFCs, oncological principles to their resection should not differ, and contemporary practices of CME and CVL to obtain adequate lymphadenectomy should be equivalently considered for both. Applying these principles to a watershed colonic region such as the SF, though, remains challenging, and future research should be directed at better understanding the heterogenous lymphatic drainage of the SF and identifying a DLP to facilitate a targeted lymphadenectomy in individual patients.

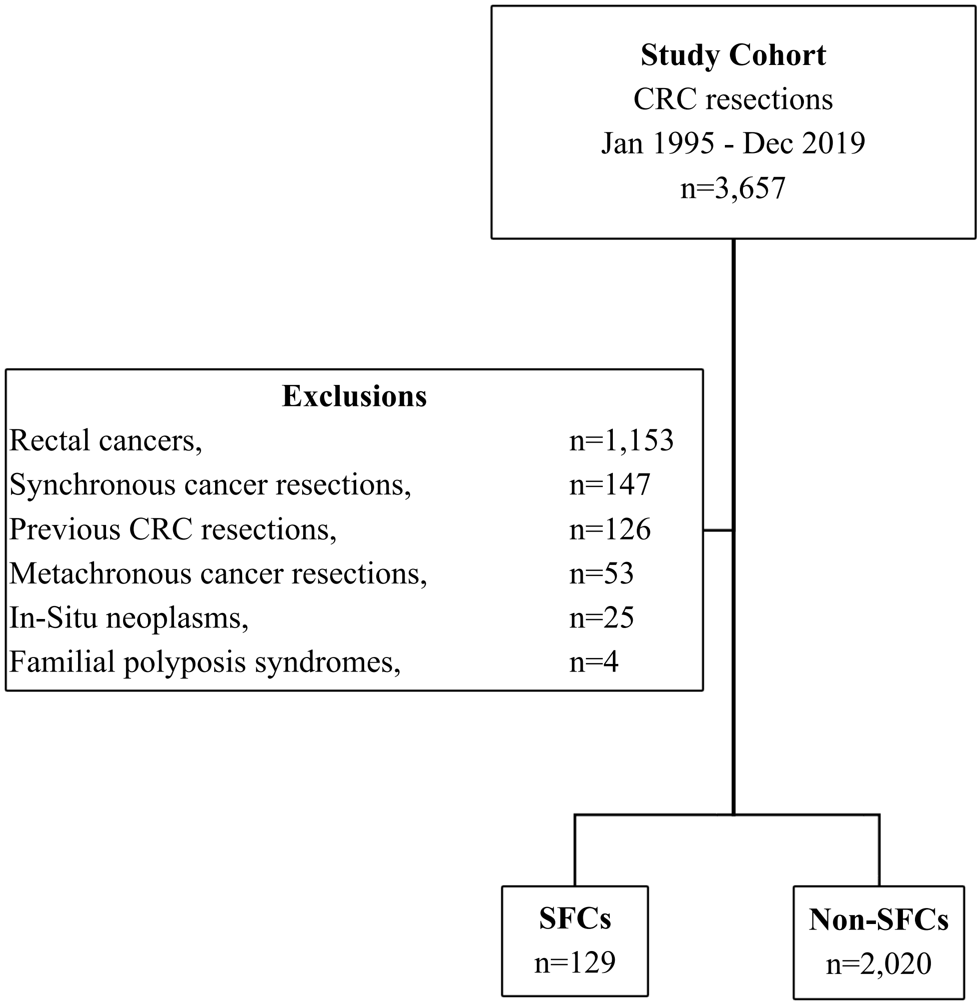

Figure 12: Flow diagram of cohort definition.

TABLE 21. Comparison of clinicopathological factors between patients with SFC and non-SFC					
	N (%) or Mean (SD) or Median (Range/ IQR)	**Non-SFC n (%) (Total=2,020)**	**SFC n (%) (Total=129)**	***P*-value**	**Odds Ratio (95% CI)**
Gender					
Male	1,150(53.5)	1,083(53.6)	67(51.9)	-	*(ref)*
Female	999(46.5)	937(46.4)	62(48.1)	0.7	1.1(0.7-1.5)
Age (Mean [SD]), years	70.5(12.4)	70.6(12.3)	69.4(14.7)	0.3	0.99(0.98-1.00)
ASA Grade					
I	351(15.3)	311(15.4)	24(18.6)	-	*(ref)*
II	1,200(52.3)	1,057(52.3)	61(47.3)	0.2	0.7(0.5-1.2)
III/IV	745(32.4)	652(32.3)	44(34.1)	0.6	0.9(0.5-1.5)
Emergency Operation					
No	1,939(90.2)	1,838(91.0)	101(78.3)	-	*(ref)*
Yes	210(9.8)	182(9.0)	28(21.7)	<0.001	2.8(1.8-4.4)
Emergency Operation (Reason)					
Non-emergency	1.939(90.2)	1,838(91.0)	101(78.3)	-	*(ref)*
Bleeding	6(0.3)	6(0.3)	-	1.0	NA
Obstruction	165(7.7)	142(7.0)	23(17.8)	<0.001	2.9(1.8-4.8)
Perforation	35(1.6)	30(1.5)	5(3.9)	0.03	3.0(1.2-8.0)
Other	4(0.2)	4(0.2)	-	1.0	NA
Operation Modality					
Laparoscopy	871(40.5)	841(41.6)	30(23.3)	-	*(ref)*
Open	1,278(59.5)	1,179(58.4)	99(76.7)	<0.001	2.3(1.6-3.6)
Procedure Conversion					
No	738(90.0)	713(90.3)	25(83.3)	-	*(ref)*
Yes	82(10.0)	77(9.7)	5(16.7)	0.2	1.9(0.7-5.0)
Splenic Injury					
No	2,125(98.9)	2,003(99.2)	122(94.6)	-	*(ref)*
Yes	24(1.1)	17(0.8)	7(5.4)	<0.001	6.8(2.8-16.6)
Splenectomy					
No	2,138(99.5)	2,013(99.7)	125(96.9)	-	*(ref)*
Yes	11(0.5)	7(0.3)	4(3.1)*	<0.001	9.2(2.7-31.9)
Anastomotic leak					
No	1,960(98.2)	1,844(98.3)	116(95.9)	-	*(ref)*
Yes	36(1.8)	31(1.7)	5(4.1)	0.06	2.56(0.98-6.72)

Blood Loss (millilitres)					
< 500	2,063(96.0)	1,949(94.5)	114(88.4)	-	*(ref)*
>500	86(4.0)	71(3.5)	15(11.6)	<0.001	3.6(2.0-6.5)
Tumour Stage (TNM AJCC)					
Stage I/II	1,219 (56.7)	1,142(56.5)	77(59.7)	-	*(ref)*
Stage III	603(28.1)	572(28.3)	31(24.0)	0.3	0.8(0.5-1.2)
Stage IV	327(15.2)	306(15.1)	21(16.3)	0.9	1.1(0.6-1.7)
Tumour Size (Mean [SD]), cm	4.8(2.4)	4.8(2.4)	4.7(2.5)	1.0	1.0(0.9-1.1)
Macroscopic Characteristic					
Pedunculated					
No	1,657(77.1)	1,544(93.2)	113(87.6)	-	*(ref)*
Yes	492(22.9)	476(23.6)	16(12.4)	0.004	0.5(0.3-0.8)
Sessile					
No	1,715(79.8)	1,625(80.4)	90(69.8)	-	*(ref)*
Yes	434(20.2)	395(19.6)	39(30.2)	0.004	1.8(1.2-2.6)
Stenosing					
No	1,820(84.7)	1,728(85.5)	92(71.3)	-	*(ref)*
Yes	329(15.3)	292(14.5)	37(28.7)	<0.001	2.4(1.6-3.6)
Ulcerating					
No	1,249(58.1)	1,173(58.1)	76(58.9)	-	*(ref)*
Yes	900(41.9)	847(41.9)	53(41.1)	0.9	0.9(0.7-1.4)
Tumour Perforation					
No	2,054(95.6)	1,930(95.5)	124(96.1)	-	*(ref)*
Yes	95(4.4)	90(4.5)	5(3.9)	0.8	0.9(0.3-2.2)
Histological Type					
Non-Mucinous/Signet ring	1,902(88.5)	1,790(88.6)	112(86.8)	-	(ref)
Mucinous	225(10.5)	209(10.3)	16(12.4)	0.5	1.2(0.7-2.1)
Signet ring	22(1.0)	21(1.1)	1(0.8)	0.8	0.8(0.1-5.7)
Histological Differentiation					
Well or Moderate	1,764(82.1)	1,657(82.0)	107(82.9)	-	*(ref)*
Poor	385(17.9)	363(18.0)	22(17.1)	0.8	0.9(0.6-1.5)
Histological Grade					
Low/Average	1,715(79.8)	1,612(79.8)	103(79.8)	-	*(ref)*
High	434(20.2)	408(20.2)	26(20.2)	1.0	1.0(0.6-1.6)
Lympho-Vascular Invasion					
No	1,511(70.3)	1,423(70.4)	88(68.2)	-	*(ref)*
Yes	638(29.7)	597(29.6)	41(31.8)	0.6	1.1(0.8-1.6)

Peri-Neural Invasion					
No	1,785(83.1)	1,680(83.2)	105(81.4)	-	*(ref)*
Yes	364(16.9)	340(16.8)	24(18.6)	0.6	1.1(0.7-1.8)
Lymph Nodes – Examined (Median [Range])	17(0-119)	17(0-119)	18(3-79)	<0.001	1.02(1.01-1.04)
Lymph Node Harvest (<12)					
No	1,692(78.7)	1,598(79.1)	94(72.9)	-	*(ref)*
Yes	457(21.3)	422(20.9)	35(27.1)	0.1	1.4(0.9-2.1)
LOS (Median [Range]), days	9.0(7.0-12.0)	9.0(7.0-12.0)	10.0(8.0-15.0)	0.003	1.02(1.01-1.04)

SFC: **splenic flexure cancers;** ***ASA:*** **American Society of Anesthesiology;** ***TNM:*** **8th edition tumour, nodes, and metastasis staging system;** ***AJCC:*** **American Joint Committee on Cancer; and** ***LOS*****: Length of Stay.**

***All four splenectomies were performed for non-oncological reasons (i.e., splenic capsular tear or haemorrhage)**

Table 21: Clinical characteristics and histopathological descriptions of SFC and non-SFC patients.

TABLE 22. Comparison of survival outcomes between clinicopathological factors within the entire cohort				
	OS		DFS	
	P-value	Hazards Ratio (95% CI)	*P*-value	Hazards Ratio (95% CI)
Splenic Flexure Cancer				
No	-	*(ref)*	-	*(ref)*
Yes	0.6	0.9(0.7-1.2)	0.5	0.9(0.7-1.2)
Gender				
Male	-	*(ref)*	-	*(ref)*
Female	0.01	0.86(0.77-0.97)	0.01	0.87(0.78-0.97)
Age(years)	<0.001	1.05(1.04-1.05)	<0.001	1.04(1.03-1.05)
ASA Grade				
I	-	*(ref)*	-	*(ref)*
II	<0.001	1.8(1.5-2.2)	<0.001	1.8(1.5-2.2)
III/IV	<0.001	4.3(3.6-5.3)	<0.001	4.1(3.3-4.9)
Emergency Operation				
No	-	*(ref)*	-	*(ref)*
Yes	<0.001	2.3(1.9-2.7)	<0.001	2.3(2.0-2.7)
Emergency Operation (Reason)				
Non-emergency				
Bleeding	-	*(ref)*	-	*(ref)*
Obstruction	0.9	1.0(0.2-4.0)	0.6	1.4(0.4-4.2)
Perforation	<0.001	2.3(1.9-2.8)	<0.001	2.3(1.9-2.8)
	<0.001	2.4(1.6-3.6)	<0.001	2.5(1.7-3.8)
Operation Modality				
Laparoscopy	-	*(ref)*	-	*(ref)*
Open	<0.001	1.3(1.2-1.5)	<0.001	1.3(1.1-1.4)
Splenic Injury				
No	-	*(ref)*	-	*(ref)*
Yes	0.6	1.1(0.7-1.8)	0.8	1.1(0.7-1.7)
Splenectomy				
No	-	*(ref)*	-	*(ref)*
Yes	0.1	1.7(0.9-3.3)	0.1	1.8(0.9-3.4)
Blood Loss (millilitres)				
< 500	-	*(ref)*	-	*(ref)*
>500	0.01	1.4(1.1-1.8)	0.01	1.4(1.1-1.8)
Tumour Stage (TNM AJCC)				
Stage I/II	-	*(ref)*	-	*(ref)*
Stage III	<0.001	1.5(1.3-1.7)	<0.001	1.6(1.4-1.8)
Stage IV	<0.001	7.2(6.2-8.4)	<0.001	7.1(6.1-8.3)
Macroscopic Characteristic				
Pedunculated				
No	-	*(ref)*	-	*(ref)*
Yes	<0.001	0.7(0.6-0.8)	<0.001	0.7(0.6-0.8)
Sessile				
No	-	*(ref)*	-	*(ref)*
Yes	0.3	0.9(0.8-1.1)	0.2	0.9(0.8-1.1)
Stenosing				
No	-	*(ref)*	-	*(ref)*
Yes	<0.001	1.4(1.2-1.6)	<0.001	1.5(1.3-1.7)
Ulcerating				
No	-	*(ref)*	-	*(ref)*
Yes	<0.001	1.3(1.1-1.4)	<0.001	1.3(1.1-1.4)
Lymph Nodes - Examined	<0.001	0.989(0.983-0.995)	0.002	0.991(0.985-0.997)
Lymph node Harvest (<12)				
No	-	*(ref)*	-	*(ref)*
Yes	0.002	1.2(1.1-1.4)	0.007	1.2(1.0-1.4)
LOS (days)	<0.001	1.026(1.022-1.031)	<0.001	1.02(1.01-1.03)

SFC: **splenic flexure cancers;** ***ASA:*** **American Society of Anesthesiology;** ***TNM:*** **8th edition tumour, nodes, and metastasis staging system;** ***AJCC:*** **American Joint Committee on Cancer; and** ***LOS*****: Length of Stay.**

Table 22: Comparison of overall survival and disease-free survival outcomes of varying clinicopathological variables.

CHAPTER VI: Assessing Lymphatic Drainage of the Splenic Flexure by SPECT: A Feasibility Study

This chapter has been prepared and submitted to the *British Journal of Surgery*. At the time of submission of this thesis, the manuscript is undergoing a peer review process.

ABSTRACT

INTRODUCTION: Areas with a heterogeneous blood supply, such as the SF, exhibit variable lymphatic drainage and lack an established consensus on optimal surgical cancer management. This study aimed to evaluate the feasibility and safety of *in vivo* endoscopic submucosal injection of Tc-99m Radiocolloid, followed by scintigraphy mapping using both planar and SPECT-CT imaging. Additionally, this study sought to determine whether a DLP of the SF could be consistently identified through this method.

METHODS: A prospective observational pilot study at CRGH, Sydney was conducted with patients who underwent a SOC colonoscopy (2022-2023). Patients were identified from their referrals to the CRGH Colorectal Outpatient Department Clinic. As positive controls, additional patients were recruited for radioisotope injection in the upper rectum, while others were recruited for lower rectal injections to understand the impact of direct digital massage of the injection site on lymphatic mapping.

RESULTS: 51 patients were approached for participation, but 23 (45.1%) declined and eight were excluded, leaving ten patients who had their SF injected, five who had their upper rectum injected, and five who received injection to their lower rectum. No adverse events were reported at the 1, 7 and 30-day post-injection reviews. No consistent lymphatic drainage pattern at the SF was discernible. No patients who received upper rectal injections showed lymphatic drainage or LN uptake, however, all patients with lower rectal injections demonstrated lymphatic drainage and focal LN activity following direct finger massage.

CONCLUSION: There is insufficient evidence to support the use of 3D-SPECT technology post-submucosal injection of Tc-99m Radiocolloid to define a DLP of the SF, as a consistent lymphatic drainage pattern at the SF was not discernible. Demonstration of tracer movement following injection into the submucosa of the colorectum appears dependent on post-injection

physical/digital massage, which cannot be performed for injections sites proximal to the lower rectum. Nevertheless, understanding the DLP of the SF remains clinically relevant, as it allows for targeted lymphadenectomy in individual patients and enables thorough operative planning with appropriate patient counselling.

INTRODUCTION

Lymph node metastasis in CRC remains one of the strongest prognostic factors for survival and the most important criterion for selecting patients for adjuvant chemotherapy[319]. Therefore, a critical element of a good quality oncological surgery relies on removal of a pre-defined lympho-neuro-vascular package that contains nodal metastases, if present. However, areas with a heterogenous blood supply, such as the SF, have variable lymphatic drainage and currently lack an established consensus with regards to their surgical management[156].

In recent non-physiological experimentation using lymphatic scintigraphy mapping in patients with non-pathological SFs, a preferential lymphatic drainage to LCA nodes in the vast majority of cases was demonstrated[157]. This would infer that the planned resection of a SFC should target the IMA pedicle. However, review of the Concord Colorectal Cancer database from 1995 to 2019 suggests that real-world practice is otherwise, showing that an ERH is performed more frequently for SFCs than a LH (46% vs. 35%)[180]. This indicates that a preference for radical lymphadenectomy of the MCA pedicle exists instead.

Such observations underscore the variable nature of SF lymphatic drainage in keeping with the watershed blood supply of this region. The specific lymphatic drainage likely differs from one individual to another and the decision to perform a D3 lymphadenectomy of the MCA or IMA pedicle is challenging. Indeed, no single operation or lymphadenectomy approach would be appropriate for all SFC patients. Of course, targeting both MCA and IMA pedicles, such as in a restorative extended LH (Deloyer's procedure) may be possible but is rarely indicated due to the excessive (and unwarranted) colonic devascularisation incurred, and complexities pervade regarding a Deloyer's reconstruction. This implies that the surgical management of SFCs should be tailored to the tumour's preferential lymphatic drainage[169,184], thus allowing a targeted lymphadenectomy to be performed. However, there is currently a lack of pre-operative parameters and feasible methods for understanding an individual's SF lymphatic outflow[84,201,202].

Given the anatomical knowledge that lymphatic channels of the bowel wall converge near the adjoining mesentery, a physiological means of mapping the native SF lymphatic system and identifying the DLP may involve *in vivo* intraluminal submucosal delivery of a radioisotope with subsequent 3-D SPECT imaging. The *in vivo* technique is preferred because it allows for the identification of aberrant lymphatic drainage, particularly in advanced-stage pathologies, and also because of the theoretical prospect of adjusting for a planned resection when required[203]. The use of SPECT-CT imaging has shown to reduce false negative results given precise anatomical localisation capacity and ability to identify aberrant lymphatic drainage. This technique is also advantageous for patients with a high BMI, overcoming the limitations of traditional lymphoscintigraphy[320]. Although alternative methods exist, such as ICG and patent blue dye, they lack quantification potential, carry significant risks and do not offer improved efficacy[321-324].

AIMS

The primary aim of this study was to address the feasibility and safety profile of *in vivo*, endoscopically placed submucosal Tc-99m Radiocolloid with subsequent scintigraphy mapping using planar and SPECT-CT technology. Following this, the secondary aim of this study was to understand if a DLP of the SF is consistently discernible.

HYPOTHESES

Specifically, this study aimed to test the hypotheses that:

(i) Endoscopically placed submucosal Tc-99m Radiocolloid at the SF is safe and feasible.

(ii) A DLP following submucosal Tc-99m Radiocolloid placement at the SF is consistently demonstrable using SPECT-CT technology.

RESEARCH ETHICS COMMITTEE APPROVAL

The basic science research contained within this Chapter was approved by the Sydney Local Health District Human Research Ethics Committee – CRGH. The following ethics committee reference covers the body of work contained within this Chapter (CH62/6/2022-012).

MATERIAL and METHODS

Patients and Recruitment

A prospective observational pilot study that included consecutive patients who underwent a SOC colonoscopy at CRGH, Sydney, Australia, between November 2022 and December 2023, was performed. Only patients whose colonoscopies resulted in normal findings, with no interventions performed, were eligible for inclusion. Patients were identified from their referrals to the CRGH Colorectal Outpatient Department Clinic, where a clinical risk assessment was used to filter those likely to have a colonoscopy yielding normal outcomes and not requiring an intervention during the SOC colonoscopy. For instance, a 60-year-old male with weight loss, dark rectal bleeding, and tenesmus was less likely be included than a 40-year-old individual experiencing outlet-type bleeding.

The consent process for these patients was conducted in two phases. First, the clinical team obtained consent for the SOC colonoscopy. After screening and referral to the research team, a separate consent specific for the study was completed. This two-step process helped minimize recruitment bias.

Those excluded were patients under 18 years of age, with claustrophobia, were lactating or pregnant, were pre-pregnancy planning, or caring for young children for at least six hours post-radioisotope injection. Patients with known allergies to either the radioisotope or its constituents were also excluded. Additionally, those with a history of colorectal resections (e.g., those with a discontinuous colon or a colostomy), were excluded. Women of child-bearing age required a negative pregnancy test prior to recruitment.

Standard of Care Colonoscopy

A SOC colonoscopy was defined as a comprehensive, systematic examination of the entire colon, including the caecum and, if possible, the distal ileum[325]. The colonoscopies performed as part of this Chapter were performed at the CRGH endoscopy suite and with proceduralist-based sedation. A SOC colonoscopy was considered complete once the endoscope was fully withdrawn. If no biopsy, tattooing, clipping, or other intervention were performed, the patient was included into the study, marking the commencement of the research phase.

Clinico-Pathological Variable of Interest – SPECT Identification of the DLP

Definition and Identification of the SF

Upon completion of the SOC colonoscopy, the colonoscope was re-inserted to the SF. The apex of the SF was defined as the highest and most angulated point of the colon, between the transverse colon and descending colon, as assessed visually by the endoscopist. The unique architecture of the transverse colon in comparison to the descending and ascending colon aided in the localisation of the SF. These assessments were further corroborated by a visual aid using the *ScopeGuide* (Olympus, Japan), which helped informed the real-time position of the endoscope (Figure 13). Once the SF was reached and confirmed, Tc-99m Radiocolloid was injected submucosally.

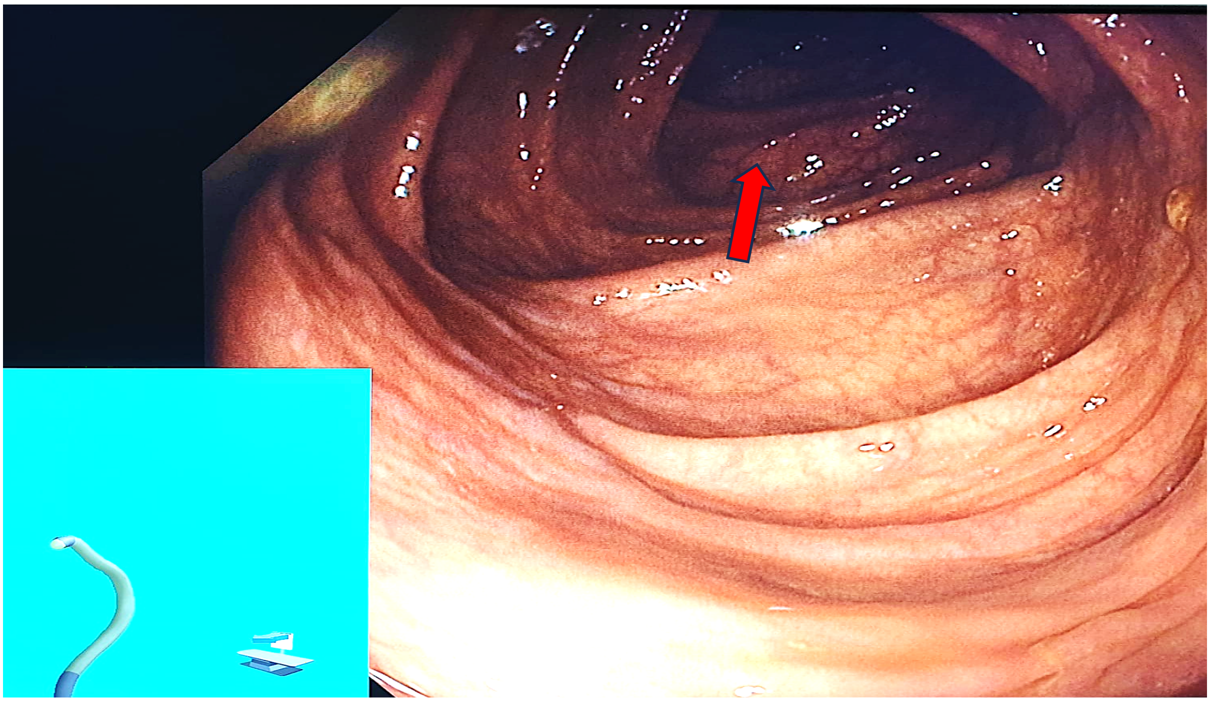

Figure 13: A PiP image from the scope guide confirms the location of the SF based on the scope's angulation. The main image corroborates this finding by displaying the triangular configuration of the bowel, consistent with the transverse colon, as indicated by the red arrow.

Definition of DLP

DLP is a novel marker that should not be confused with SLN identification. Pathological cells disseminate in an orderly, centrifugal manner, resulting in a predictable lymphatic drainage pattern from the primary disease site, through regional lymphatic channels, to the principal or 'sentinel' node(s)[326-330], thereby guiding accurate sentinel lymphadenectomy. In considering colonic lymphatic drainage, SLN's would likely be pericolic (or D1) LNs. In contrast, DLP focuses on understanding the unique lymphatic drainage pathway(s) in a specific area, rather than aiming to identify a single draining LN or group of nodes. The DLP therefore considers drainage patterns beyond the SLN, which are relevant in planning a surgical lymphadenectomy (as opposed to a SLN biopsy). This distinction is particularly relevant in regions with a watershed blood supply, such as the SF. Consequently, understanding the difference between DLP and SLN is crucial, as the DLP approach can offer a more individualized strategy for lymphadenectomy in areas like the SF that have variable blood supply and lymphatic drainage.

Radio Isotope - Tc-99m Radiocolloid - Preparation and Transport

The radiopharmaceutical (Tc-99m Nanocis) was prepared in the Nuclear Medicine Department at CRGH using Nanoscan (Media Radiopharma, Hungary), a nano-colloid containing 500 µg of nano-colloidal human albumin per vial. After confirming that the radiopharmaceutical met quality control standards—exceeding 95% purity at 15–25 °C—40 MBq of Tc-99m Nanoscan was reconstituted to 1.3mL with NaCl and 0.2mL of methylene blue in a 1.5mL syringe. The final product was then stored and transported in a lead-shielded carrying case to the endoscopy suite. If Tc-99m Nanoscan was not available, the alternate nano-colloid, Tc-99m Antimony Trisulfide Colloid was prepared instead, and similar process of reconstitution was undertaken with the same radioactive dose as Tc-99m Nanoscan.

Methylene blue (0.2mL) was used to confirm that the radioisotope was correctly deposited in the submucosal plane, as demonstrated by the appearance of a superficial, raised and blue bleb (Figure 14). In contrast, radioisotope instillation into the muscular or mucosal layers was indicated by the absence of a blue hue, no visible raised bleb, and increased resistance during injection.

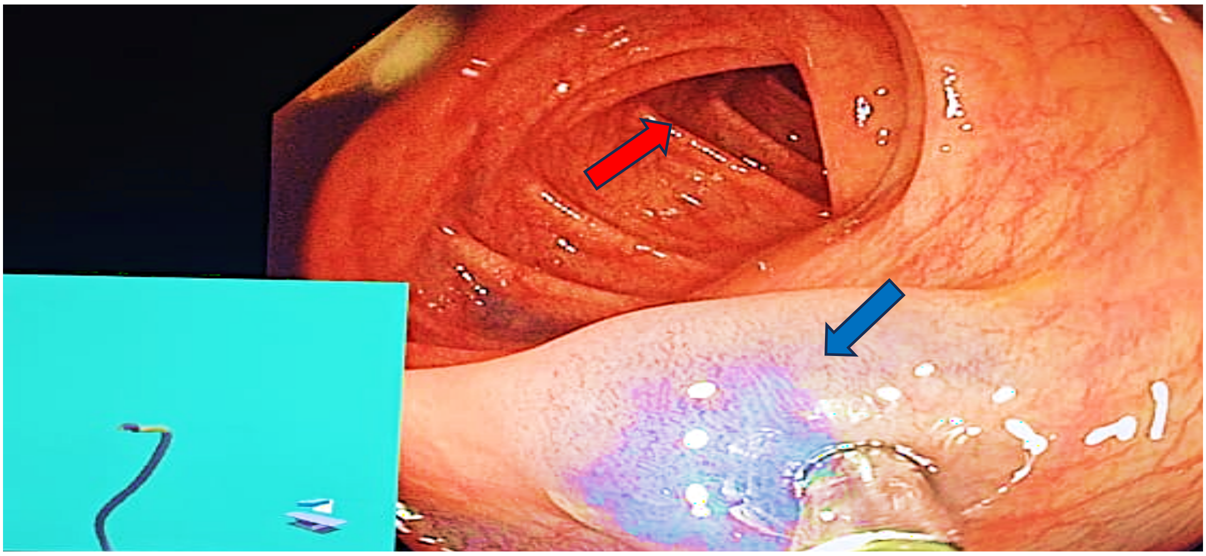

Figure 14: A PiP image of the scope guide confirming the location of the SF. The main image shows the transverse colon as marked by the red arrow and a SF submucosal blue bleb being raised (blue arrow).

Tc-99m Radiocolloid Submucosal Injection

A 23-gauge (0.6mm diameter), 5mm Carr-Locke injection needle (Steris Endoscopy, Device Technologies, USA) was used to instil the radioisotope through the endoscopic working port. This needle features automatic retraction technology and thus minimizes the risk of needle stick injuries and spillage. It is housed within a 2.5mm diameter sheath and attached to a 2300mm long conduit with a fixed volume of 1.38mL. This setup was relevant for accounting not only for the volume of the radioisotope used for luminal priming but also for the volume of 0.9% NaCl required to follow through after radioisotope administration, ensuring optimal radioisotope delivery and deposition. Correct needle placement was confirmed by raising a submucosal blue bleb. At that point, the time of injection was recorded. The system was then flushed with 1.5mL of 0.9% NaCl to maximize radioisotope administration and minimize colonoscope radioactivity. This methodology was initially piloted on a single patient to better understand the sequence and optimize the study's logistical flow.

Throughout the research phase, the investigator was reminded not to use the suction function on the endoscope to prevent potential radioactive contamination of the system. A *Resolution Clip* (Boston Scientific, USA) was deployed at the site of radioisotope infiltration to serve as an additional marker for SF identification on SPECT-CT given its radio-opacity (Figure 15). It is widely accepted that endoscopic clips naturally detach within a 2-week period[331].

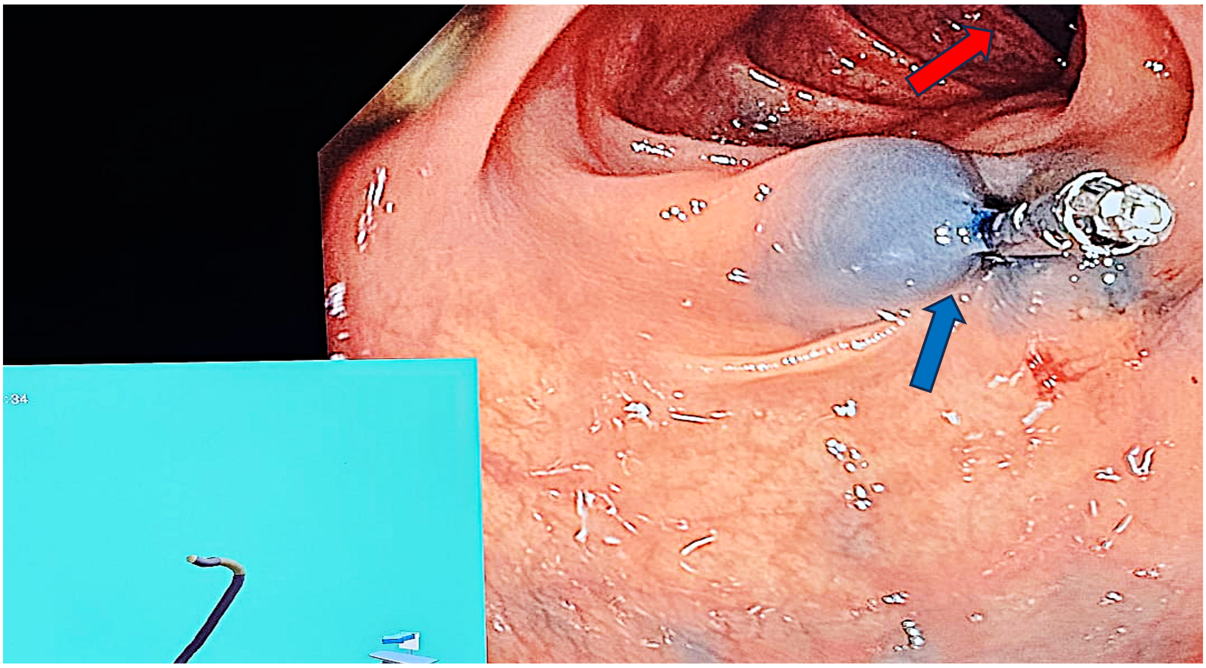

Figure 15: The main image shows the transverse colon as marked by the red arrow and SF injected and clipped as marked by a blue arrow.

After the procedure, several items were screened with a Gieger counter (Figure 16) including the colonoscope, consumables and the hands of staff who handled the radioisotope in the endoscopy suite. In accordance with the CRGH Radiation Safety Officer's advice, the colonoscope was then cleaned, sterilized, and isolated for 48 hours.

Figure 16:An example of a device used to monitor radiation levels in the Endoscopy Suite.

3D-SPECT Imaging Post-Tc-99m Radiocolloid Injection

The patients were then monitored in the endoscopy suite's recovery area with appropriate precautions. Any symptoms or signs of a reaction were documented. Once adequately recovered, patients were transferred to the Nuclear Medicine suite for the SPECT-CT imaging at time points described below. The dosage and radioactivity of Tc-99m Radiocolloid were documented in the SPECT-CT report.

Imaging Protocol

Either a General Electric (GE) Discovery 670 16-slice CT (2012) or a GE Discovery 8-slice CT (2016) system (Figure 17) was used for imaging, and their processing was performed using an Xeleris Functional Imaging workstation (GE). A 1-hour, 2-hours, and 4-hours timepoint post-radioisotope injection were used for both Planar imaging and SPECT, but an additional 24-hour and 48-hour SPECT imaging was also undertaken (dependent on the initial timepoint findings), to assess for any evidence of delayed drainage. Planar and SPECT images were displayed on the Xeleris Functional imaging workstations as shown in Figure 18 and 19 respectively.

CT was performed for attenuation correction of the images, which also provided anatomical reference regarding the radioactivity present. CT scans were only performed at the 1-hour timepoint, which was then used for attenuation correction and anatomical reference for the 2-hour and 4-hour SPECT images. The *Resolution* clip used to secure the site of injection was used as the reference point when fusing the SPECT and CT datasets. The same scanner was used to image all timepoints in each individual subject. The acquisition parameters are shown in Table 23 below.

Figure 17: GE Discovery 670 16-slice CT scanner used for capturing planar and SPECT images for this study.

TABLE 23. Planar and SPECT CT Imaging Acquisition Parameters			
Protocol Overview	**Image**	**Phase**	**Positioning**
Time: 5 min and 10 min (24hr images) **Matrix:** 256 x 256 **Zoom:** 1.0 **View:** Anterior & Posterior	**Static**	1, 2 & 4 hours +/- 24 hour	Feet First Supine Arms raised
Scan Speed: 30 sec/view **Matrix:** 128 x 128 **Zoom:** 1.0 **Views:** 60	**SPECT/CT**	1 hour	Feet First Supine Arms raised
mA: 10 **kV:** 120	**Topogram**	1 hour	Feet First Supine Arms raised
mA: Smart mA 80-160 **kV:** 120	**CT**	1 hour	Feet First Supine Arms raised
Scan Speed: 30 sec/view **Matrix:** 128 x 128 **Zoom:** 1.0 **Views:** 60	**SPECT Only**	2 & 4 hours	Feet First Supine Arms raised

Table 23: Imaging Protocol and Acquisition Parameters.

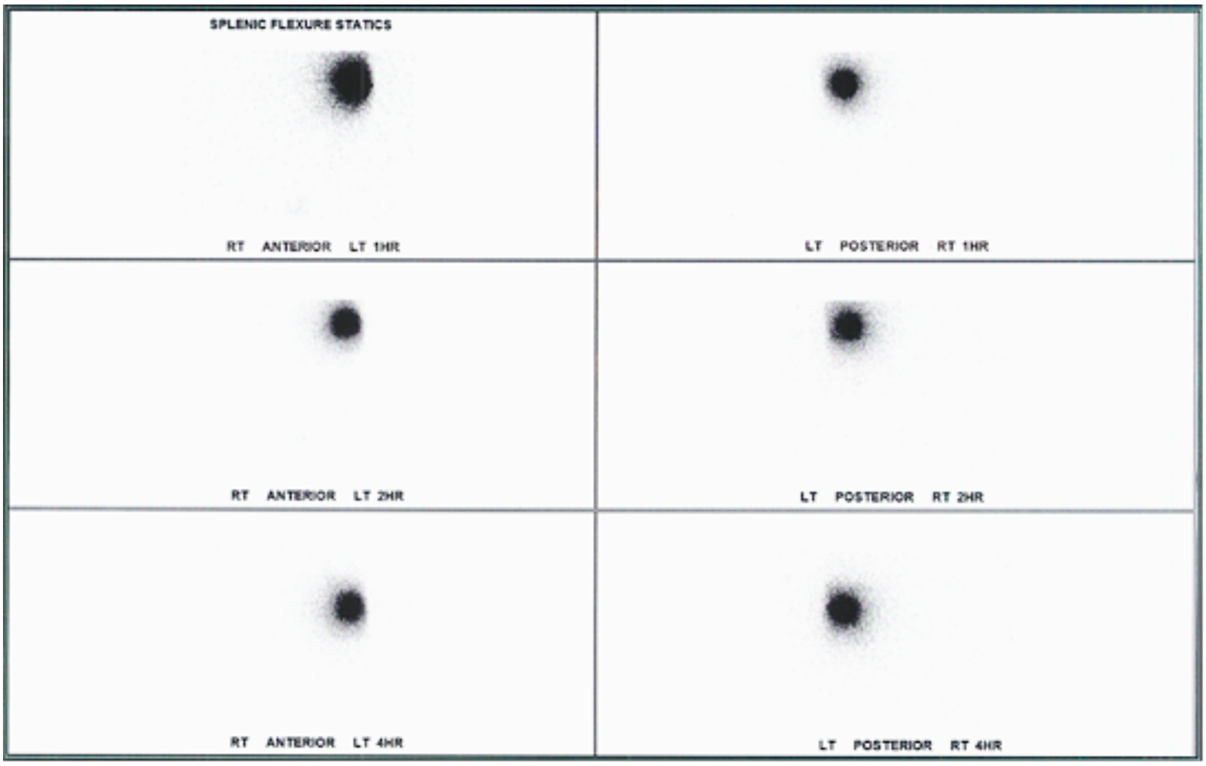

Figure 18: Example of planar imaging following SF injection with radioisotope at the 1-hour, 2-hour and 4-hour time points where focal avidity is seen only at the injection site.

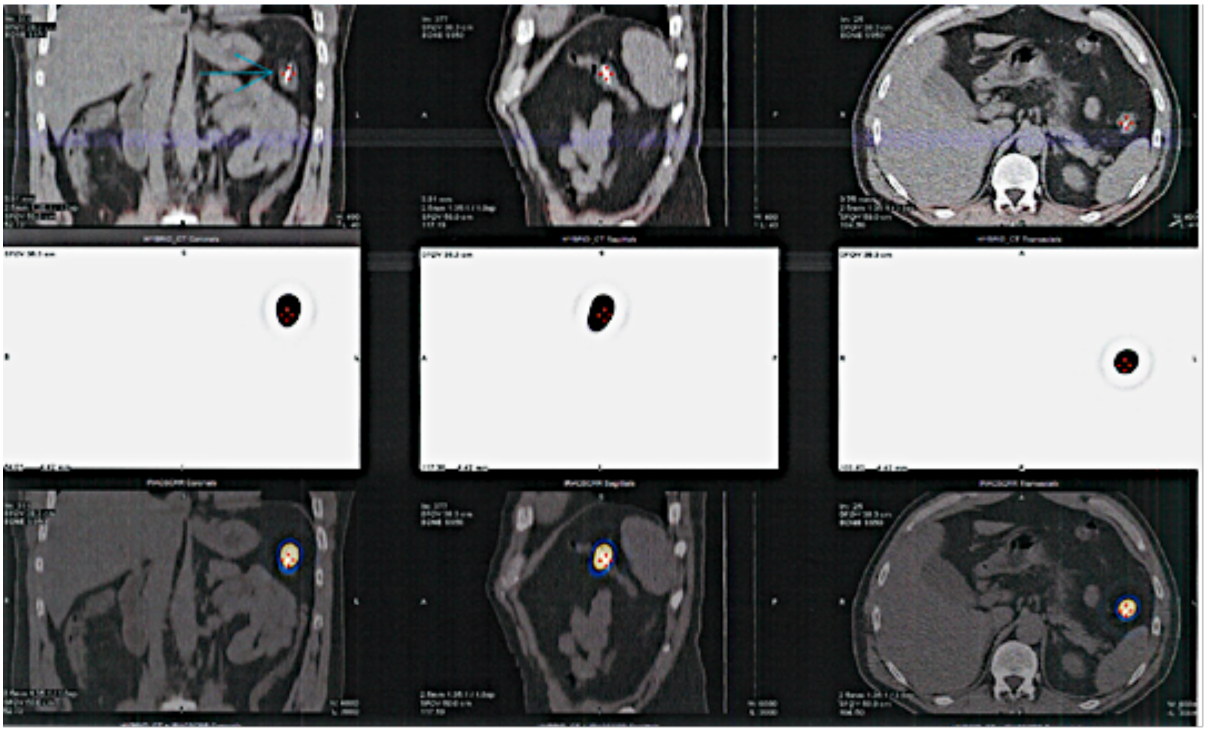

Figure 19: Example of coronal, sagittal and axial CT with superimposed SPECT imaging following radioisotope injection at the SF, captured at the 1-hour, 2-hour, and 4-hour time points. The blue arrow (top left) indicates the SF, identifiable by the focal uptake and the radio-dense Resolution clip.

Tc-99m Radiocolloid Injection at Other Regions of the Colorectum

Depending on DLP identification following SF injection, consideration was given to injection at other regions of the colorectum, such as the upper rectum (a non-watershed area) and anorectum (for which digital massage of radioisotope could be applied). The injection protocol for the upper and lower rectum are detailed later in this Chapter.

Follow-up

At the conclusion of the scan(s), patients were discharged in accordance with standard post-endoscopic recovery and nuclear medicine protocol. On days 1, 7 and 30 following the SPECT-CT, follow-up phone calls were made to individual patients to assess for any delayed post-colonoscopy complications or adverse reactions.

Outcome Measures

The primary outcome measures were the feasibility and safety of submucosal radioisotope injection, specifically assessing recruitment rate, drop-out rate, adverse reaction profile and rate, and protocol adherence. Secondary outcomes included establishing a DLP by describing lymphatic drainage patterns in the native, non-pathological SF using lymphoscintigraphy, including both planar and SPECT-CT imaging. Only patients who underwent image acquisition within two hours of radioisotope injection were analysed. Beyond this two-hour window period, the radioisotope may diffuse or redistribute, potentially obscuring the primary drainage pathways and increasing variability in the data. This time restriction helped maintain consistency and reliability in the analysis.

Statistical Analyses

As this was a pragmatic feasibility study, a power calculation was not required. A sample size of ten patients was considered appropriate. Continuous variables were reported as median (IQR or range [minimum to maximum values]) while categorical variables were reported as frequencies and percentages.

RESULTS

Description of All Patients Included in This Study

During the study period from November 2022 to December 2023, 51 patients were approached for participation and injection into their SF, upper rectum, or lower rectum. Of these, 23 (45.1%) declined, leaving 28 patients (54.9%) who underwent a SOC colonoscopy. However, eight patients were subsequently excluded: five required a polypectomy, two had a traversable malignancy identified, and one had the SOC colonoscopy abandoned due to difficulties traversing the sigmoid colon.

Specific descriptions of patients who received SF injections are provided below. Following that, the results for patients who had their upper or lower rectum injected—including their corresponding lymphatic drainage patterns—are presented, after discussing aspects of protocol refinement.

Description of Patients Who Had Their SF Injected

From November 2022 to September 2023, 29 patients were approached for participation and injection of their SF. Of these, 14 (48.2%) declined, leaving 15 patients (51.8%) who underwent a SOC colonoscopy (Figure 20). The reasons cited for declining included concerns about procedure-related risks, particularly the radioisotope injection (*n*=8), refusal of procedural sedation for the SOC (*n*=4), and the need to care for young children (*n*=2). Of the 15 patients who initially agreed, five (33.3%) were subsequently excluded. Specifically, four patients (72.5%) required a polypectomy, and one patient (12.5%) had their SOC colonoscopy abandoned due to difficulty traversing the sigmoid colon.

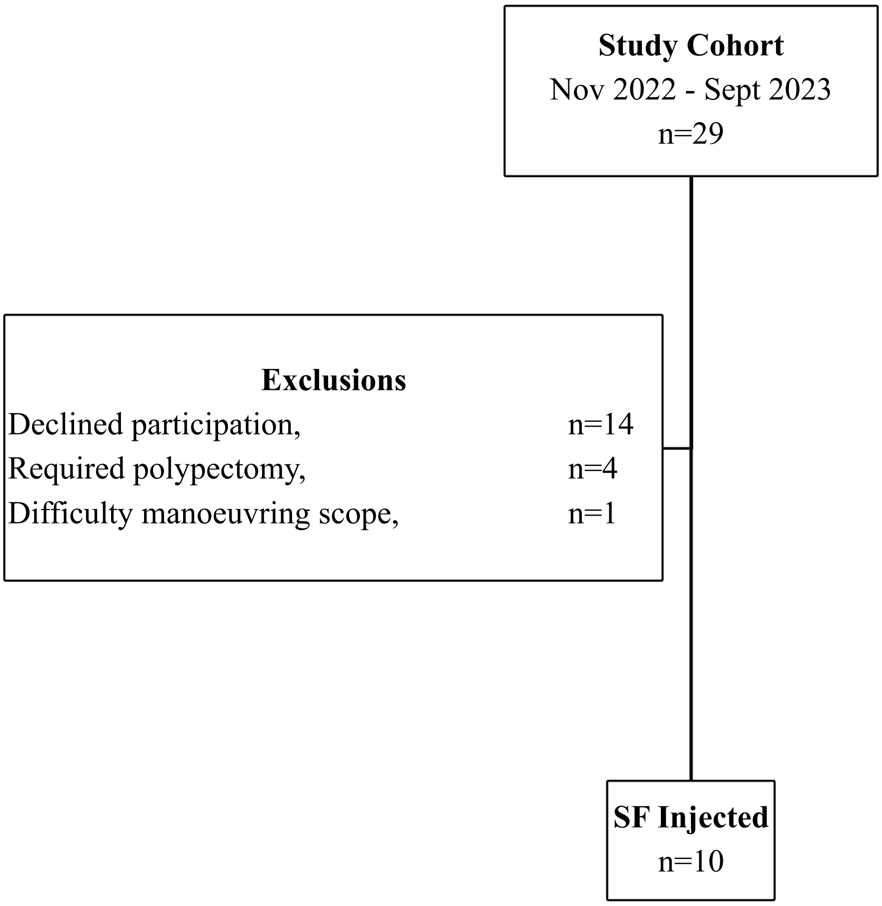

Figure 20: Flow diagram of cohort definition for patients who had their SF injected.

Among the patients included, six (60%) were male, with a median age of 47.2 years (range 26.4-69.8) and a median BMI of 24.3 kg/m^2 (range 19.7-33.7). The median duration of the research colonoscopy phase—which included radioisotope injection, NaCl flush, clip placement, and colonoscope withdrawal—was 12.9 minutes (range 9.7-21.4). There was no extravasation of radioisotope with any of the patients who had their SF injected on visual inspection at the time of the colonoscopy. The median time from radioisotope injection to scanning (including recovery in the endoscopy suite) was 77.5 minutes (range 67.9–93.7). All patients tolerated at least one high-calorie meal prior to being scanned, and none experienced immediate or delayed side effects or complications following the radioisotope injection. The features of all the included patients are summarised in Table 24.

TABLE 24. Clinical Features of SF Patients Studied				
Study Patients	**Date of Scope**	**Gender**	**Age (Years)**	**BMI (kg/m^2)**
Patient 1	13/10/2022	Male	27.3	23.3
Patient 2	16/11/2022	Male	52.1	25.3
Patient 3	08/02/2023	Male	66.0	32.5
Patient 4	03/05/2023	Male	53.7	33.7
Patient 5	05/05/2023	Female	29.9	19.7
Patient 6	19/07/2023	Female	44.6	21.4
Patient 7	26/07/2023	Female	43.0	22.1
Patient 8	23/08/2023	Male	26.4	22.4
Patient 9	20/09/2023	Female	69.8	29.7
Patient 10	20/09/2023	Male	49.7	30.4
TP: ***Time Point;*** **BMI:** ***Body Mass Index;*** **SF:** ***Splenic Flexure***				

Table 24:Clinical summary of SF patients suitable for radioisotope injection.

General Observations

Upon preliminary review of the first seven patients who received Tc-99m Radiocolloid injections to their SF, planar imaging showed no discernible DLP or focal LN uptake over multiple time points. This finding suggests that planar imaging has limited utility in SF scintigraphy.

Additionally, on Patient 7's imaging (Figure 21), the injected SF was identified close to the midline. This positioning rendered it difficult to assess the lymphatic pedicle for separate focal activity due to the presence of scatter and ring artefacts arising from the injection site.

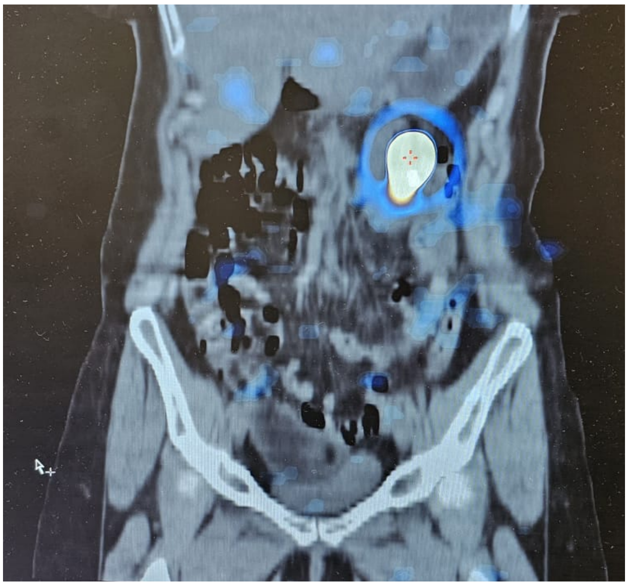

Figure 21: SPECT-CT image of Patient 7 showing a halo around the injection site (red crosshair) at the SF, situated close to the midline abdominal vasculature.

Lymphatic Drainage Patterns Following Injection of First Seven SF Patients with Tc-99m Radiocolloid (Figure 22, Figure 23 and Table 25)

Table 25 summarises the lymphatic drainage patterns of the first seven SF patients who had the radioisotope injected.

TABLE 25. Comparison of Lymphatic Drainage Patterns of the First Seven SF Patients								
Study Patients Gender Age	**Date of Scope**	**BMI (kg/m^2)**	**Imaging TPs**	**PI Pattern**	**DLP or LN Uptake**	**SPECT-CT Imaging Patterns**	**DLP or LN Uptake**	**Comments**
Patient 1 Male 27.3years	13/10/22	23.3	1hr and 2hr	F	No	F+D	No	Intracolonic movement on SPECT
Patient 2 Male 52.1years	16/11/22	25.3	1hr, 2hr, 4hr, 24hr	F	No	F	No	NA
Patient 3 Male 66.0years	08/02/23	32.5	1hr, 2hr, 4hr, 24hr, 48hr	F	No	F+D	No	Intracolonic movement on SPECT
Patient 4 Male 53.7years	03/05/23	33.7	1hr, 2hr, 4hr, 24hr	F	No	F+D	No	Within bowel wall
Patient 5 Female 29.9years	05/05/23	19.7	1hr, 2hr, 4hr, 24hr	F	No	F+D	No	Planar = Prompt dispersal distally ? mucosal extravasation
Patient 6 Female 44.6years	19/07/23	21.4	1hr, 2hr, 4hr, 24hr	F	No	F+D	No	Dispersion to sigmoid
Patient 7 Female 43.0years	26/07/23	22.1	1hr, 2hr, 4hr, 24hr	F	No	F	No	Injection site close to midline
LN: ***Lymph Node;*** **TP:** ***Time Point;*** **BMI:** ***Body Mass Index;*** **DLP:** ***Dominant Lymphatic Pedicle;*** **Imaging Patterns - F:** ***Focal;*** **D:** ***Dispersed;*** **D+N:** ***Dispersed with LN uptake;*** **- F+D+N:** ***Focal uptake at injection site, dispersion of radioisotope and LN uptake;*** **PI:** ***Planar Imaging;*** **SPECT:** ***Single photon emission computerised tomography*****; NA:** ***Not Applicable***								

Table 25: Comparison of lymphatic drainage patterns in the first seven SF patients.

Two distinct radioisotope distribution patterns were observed on SPECT imaging:

i. Focal pattern (*n*=2; Patients 2 and 7), where no radioisotope movement was observed throughout the imaging period, and focal activity remained localized at the SF injection site (Figure 22).

ii. Dispersed pattern (*n*=5; Patients 1, 3, 4, 5 and 6), where no DLP or focal LN uptake was detected. Radioactivity was dispersed along the bowel wall from the SF injection site, moving anterograde in Patients 4 and 6, retrograde in Patients 1 and 5 and both ways in Patient EB (Figure 23). The clinical significance of this finding is unclear.

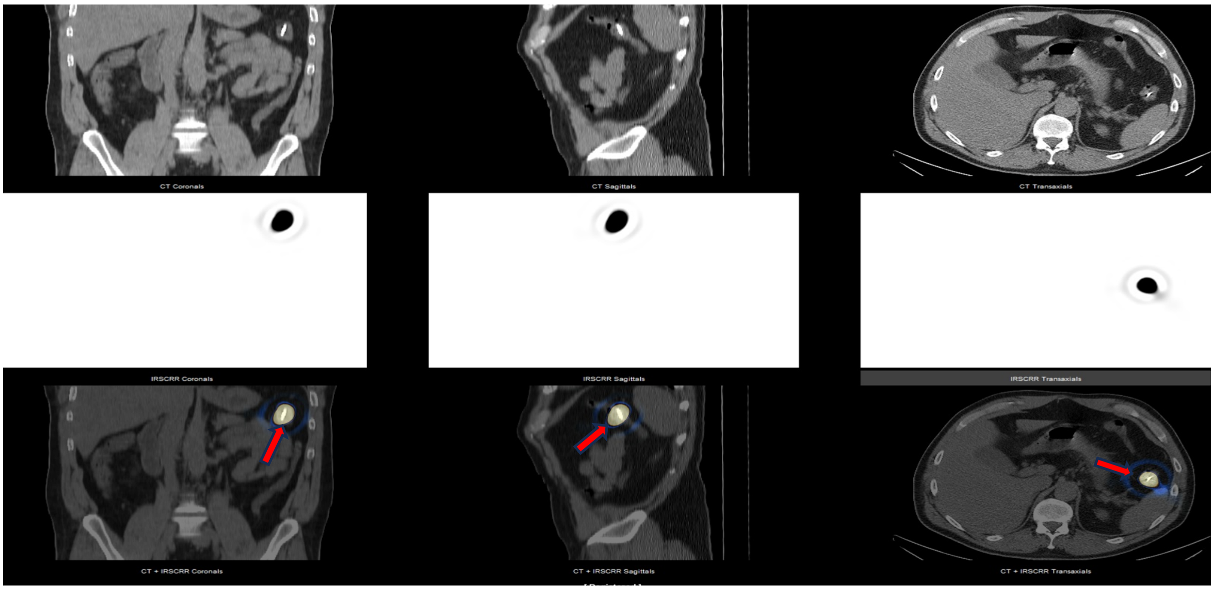

Figure 22: Planar and SPECT-CT imaging over coronal, sagittal and axial planes that only depict focal radioactivity at the SF injection site (red arrow).

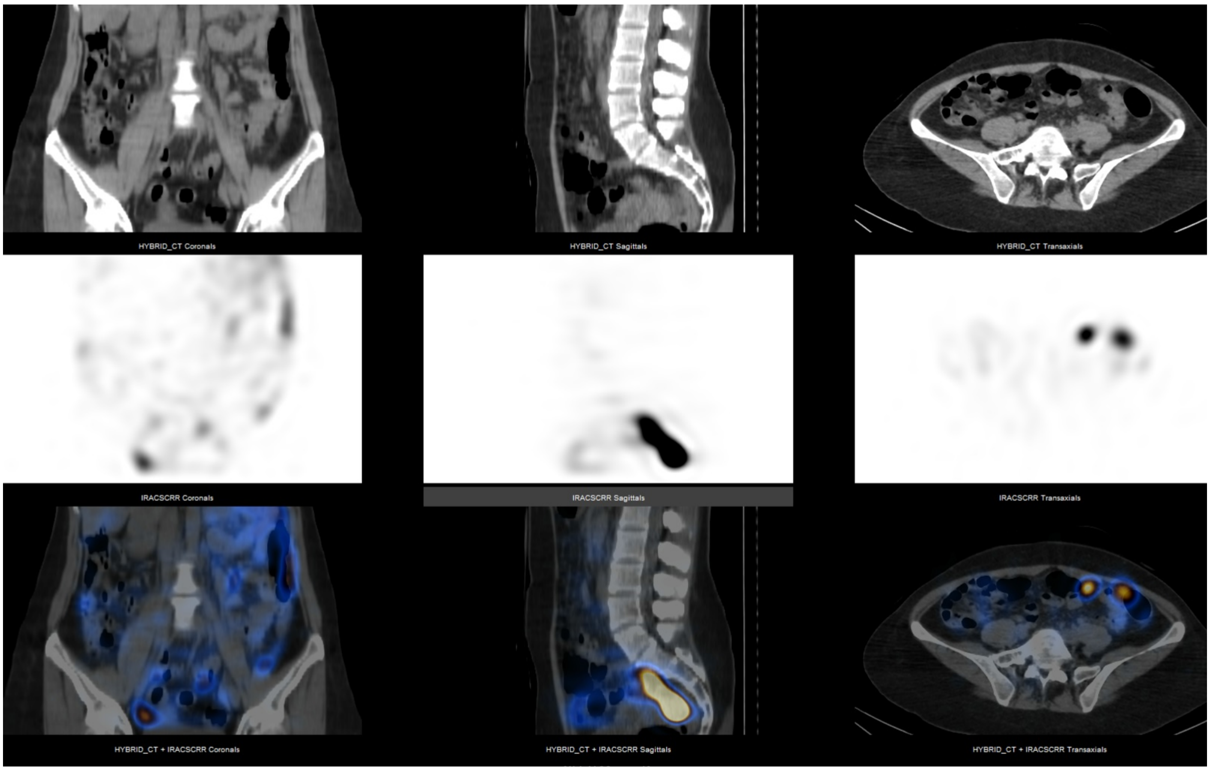

Figure 23: Planar and SPECT imaging of Patient 3 over coronal, sagittal and axial planes depicting dispersion of radioactivity both anterograde and retrograde from the SF injection site.

Protocol Refinement for SF Injection – Water-Jet Massage

The findings from the first seven patients studied contradicted the initial hypothesis, as no consistent DLP was identified following submucosal SF injection with Tc-99m Radiocolloid. However, in the management of malignancies at extracolonic sites (e.g., breast and skin), radioisotope injections routinely involve massaging the injection site to facilitate lymphatic drainage[332-336]. Drawing on this practice, it was hypothesized that mucosal massaging post-SF injection might have promoted a more consistent DLP to be identified.

Therefore, in the subsequent three recruited patients, a massage technique was employed to mimic local radioisotope manipulation. Specifically, after Tc-99m Radiocolloid injection and clip placement, the injected bleb was "massaged" with water-jets through the endoscope using a foot pump for two minutes.

Lymphatic Drainage Pattern of Subsequent Three Patients Who Had Their SF Massaged Post-Injection of Tc-99m Radiocolloid (Table 26 and Figure 24)

Table 26 summarises the lymphatic drainage pattern of the subsequent three SF patients who had their SF massaged with water-jet.

TABLE 26. Comparison of Lymphatic Drainage Patterns of the Last Three SF Patients							
Study Patients; Sex; Age	**Date of Scope**	**Imaging TPs**	**PI Pattern**	**DLP or LN Uptake**	**SPECT-CT Imaging Pattern**	**DLP or LN Uptake**	**Comments**
Patient 8; Male; 26.4 years	23/08/23	1hr, 2hr, 4hr, 24hr	F +D	No	F +D +N	DLP - IMA	LN uptake along IMA at 1hr (SPECT)
Patient 9; Female; 69.8 years	20/09/23	1hr, 2hr, 4hr, 24hr	F +D	No	F +D +N	DLP - IMA	Retrograde radioisotope movement and uptake along IMA at 1hr (SPECT)
Patient 10; Male; 49.7 years	20/09/23	1hr, 2hr, 4hr, 24hr	F	No	F	No	Injection site close to midline (SPECT and Planar)
LN: *Lymph Node;* TP: *Time Point;* DLP: *Dominant Lymphatic Pedicle;* Imaging Patterns - F: *Focal;* D*: Dispersed;* D+N*: Dispersed with LN uptake;* - F+D+N*: Focal uptake at injection site, dispersion of radioisotope and LN uptake;* PI: *Planar Imaging;* SPECT: *Single photon emission computerised tomography*							

Table 26: Comparison of lymphatic drainage patterns in the last three SF patients following water-jet massage.

As seen in Table 26 and consistent with the first seven SF patients, none of the subsequent three who received SF massage exhibited any discernible DLP or focal LN uptake on planar imaging, even when assessed at multiple time points. However, SPECT imaging revealed a dispersion pattern with lymphatic uptake characteristic of a DLP confined exclusively to the IMA region—with no activity noted at the MCA or SMA pedicles—in two of the three patients ([66.7%] Patients 8 and 9) who received SF massaging with a water-jet (Figure 24). Of note, Patient 9 exhibited a retrograde dispersion pattern alongside the DLP identification. Notably, the DLPs for both patients were observed at the 1-hour mark, with no further radioisotope distribution changes at later time points, suggesting limited value in multi-timepoint SPECT imaging for those patients.

Similar to Patient 7's finding, in the final patient (Patient 10) who underwent SF massage following radioisotope injection, the injected SF was seen placed close to the midline—likely due to colonic redundancy—and showed no DLP or focal LN uptake beyond the injection site.

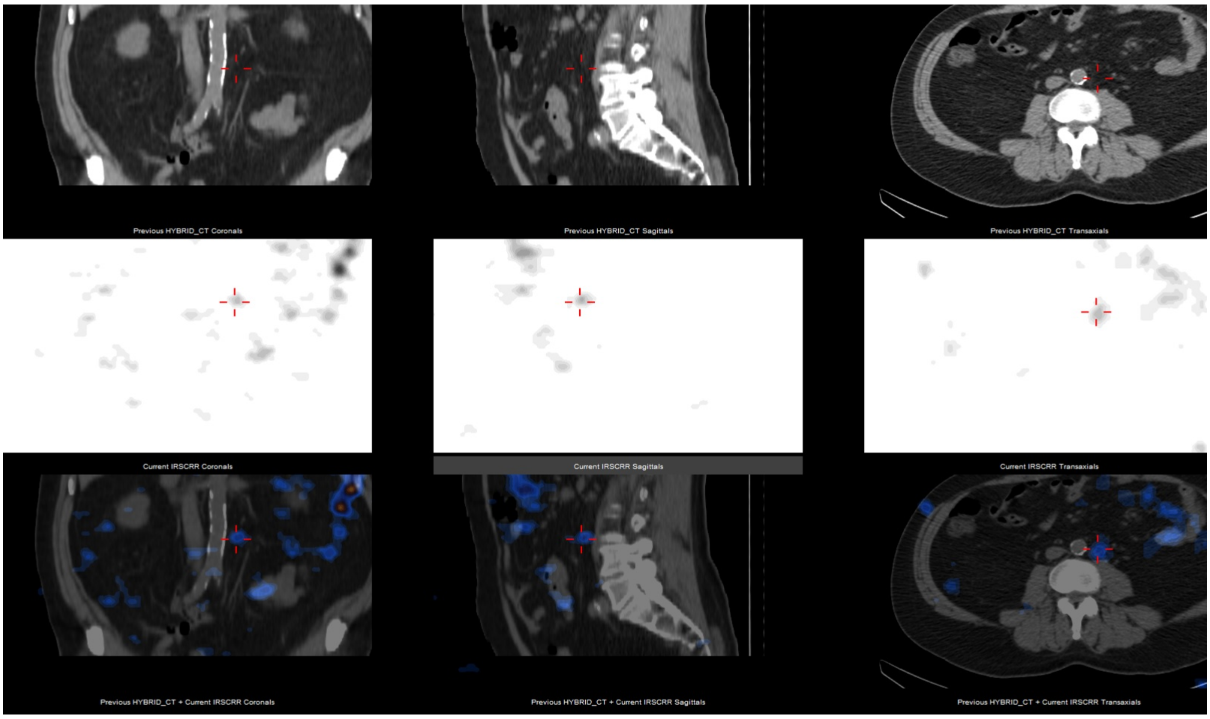

Figure 24: Planar and SPECT imaging over coronal, sagittal and axial planes depicting an IMA DLP in Patient 8, who underwent water-jet massage following SF injection. The crosshairs are centred over a LN with focal uptake, located in the para-aortic area along the IMA trajectory.

Protocol Refinement for Rectal Injection with Tc-99m Radiocolloid and Direct Mucosal Massage

Following injections in ten SF patients, an IMA DLP was identified in only two out of three patients who underwent mucosal water-jet massage. This raised uncertainty about whether a DLP could truly be identified in a watershed region. In addition, these results highlighted the need to ascertain whether direct (i.e. digital) massage could influence radioisotope drainage and facilitate DLP identification[332-336].

Accordingly, the study was extended to include patients receiving Tc-99m Radiocolloid injections in the upper rectum, followed by similar injections in the lower rectum. Specifically, recruiting patients for injection into the upper rectum was intended to demonstrate a "positive control", in the hope that a DLP could be demonstrated in a non-watershed area, as the upper rectum has a well-established singular blood supply from the IMA and lymphatic drainage through its nodal basins. Subsequent injection to the lower rectum was intended to investigate the effect of direct digital massage on tracer drainage.

Tc-99m Radiocolloid Injection of the Rectum (Figure 25 and Figure 26)

Five patients were recruited for injections in the upper rectum using the same method previously described for the SF patients who had the injection site massaged with water-jet. In addition to potentially serving as a 'positive control', this region was selected for other practical reasons. First, the upper rectum is easily identified endoscopically, aligning with the third rectal valve. Second, because the research phase takes place after the SOC colonoscopy, re-inserting the colonoscope into the rectum is simpler for both the endoscopist and the patient, and it readily facilitates water-jet massaging (Figure 25).

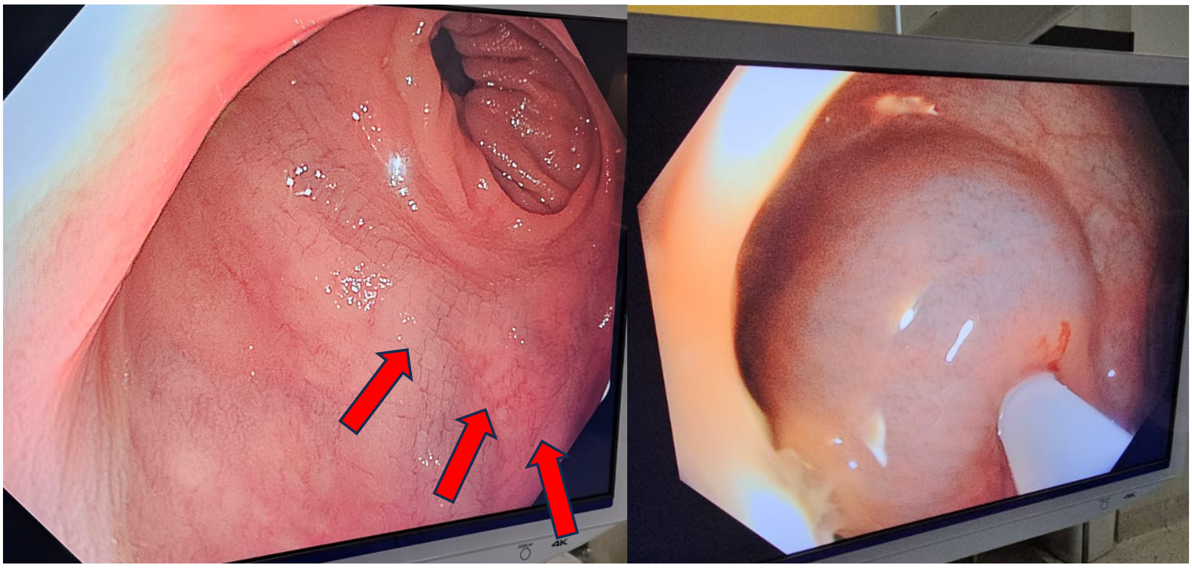

Figure 25: Third rectal fold identification (Left; Red arrows) and submucosal injection (Right) of the upper rectum.

Finally, to investigate how digital massaging the Tc-99m Radiocolloid injection site affects lymphatic drainage, five additional patients were recruited following the previously described protocol. In these patients, the radioisotope was injected into the lower rectum—an area above the dentate line at the anorectal junction (Figure 26). Because the posterior aspect of the lower rectal lumen can be easily palpated and compressed against the coccyx, it was chosen as the injection site in this group of patients. The area was digitally massaged in a gentle circular motion for two minutes. These patients were then subjected to SPECT-CT imaging as described for the SF patients.

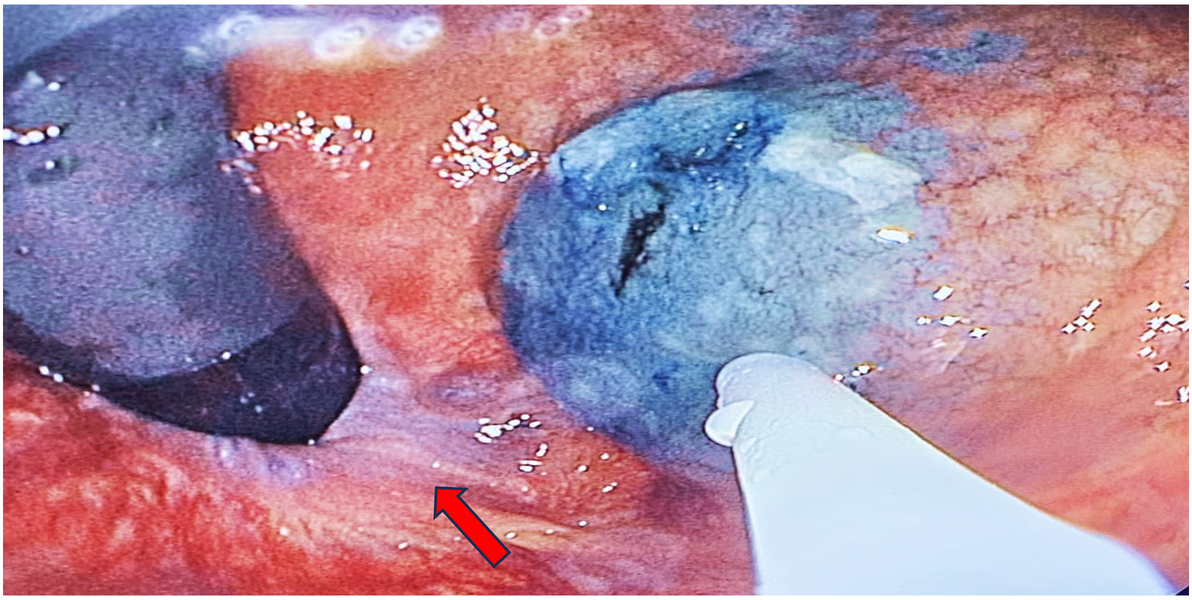

Figure 26: Endoscopic retroflexed image of radioisotope injection at the lower rectum at its posterior location. The red arrow highlights the haemorrhoidal plexus.

Description of Patients Who Had Their Rectum Injected with Tc-99m Radiocolloid (Figure 27)

For the subsequent phase of this study, an additional 22 patients were approached for participation. Of these, nine (40.9%) declined study participation, leaving 13 (59.1%) who underwent an SOC colonoscopy (Figure 27). The reasons cited for declining included concerns about procedure-related risks, particularly the radioisotope injection (*n*=5) and refusal of procedural sedation for the SOC (*n*=4). Of the 13 patients who initially agreed, three (23.1%) were subsequently excluded. Specifically, one patient required a polypectomy and two had a traversable malignancy identified.

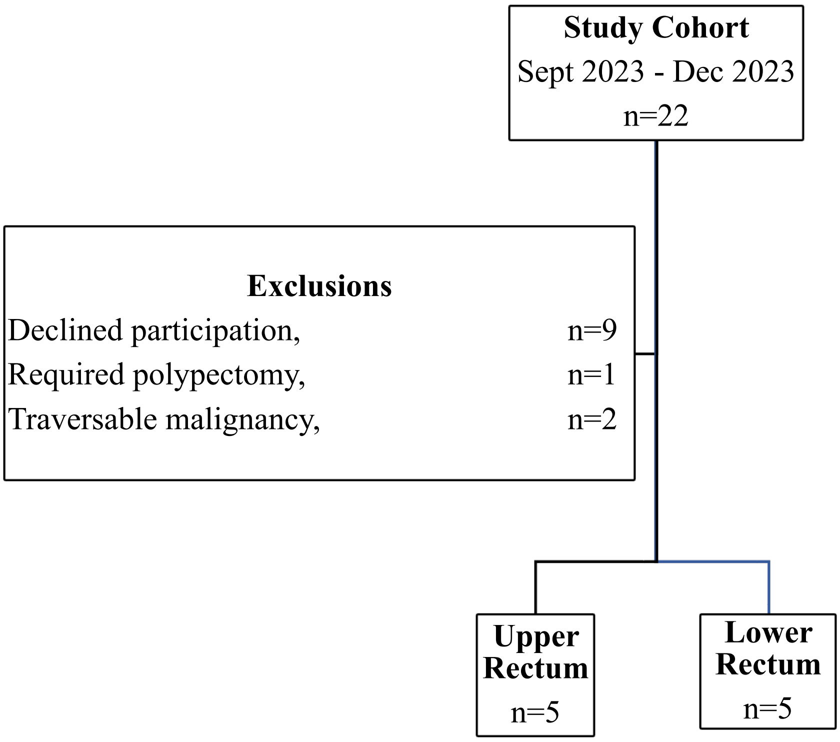

Figure 27: Flow diagram of cohort definition for patients who had their rectum injected.

Among the patients included, six (60%) were male, with a median age of 35.8 years (range 26.4-51.3) and median BMI of 27.3 kg/m^2 (range 19.5-30.3). The median duration of the research colonoscopy phase—which include radioisotope injection, NaCl flush, clip placement, and colonoscope withdrawal—was 8.0 minutes (range 5.9-10.0). There was no extravasation of radioisotope with any of the patients who had their rectum injected upon visual inspection at the time of the colonoscopy. The median time from radioisotope injection to scanning (including recovery in the endoscopy suite) was 74.3 minutes (range 63.9–83.7). All patients tolerated at least one high-calorie meal prior to being scanned, and none experienced immediate or delayed side effects or complication following the radioisotope injection.

Lymphatic Drainage Patterns of Patients Who Had Their Rectum Injected with Tc-99m Radiocolloid (Table 27, Figure 28 and Figure 29).

Table 27 summarises the lymphatic drainage patterns of the ten patients who had their rectum injected and subsequently massaged with either water-jet or directly with a finger.

TABLE 27. Comparison of Lymphatic Drainage Patterns of Patients who had their Rectum Injected

Study Patients	Date of Scope	Gender	Age (Years)	BMI (kg/m^2)	Injected Site	Massage	Imaging TPs	PI Pattern	DLP or LN Uptake	SPECT-CT Imaging Patterns	DLP or LN Uptake	Comments
Patient 11	18/10/23	Male	46.4	30.3	Upper Rectum	Water-Jet	1hr, 2hr, 4hr, 24hr	F	No	F	No	At 4hr localises close to the bowel wall (SPECT and Planar)
Patient 12	29/11/23	Male	31.8	29.4	Upper Rectum	Water-Jet	1hr, 2hr, 4hr	F	No	F	No	NA
Patient 13	13/12/23	Male	37.8	23.9	Upper Rectum	Water-Jet	1hr, 2hr and 4hr	F	No	F	No	NA
Patient 14	14/12/23	Male	33.0	27.4	Upper Rectum	Water-Jet	1hr, 2hr and 4hr	F	No	F	No	NA
Patient 15	14/12/23	Female	32.9	23.7	Upper Rectum	Water-Jet	1hr, 2hr, 4hr	F	No	F	No	At 1hr: ? LN in proximity to injection site (SPECT)
Patient 16	18/10/23	Female	26.4	20.8	Lower Rectum	Direct Finger	1hr, 2hr, 4hr, 24hr	F +D +N	LN	F +D +N	Both	At 1hr and 2hr – Left side wall LN (Planar) Ext Iliac LN (SPECT) At 4hr – Paraaortic (SPECT and Planar)
Patient 17	18/10/23	Female	37.0	27.2	Lower Rectum	Direct Finger	1hr, 2hr, 4hr, 24hr	F +D +N	LN	F +D +N	LN	At 1hr – left side wall LN x2

												(SPECT and Planar) At 2hr and 4hr show increased uptake (SPECT and Planar)
Patient 18	29/11/23	Female	37.0	19.5	Lower Rectum	Direct Finger	1hr, 2hr, 4hr, 24hr	F +D +N	LN	F +D +N	LN	Bilateral presacral drainage/LN at 1 hour (SPECT and Planar)
Patient 19	13/12/23	Male	34.5	27.5	Lower Rectum	Direct Finger	2hr, 4hr	F +D +N	Both	F +D +N	Both	At 2hr and 4hr - Left external iliac (SPECT) At 2hr and 4hr - Left pelvic side wall (Planar)
Patient 20	13/12/23	Male	51.3	29.7	Lower Rectum	Direct Finger	2hr, 4hr, 24hr	F +D +N	Both	F +D +N	Both	At 2hr - Right sidewall (Planar) Right Comm Iliac (SPECT) - Left Presacral (SPECT and Planar) - Para-aortic (SPECT and Planar) At 4hr - Right Ext Iliac (SPECT)
LN: ***Lymph Node;*** **TP:** ***Time Point;*** **BMI:** ***Body Mass Index;*** **DLP:** ***Dominant Lymphatic Pedicle;*** **Imaging Patterns - F:** ***Focal;*** **D:** ***Dispersed;*** **D+N:** ***Dispersed with LN uptake;*** **- F+D+N:** ***Focal uptake at injection site, dispersion of radioisotope and LN uptake;*** **PI:** ***Planar Imaging;*** **SPECT:** ***Single photon emission computerised tomography*****; NA:** ***Not Applicable***												

Table 27: Comparison of lymphatic drainage patterns of the patients who had their rectum injected and massaged with either water-jet or directly with a finger.

When comparing patients who received injections in the upper rectum with those who received injections in the lower rectum (i.e., the anorectal junction), no DLP or focal LN activity was detected in the upper rectal group, except for at the injection site, either on planar or SPECT imaging (Figure 28). Of note, in patient 11 and 15, the radioisotope uptake observed near the bowel wall on the 1-hour and 4-hour images, respectively, were likely artefacts resulting from its proximity to the injection site.

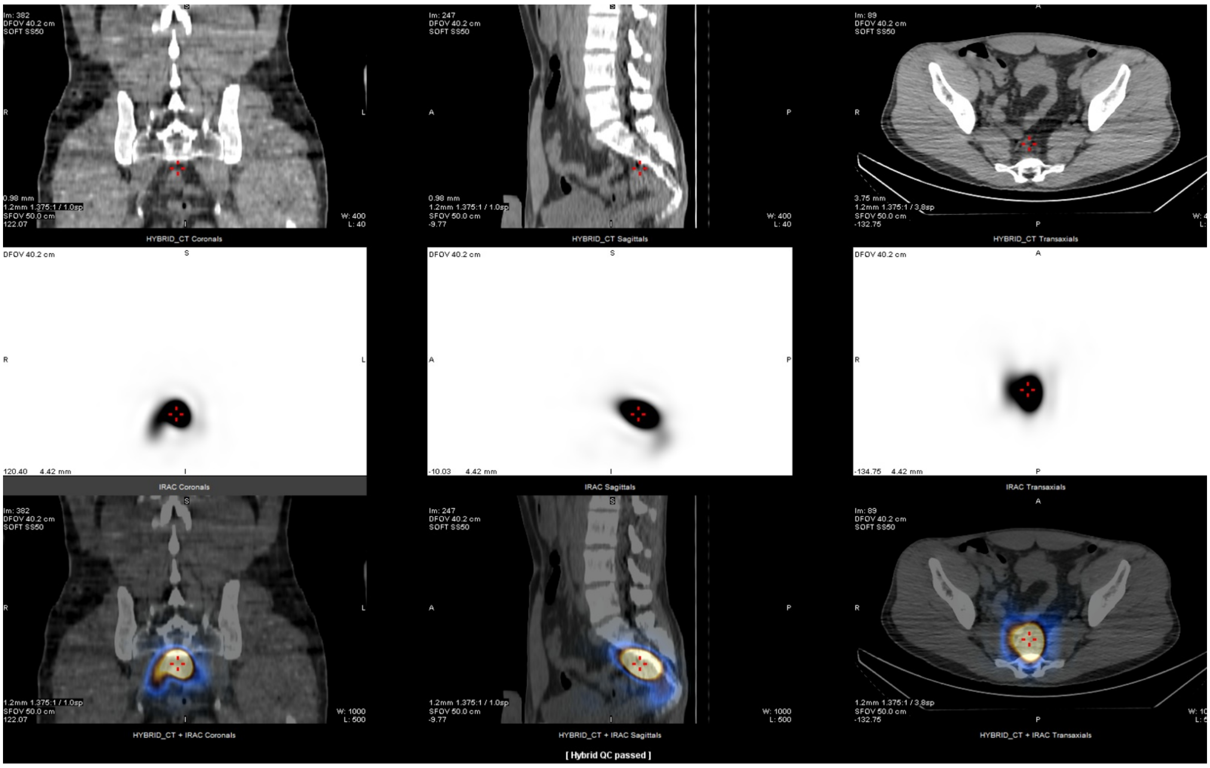

Figure 28: Planar and SPECT-CT imaging over coronal, sagittal and axial planes show no migration of the radioisotope from the injection site in a patient who received an upper rectal injection. The crosshairs highlight focal activity at the injection site.

In contrast, all patients with lower rectal injections showed lymphatic drainage and focal LN activity after direct massage, specifically in the right common iliac (Patient 20), presacral (Patients 18, 20), para-aortic (Patients 16, 20), left external iliac (Patients 16, 17, 19), and right external iliac (Patient 20) regions. Notably, in all cases with observed activity, it appeared early (i.e., 1-hour, 2-hour or 4-hour images) and remained unchanged on delayed imaging. Planar imaging was as sensitive as SPECT for detecting LN uptake, although SPECT provided more detailed information regarding the specific site of LN involvement. Figure 29 illustrates the planar and SPECT imaging in a patient who had their lower rectum injected.

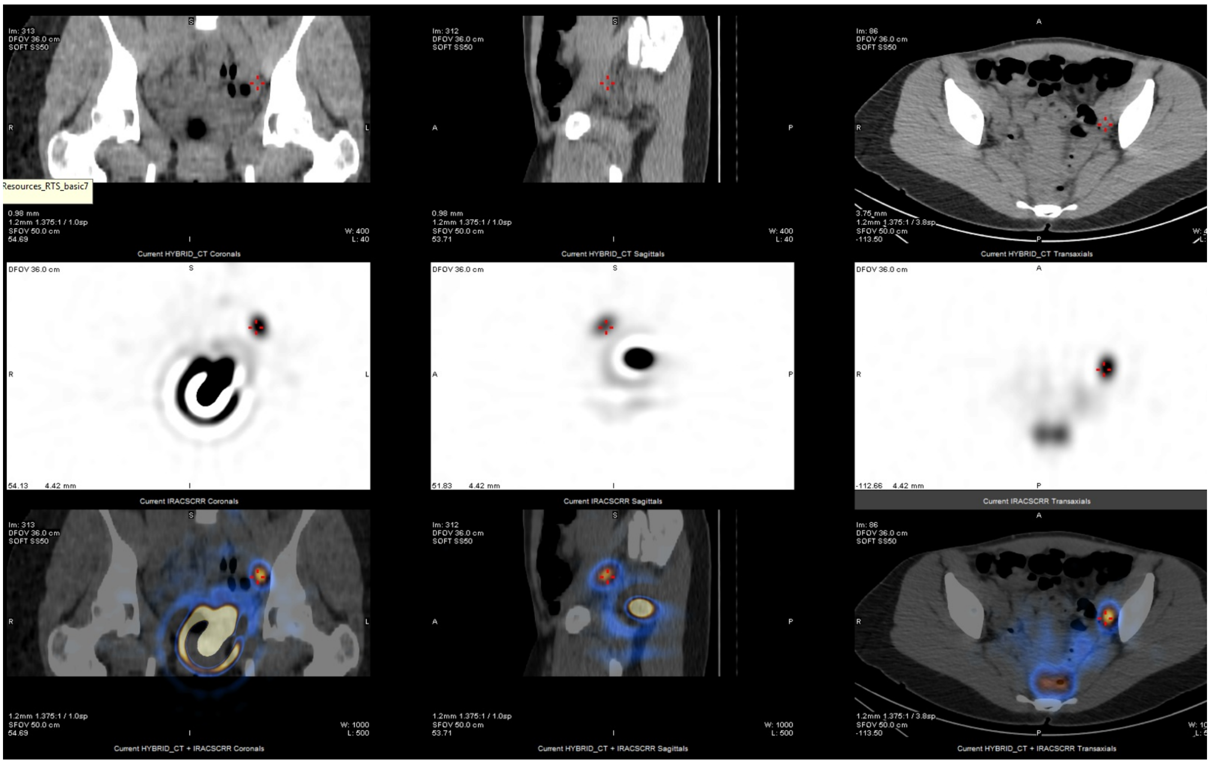

Figure 29: Planar and SPECT-CT imaging over coronal, sagittal and axial planes demonstrate the transit of the radioisotope from the injection site at the lower rectum to the left pelvic side wall in Patient 13, with the crosshairs indicating focal LN uptake in that region.

DISCUSSION

Over a one-year period, this prospective pilot study evaluated the lymphatic drainage of the SF by integrating endoscopic and nuclear medicine technologies. Specifically, Tc-99m Radiocolloid was delivered *in vivo* via intraluminal submucosal injection at the SF using a colonoscope, with subsequent scintigraphy performed via planar and SPECT imaging. Through this collaborative approach, the study aimed to confirm both the feasibility and safety profile of this technique, which mimics physiological lymphatic drainage through the bowel wall. As a secondary objective, and given the lack of established pre-operative parameters or feasible methods for assessing individual SF lymphatic drainage[84,201,202], the study sought to define a DLP for the SF. With this pre-operative insight, it was extrapolated that this lymphoscintigraphy technique could allow for a better understanding of a SFC's preferential lymphatic drainage thus informing targeted lymphadenectomy for patients with SFCs. This approach may be especially useful in laparoscopic surgeries where efficient port placement is possible allowing a more streamlined procedure and reducing unnecessary anaesthetic or operative time.

Although the protocol proved to be feasible, reproducible, and safe, a consistent lymphatic drainage pattern at the SF was not discernible. Notably, an IMA drainage pattern was observed in only a small proportion of patients (n=2) when the SF injection site was endoscopically massaged with a water-jet. To address the inconsistencies in lymphatic drainage patterns and control for potential confounders, the study was extended to include *positive* controls by way of upper rectal injections because of their non-watershed blood supply. Next, lower rectal injections were performed to assess the impact of direct finger massage—a standard technique in cutaneous and mammary malignancies—on lymphatic drainage. Although no patients who received upper rectal injections showed lymphatic drainage or LN uptake, all patients with lower rectal injections demonstrated lymphatic drainage and focal LN activity following direct finger massage. However, the observed drainage pathways were inconsistent, involving both the pelvic side wall and intra-abdominal regions. Originally, the upper rectal group was designated as the *positive* control cohort; however, only the lower rectal group exhibited lymphatic drainage and LN uptake. As a result—likely due to the role of direct finger massage—the lower rectal group became the effective *positive* controls, even though no consistent pattern emerged.

Understanding the literature on DLP identification of the colon is challenging because most studies have focused on the SLN concept[324,327,337-354], paying little attention to delineating the DLP—especially in regions with a watershed blood supply such as the SF[166]. It is prudent to emphasise that the definition of a DLP and SLN identification are fundamentally different. The identification of SLNs is fundamentally used in malignancies such as breast and cutaneous cancers, where the intention is to identify node negative patients to avoid a morbid (wider) lymphadenectomy. Whereas, in CC surgery, defining a DLP allows for a more precise lymphadenectomy tailored to an individual tumour's unique lymphatic drainage pattern. Thus, a lymphadenectomy in this setting includes ligating the tumour supplying vessel within an intact mesocolic envelope with the goal of identifying patients who have nodal metastases and would likely benefit from adjuvant systemic therapy. Such an individualised strategy is particularly appealing in a watershed region like the SF, where the dual blood supply from the SMA and IMA tributaries complicates ligation of the dominant tumour supplying vessel and identification of positive LNs[166]. Therefore, relevance is improved when focussing on DLP rather than SLN identification in these regions (in which colonic lymphatic drainage would likely be limited to pericolic [or D1] LNs). Rather than attempting to identify a single draining

LN or small group of nodes, the DLP approach acknowledges the unique lymphatic drainage pathways, which provides a more accurate representation of lymphatic drainage in watershed regions. This is particularly relevant given the current contemporary practice with regards to CME and CVL principles in CC surgery.

The mucosal origin of CRC significantly influenced this Chapter's study design, prompting the administration of the radioisotope submucosally rather than subserosally, as has been done in other studies[153,339,340,351,355-360]. Submucosal injection most closely mirrors the physiological route of colonic lymphatic drainage, given that there is ordered orthodromic lymphatic flow from the pericolic tissue toward the mesentery after the convergence of two distinct lymphatic networks within the muscular and mucosal layer into a common collecting duct system[329,330]. Therefore, considering the mucosal origin of CRC adenocarcinomas, LNs draining this region—and consequently the tumour—may be overlooked if the radioisotope is injected subserosally. This was supported by a 2023 meta-analysis by Lucas *et al*.[354], where the pooled estimate for tracer-positive LNs was higher for submucosal injections compared to subserosal injections (22.9% vs. 14.3%), and submucosal tracer application resulted in a significantly higher aberrant drainage detection rate (18.5% vs. 2.0%).

To date, only a small proportion of studies have investigated the lymphatic drainage of the SF[153,157,355,359,361]. Most of these have focused on patients with SFCs[153,355,359,361], using heterogeneous methods in terms of study design, injection techniques, injection locations, radiopharmaceutical type and volume and imaging protocols. The complexity of the available results is further reinforced by an intercontinental survey which found that although clinicians agreed lymphangiography might increase LNY, they did not reach a consensus regarding lymphatic mapping or the impact of lymphatic evaluation on resection plans[362,363].

Notably, one study has investigated the preferential lymphatic drainage of the non-pathological SF in a non-cadaveric setting[157]. In that study, Vasey *et al*.[157] conducted a single-centre clinical trial using laparoscopic scintigraphic mapping to evaluate normal lymphatic drainage of the SF. The trial involved 30 patients undergoing elective colorectal resections for malignancies not involving the SF and demonstrated that drainage was preferentially directed to the LCA pedicle (a tributary of the IMA) in the vast majority of cases. However, the injection was placed subserosally, and during the initial 15- and 30-minute intervals, dissection was limited to mobilizing the sigmoid colon laterally and "initiating rectal dissection in the mesorectal plane below the IMA pedicle".

It is difficult to understand how mobilizing these key structures would not inadvertently encourage *in vivo* lymphatic flow surrounding the IMA tributaries as the aforementioned key structures are intimately related to the IMA. Additionally, dissection in this area requires triangulation using at least three points of contact— two of which are likely on the colon or mesentery itself. Moreover, the intra-operative timing of radioisotope injection in Vasey *et al*'s.[157] study may have a bearing on LNY and aberrant drainage rates compared to this Chapter's methodology. For instance, Lucas *et al*.[354] found that tracer application timing (i.e., pre-operative vs intra-operative) was the strongest predictor of effective LN mapping and LNY, with intraoperative tracer injection (regardless of type) resulting in lower rates of tracer-laden LNY (14.1% vs 30%) and aberrant drainage (2.5% vs. 26.3%) compared to those that received a pre-operative injection. It is likely that "pre-operative" submucosal injection of radioisotope (as performed in this Chapter) may allow more time for the tracer to traverse the lymphatic system, reach more distant LNs, and enable more effective mapping.

Planar imaging was unable to discern any focal LN uptake in all of those who had their SF injected, irrespective of the time point—even in cases where activity was noted at the IMA pedicle on SPECT imaging. Similarly, in those who had their upper rectum injected, no focal LN uptake or lymphatic drainage was seen. This is contrary to planar images obtained following lower rectal injection, where a lymphatic drainage pattern and LN uptake were seen in all patients, albeit disparate. The observed discrepancy may be related to the fact that in the SF, redundant bowel loops likely contributed to increased scatter and ring artefact, thereby obscuring the assessment of vascular pedicles. Additionally, the focal activity at the injection site in those who had their upper rectum injected may have produced artefacts that obscured potential LN drainage. Notably, these artefacts were not observed in those who had their lower rectum injected. In fact, in those who had their lower rectum injected, planar imaging was as sensitive as SPECT for detecting LN uptake, although SPECT provided more detailed information regarding the specific site of LN avidity. The successful drainage and LN uptake in the lower rectum, despite its variable blood supply, may be attributed to its location within the concavity of the sacrum and coccyx. When digital massage was applied to the posterior aspect of the lower rectum, the sacrum and coccyx served as a firm counterpressure, enabling more effective massaging and dissipation of the radioisotope, unlike water jet massage to the upper rectum and SF that could be regarded as *less contained or bounded*.

Differences in tissue architecture and interstitial pressures may underlie the distinct patterns of radioisotope dispersion and lymphatic drainage observed in the colorectal submucosa in this study compared to the breast and skin. Focal uptake at the injection site was noted in the bowel wall among patients who received upper-rectal injections and water-jet massage, whereas those injected in the lower rectum displayed more varied lymphatic drainage and LN uptake—unlike the more uniform uptake typically seen in regional nodes after subdermal injections in the breast and skin. These findings suggest that understanding the differences in interstitial tissue pressures between the colorectal submucosa and the dermis/sub-dermis of the breast and skin could be key to elucidating the pharmacodynamics of radioisotopes in relation to the lymphatic system. Moreover, understanding these differences may help explain why submucosal radioisotope delivery often disperses as a bleb—a phenomenon not commonly observed with subdermal injections.

Furthermore, while lymphoscintigraphy in cutaneous and mammary malignancies reliably identifies a drainage pathway leading to a finite number of sentinel nodes, this pattern was not observed in colon lymphatics. Several factors may account for this discrepancy: (i) LNs in the colon tend to be less than 1cm and buried by mesenteric adipose tissue[357], (ii) the colon typically contains a larger and more variable number of LNs compared to a region like the breast which may drain to only about 20–40 nodes[364], (iii) colonic LNs are less predictable, and more than one LN can frequently be assigned as a D1, pericolic or sentinel node[342] and (iv) histopathological differences between colorectal nodes and those in extra-colonic sites may make pathological nodes from the latter sites more readily identifiable—often being solitary and distinct on lymphoscintigraphy—than their colorectal counterparts.

As this is a pilot study, its proof of concept and scientific applicability require validation before fully appreciating its limitations. Regardless, it is important to note that this trial did not include individuals with SFCs for several reasons: (i) the recruitment period would be lengthy due to a 25-year incidence rate of only 6%[180], (ii) the presence of cancer could alter lymphatic flow due to mass effect, and (iii) it would be inappropriate, both psychologically and oncologically, to subject patients with a cancer diagnosis to this untested preoperative technique.

In summary, based on the results of this study, there is insufficient evidence to support the use of SPECT technology post-submucosal injection of Tc-99m Radiocolloid to define a DLP of the SF. However, understanding the DLP of a watershed region like the SF remains clinically relevant, as it would allow for targeted lymphadenectomy in individual patients and enables thorough operative planning with appropriate patient counselling. Establishing such an imaging modality that informs which pedicle should be targeted for radical lymphadenectomy should remain a topic for further research.

CHAPTER VII: SUMMARY

In **Part I** of this thesis, novel markers of EoL (i.e., RAPL and TM measurements) were investigated to determine whether they could provide insight into the *unresected* nodal tissue left behind and, by extension, assess their potential survival associations to appraise their utility as indicators of high-quality CRC surgery. Additionally, the importance of EoL in a unique, and often under-studied, population of patients with mCRC was investigated, with LNR used to prognosticate outcomes in these patients.

Chapter II confirmed that while RAPL measurements were feasible and reproducible in a non-routine CVL cohort, no demonstratable association was found between RAPL and survival outcomes. This is in contradistinction with previous published literature; in a cohort where CVL was routinely intended to be performed, Vogelsang *et al.*[81] observed a survival benefit associated with a shorter RAPL. The inability of the results in **Chapter II** to replicate Vogelsang *et al.*'s[81] findings in a non-routine CVL cohort suggests that the benefits of a shorter RAPL may only be realised in the context of routine CVL being performed (RAPL≤10mm), reinforcing the idea that CVL is an 'all or nothing phenomenon'.

Chapter III investigated *ex vivo* TM measurements in the setting of non-routine CVL surgery but, contrary to the initial hypothesis, no associations between these measurements and either OS or DFS in the AR or RH surgery cohorts were found. The absence of survival associations were consistent across all TM measurements, including those 'oncologically relevant' (i.e., TM A, TM B, and TM C) or otherwise (i.e., TM D). Moreover, the lack of association persisted even in the AJCC Stage III group, where an increased EoL would have been expected to yield the greatest survival advantage.

In **<u>Chapter IV</u>**, the aim was to investigate whether LNR=0 and LNR>0 could serve as independent predictors of OS in a unique and often under-studied population of patients—those with mCRC who have undergone resection of the primary tumour. This population has been historically overlooked due to the perception of futility in performing standard oncological surgery in these patients, given their presumed poor prognosis. The hypothesis was that a rising LNR is associated with poorer survival, while LNR=0 would be associated with better outcome. The results of **<u>Chapter IV</u>** highlight that the dichotomization of LNR into LNR=0 and LNR>0 holds useful prognostic information, with LNR=0 (i.e., N0 status) identifying a unique subgroup of patients with an improved prognosis. This finding underscores the importance of performing an adequate lymphadenectomy (i.e., harvesting at least twelve LNs) to provide accurate nodal staging of mCRC patients. It is argued that the demonstrable prognostic importance of LNR (specifically LNR=0, or N0 status) can only be reliably ascertained in the presence of an adequate lymphadenectomy (i.e., LNY of at least twelve nodes).

In summary, the findings of **<u>Part I</u>** suggest that while RAPL and TM measurements are feasible and reproducible in a cohort where CVL is not routinely performed, their utility as quality metrics for good CRC surgery is this 'non-routine' CVL cohort is not supported. Interpreting these results in the context of previous published literature, the role of RAPL and TM measurements as quality metrics may be confined to cohorts where CVL surgery is routinely intended, and indeed, may be one critical metric to *verify* that CVL surgery has been performed. Additionally, the prognostic value of LNR, as demonstrated in a mCRC cohort, is contingent upon an adequate lymphadenectomy so as to allow an accurate calculation of LNR. Together, these findings underscore the importance of surgical standardization when evaluating potential quality indicators for CRC surgery. Until such standardisation is achieved, accurate and structured pathology assessment of resected specimens remains crucial for disease prognostication. Of these, traditional pathology assessment methods by way of accurate LN count remains essential to accurate cancer staging and guiding the decision for or against adjuvant therapy.

Part II of this thesis explored the twin concept of quality of CRC surgery termed “appropriateness” or “correctness” of lymphadenectomy as seen at watershed areas of the colon and rectum. This section focused exclusively on the SF watershed area.

In **Chapter V**, the aim was to advance knowledge of SFC management by examining the Concord experience, with the goal of establishing an SFC phenotype and assessing long-term oncological outcomes in relation to clinicopathological factors. This Chapter found that SFC did not exhibit worse survival outcomes than those with CC at other sites, even though adverse clinicopathological features were more common in the SFC group. The similarity in survival between SFC and non-SFC patients likely reflects the fact that tumour stage—a key determinant of oncological outcome—was comparable in both groups. Recognizing that SFCs do not differ from non-SFCs in their propensity for metastasis, it follows that the fundamental oncological principles for surgical resection should likewise remain the same. Consequently, it could be argued that contemporary approaches such as CME and CVL to achieve adequate lymphadenectomy should be applied to SFCs, just as they are to non-SFCs. Nevertheless, implementing these principles in the SF watershed region continues to pose significant challenges. Indeed, CVL surgery for SFCs has not been standardised, and high ligation of MCA and IMA pedicles would result in excessive (and unwarranted) devascularisation of the colon.

Therefore, **<u>Chapter VI</u>** aimed to gain a more comprehensive understanding of the heterogeneous lymphatic drainage of the SF and to identify a DLP that would facilitate targeted lymphadenectomy in individual patients. This would potentially allow a method by which CVL surgery for SFCs could be performed while preserving vascular supply of a (presumably) uninvolved pedicle. Although submucosal injection of *in vivo* intraluminal Tc-99m Radiocolloid via an endoscope, followed by planar and 3D-SPECT-CT imaging, proved both feasible and reproducible, it failed to demonstrate a consistent DLP originating from the SF. This finding remained unchanged even after controlling for potential confounders and extending the study to include upper rectal injections (chosen for their fixed blood supply), followed by lower rectal injections after which the impact of digitally massaging the injection site, as is the standard with other cutaneous and mammary malignancies, was assessed. Ultimately, no reliable lymphatic drainage pathway was observed at the SF, with or without massaging. Thus, at this stage there is insufficient evidence to support the use of 3D-SPECT technology to define a DLP following SF injection of Tc-99m Radiocolloid.

CHAPTER VIII: FUTURE DIRECTIONS

There remains a pressing need for rigorous clinical research on EoL metrics—particularly focusing on *unresected* LN tissue—to clarify their potential impact on survival. Although the findings from **Chapters II** and **III** confirm that RAPL and TM measurements are both feasible and reproducible, their value as quality metrics for EoL in CRC surgery may be limited unless implemented specifically within the framework of routine CVL surgery.

However, the long-term survival benefits reported for CVL surgery itself have thus far been derived exclusively from non-trial settings and remain contentious. For example, a meta-analysis in 2021 by Crane *et al.*[211] reported significantly higher OS (3-year and 5-year) and DFS (1-year and 3-year) with CVL/D3 surgery[6,365]. Contrarily, Olofsson *et al.*'s[366,367] retrospective cohort studies in 2016 and 2019 investigated right and left sided CC resections and found no difference in OS or DFS between D3/CVL and conventional/D2 lymphadenectomy. Similarly, in 2016, Gouvas *et al.*'s[218] meta-analysis failed to identify evidence linking D3 lymphadenectomy to improved survival[368]. When synthesizing the varied results, it becomes clear that specimen quality *viz.* plane of excision was not consistently comparable across studies such that in some, a control group, was missing[369]. Consequently, it remains uncertain whether any observed survival benefits arise from central lymphadenectomy/CVL surgery or superior specimen quality with respect to plane of excision[369].

A rigorous prospective clinical trial is essential to accurately assess the true impact of CVL on survival outcomes. In this randomized trial, both the CVL and non-CVL arms should include the prospective collection of RAPL and TM measurements in all patients undergoing CME surgery. This approach would allow for a comprehensive evaluation of these novel EoL metrics and their long-term effects on survival in both CVL and non-CVL groups, while ensuring that the plane of excision (i.e., CME) does not serve as a confounding factor.

With the growing trend of leveraging AI in clinical research, integrating an AI-driven protocol for measuring RAPL and TM metrics could be highly beneficial, particularly given the absence of published research applying AI to these endpoints. Although these protocols have not been formally described, they hold the potential to significantly improve the accuracy and reproducibility of RAPL and TM measurements both in the regions discussed in this thesis and in other areas of the colon with challenging anatomy. For instance, in patients with transverse CCs, accurately measuring MCA RAPL is particularly challenging due to anatomical variations in the region, which stem from individual differences in the colonic configuration, topography and mesenteric vasculature. Consequently, further investigation into the development and validation of an AI protocol for quantifying RAPL and TM is warranted, as it could improve measurement precision and overall utility in the clinical setting.

Chapter IV's investigation provides compelling evidence for the prognostic value of LNR, and particularly the distinction between LNR=0 and LNR>0 in mCRC patients undergoing upfront surgery. However, there is an increased complexity when applying these findings to patients who receive nCT. In retrospective cohort studies, nCT has been shown to induce tumour and nodal downstaging, which potentially reduces the number of retrievable LNs, thus altering the LNR[26,259]. This in turn may affect the accuracy of nodal staging as well as the prognostic significance of LNR. Thus, it is necessary to pursue further prospective research to determine whether the prognostic value of LNR observed in the upfront surgery setting applies to patients treated with nCT. Ideally, future prospective studies should explore how nCT affects the calculation and interpretation of LNR, and whether the dichotomization into LNR=0 and LNR>0 continues to provide reliable prognostic information.

The results of **Part II** of this thesis did not support the use of submucosal injection of Tc-99m Radiocolloid and subsequent SPECT imaging to determine a DLP for the SF. However, understanding the DLP of a watershed region like the SF remains clinically relevant, as it allows for targeted lymphadenectomy in individual patients and enables thorough operative planning with appropriate patient counselling.

A comprehensive investigation could adopt a three-pronged strategy. First, using Python (PSF, Delaware, USA) programming language, maximum intensity projection images could be constructed from original DICOM images. Subsequently, VOI and decay correction calculations and tools could be used to delineate the amount of radioactivity along the IMA, MCA and ICA (as a control) to potentially identify the DLP of the SF. This objective process could better determine whether SPECT technology has the sensitivity to identify lymphatic drainage in the colon, where radioisotope diffusion may be greater compared to regions such as the breast and skin.

Second, a basic science research model would likely be useful to clarify the histopathology, physiology, and biomechanics of the colonic bowel wall and its lymphatic drainage—particularly in light of the inconsistent patterns noted in this thesis. It remains puzzling why despite utilising the same radioisotope (Tc-99m Radiocolloid) and performing similar protocols of image acquisition to lymphoscintigraphy investigations for breast cancer and melanoma, reliable lymphatic drainage patterns could not be demonstrated with colonic radioisotope injection. While no histological differences in lymphatic channels between the different anatomical sites have previously been described, this could be more comprehensively investigated with prospective studies using human tissue, and even investigation using electron microscopy technology. Thought should be given to objectively comparing interstitial tissue pressures within the colonic submucosa and dermis/subdermis, as this may give an understanding as to why the radioisotope seems to be readily 'pushed' into lymphatic channels when injected into the dermis/subdermis, while tending to disperse as a bleb when injected into the colonic submucosa. Such foundational work may inform decisions about the most appropriate radioisotope type, concentration, and nuclear imaging modality to evaluate colonic lymphatic drainage, focusing on the SF DLP.

Thirdly, it is conceivable that a study with a 'positive control' using another method of radioisotope detection could be designed to help ascertain whether the challenges with colonic lymphoscintigraphy relate to the sensitivity of SPECT image acquisition, or whether there is a true lack of radioisotope drainage following colonic submucosal injection. From this perspective, one such prospective cohort study could enlist patients undergoing any colorectal resection, for any indication (i.e. benign or malignant), to receive submucosal Tc-99m Radiocolloid injection within the bowel intended to be resected. SPECT imaging, as per the established protocol in **Chapter VI**, should be performed one day before surgery. On the day of the operation, the IMA, MCA and ICA (as a control) vascular pedicles would be surveyed with a Gamma probe to measure radioactivity along each pedicle. Matching results between the Gamma probe assessment and the earlier SPECT imaging would be a means of validating

SPECT as a useful modality for delineating the DLP; conversely, significant discrepancies would cast doubt on its utility in this context.

Finally, although exploring the rectal watershed area lies beyond the scope of this thesis, the same methodology could ultimately be applied to the rectum, potentially offering additional insights into the role of LPLND for pathologically enlarged lateral pelvic LNs or those LNs persistently enlarged despite neoadjuvant therapy in patients with RC. This is particularly relevant as the landscape of LPLND is currently complex, contentious, and lacks uniformity worldwide[370]. Briefly, the indication criteria for LPND, especially size criteria with and without neoadjuvant therapy and the timing of imaging studies both in the pre-operative and post-operative setting remains controversial[370].

Western classification systems consider pelvic side-wall involvement (i.e., external iliac and obturator nodes) to be metastatic disease, whereas in the East such disease is treated as locoregional[370]. So much so that, in Japan, prophylactic LPLND has been routinely recommended for advanced lower RC to reduce LR and SR[371]. Long-term results from the JCOG0212 trial which evaluated 701 patients with AJCC Stage III RC showed that while TME and prophylactic LPLND confers significantly better local control, the improvement did not translate to an OS benefit in favour of TME and LPLND[371].

Nevertheless, LPLND is associated with longer operative times, higher morbidity rates than TME due its learning curve and complex anatomy[370]. In this context, ICG has been utilized innovatively intraoperatively to improve visualization during LPLND for locally advanced RC. Based on a recent systematic review by Kehagias *et al.*[370], ICG in LPLND is associated with an increase in the number of lateral pelvic LNs harvested, but with decreased blood loss and shortened hospital stay. These benefits are ascribed to the improved accuracy and efficacy ICG lends to defining LNs in the pelvic side walls[370]. However, long term outcomes regarding ICG use are scarce and there is poor definition of a dominant lymphatic drainage pattern seen with ICG in the literature thus far. This may be related to the fact that ICG is limited by its tissue penetration, which is only 1-2cm[370]. Therefore, as demonstrated in **Chapter VI**, SPECT imaging following radioisotope injection may be quantified to determine the DLP of a RC—whether it drains to the lateral pelvic sidewall nodes or the IMA nodes—thus guiding an appropriate lymphadenectomy.

CHAPTER IX: APPENDIXES

APPENDIX I

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ORIGINAL ARTICLE

Is computed tomography assessment of residual arterial pedicle length following colorectal cancer surgery a useful marker of surgical quality?

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Abstract

Background *In vivo* residual arterial pedicle length (RAPL) has been proposed as a quality indicator for central vascular ligation (CVL [i.e., RAPL ≤ 10 mm]) in colorectal cancer (CRC) surgery. However, its survival association in non-routine CVL practice requires clarification. This study aimed to assess the feasibility and reproducibility of measuring RAPL alongside its oncological associations in non-routine CVL surgery.

Methods A prospective cohort study at Concord Hospital was conducted on anterior resection (AR) or right hemicolectomy (RH) patients with stage I to III CRC (1995–2019). Using surveillance computed tomography (CT), RAPL of the inferior mesenteric artery (IMA) or ileo-colic artery (ICA) pedicle was measured independently by two observers. The intra-class correlation coefficient assessed the reproducibility of the measurements. Kaplan-Meier and univariate Cox regression analyses estimated overall survival (OS) and disease-free survival (DFS), while univariate and multivariate linear regression models tested correlations between RAPL and clinicopathological features.

Results A total of 1425 patients underwent a CRC operation. Post-operative CTs were reviewed in 424 patients, with 422 (mean age 69.0 years [SD 12.3]; 54.0% males) RAPLs measured. The majority studied underwent an AR (59.2%). Excellent inter-rater reliability was noted in AR (ICC = 0.97; $P < 0.001$) and RH (ICC = 0.89; $P < 0.001$) patients. No association was observed between RAPL and OS or DFS in either group. Also, RAPL lacked association with nodal harvest in either AR ($P = 0.54$) or RH ($P = 0.16$) patients.

Conclusion The value of RAPL as a quality marker of CRC surgery in non-routine CVL practice has not been confirmed. Furthermore, its lack of association with nodal harvest emphasizes the importance and the need for comprehensive pathology examination of the specimen following resection of CRC.

Keywords CME · CVL · Residual arterial pedicle length · Tissue morphometry

Introduction

Central tenets of 'good quality' CRC surgery include operating in the correct anatomical plane, complete primary tumour removal with clear margins, and performing an adequate lymphadenectomy. Most early studies appraising surgical quality have centred on the correct plane of excision (total mesorectal excision [TME] for rectal cancer or complete mesocolic excision [CME] for colon cancer) and avoidance of tumour transection [1–5]. However, the extent of lymphadenectomy (EoL) has arguably received less attention. This may be due to challenges in its objective measurement, which is so far limited to the rudimentary assessment of lymph node (LN) harvest in the excised specimen [6], when perhaps the assessment of unresected

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LNs may be more meaningful. Indeed, recent interest in CVL as a counterpart to CME suggests that an alternative approach to appraising the EoL would be useful [7, 8].

Based on the level of vascular ligation from the origin of the arterial vessel(s) supplying a particular segment of colon, CVL contributes to the completeness of a cancer operation [5]. This demarcates the maximum EoL by completely clearing regional LNs and those located centrally (to minimise any residual LN tissue potentially involved with cancer) [5, 9]. In cases where CVL is not strictly practised, pedicle ligation as high as possible is the usual aim. In both practices, the quality of lymphadenectomy may more accurately be determined by assessment of unresected residual LN tissue rather than the number of LNs found in the resected specimen.

The determination of whether CVL surgery has been performed remains largely dependent on corroboration by the surgical team. This practice does not allow for the level of pedicle transection to be assessed based upon pathological assessment of the excised specimen. Alternatively, in vivo RAPL measured on post-operative imaging has been proposed a more robust method to verify CVL surgery [7, 10, 11]. However, its role as a surrogate of the EoL and as a quality measure of survival outcomes in non-routine CVL practice has not been studied.

Therefore, the primary aim of this study was to assess the feasibility and reproducibility of measuring RAPL using surveillance CT in patients who had an AR or RH operation for CRC for which CVL was not routinely practised. A secondary aim of the study was to investigate whether RAPL was associated with survival outcomes or standard clinicopathological variables. We posited two hypotheses: (1) that measuring RAPL would prove to be both feasible and reliably reproducible and (2) a longer RAPL was associated with poorer oncological outcome and less radical lymphadenectomy.

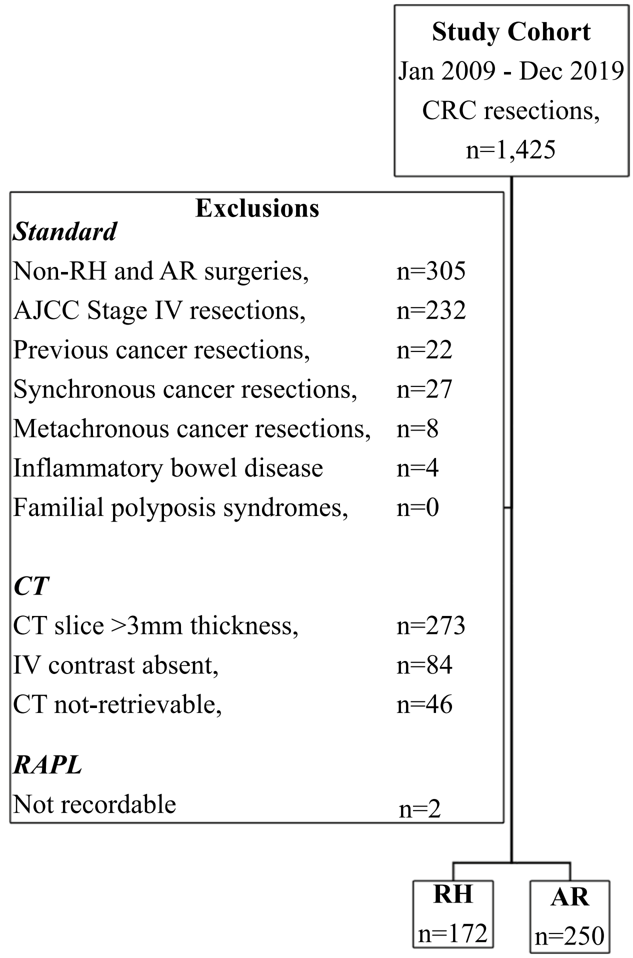

Fig. 1 Flow diagram of cohort definition

Materials and methods

A prospective observational cohort study of consecutive patients who underwent a potentially curative resection for a rectal or colon adenocarcinoma was performed. Patients included were those who had either an AR or RH performed between January 2009 and December 2019 at Concord Hospital, Sydney, Australia. Patients were identified from a prospectively maintained institutional database. Excluded were patients with American Joint Committee on Cancer (AJCC) Stage IV cancer, inflammatory bowel disease, polyposis coli, and a synchronous or metachronous CRC (Fig. 1).

Surgical procedures

An AR operation was performed with adherence to TME principles and ligation of the IMA proximal to the origin of the ascending left colic artery (LCA), without abdominal aorta exposure [12]. A RH operation was performed according to the technique described by Bokey et al. [12], which involved ligation of the ICA without routine exposure of the superior mesenteric artery (SMA) or vein (SMV). In our unit, some surgeons perform a full lymphadenectomy but preserve the vascular stump by sweeping potential central nodes from the pedicle root distally in patients with suspected central lymphadenectomy. The precise tumour location was marked by the surgeon on a diagram including whether there was distant metastasis or unresected tumour remaining at the completion of the operation.

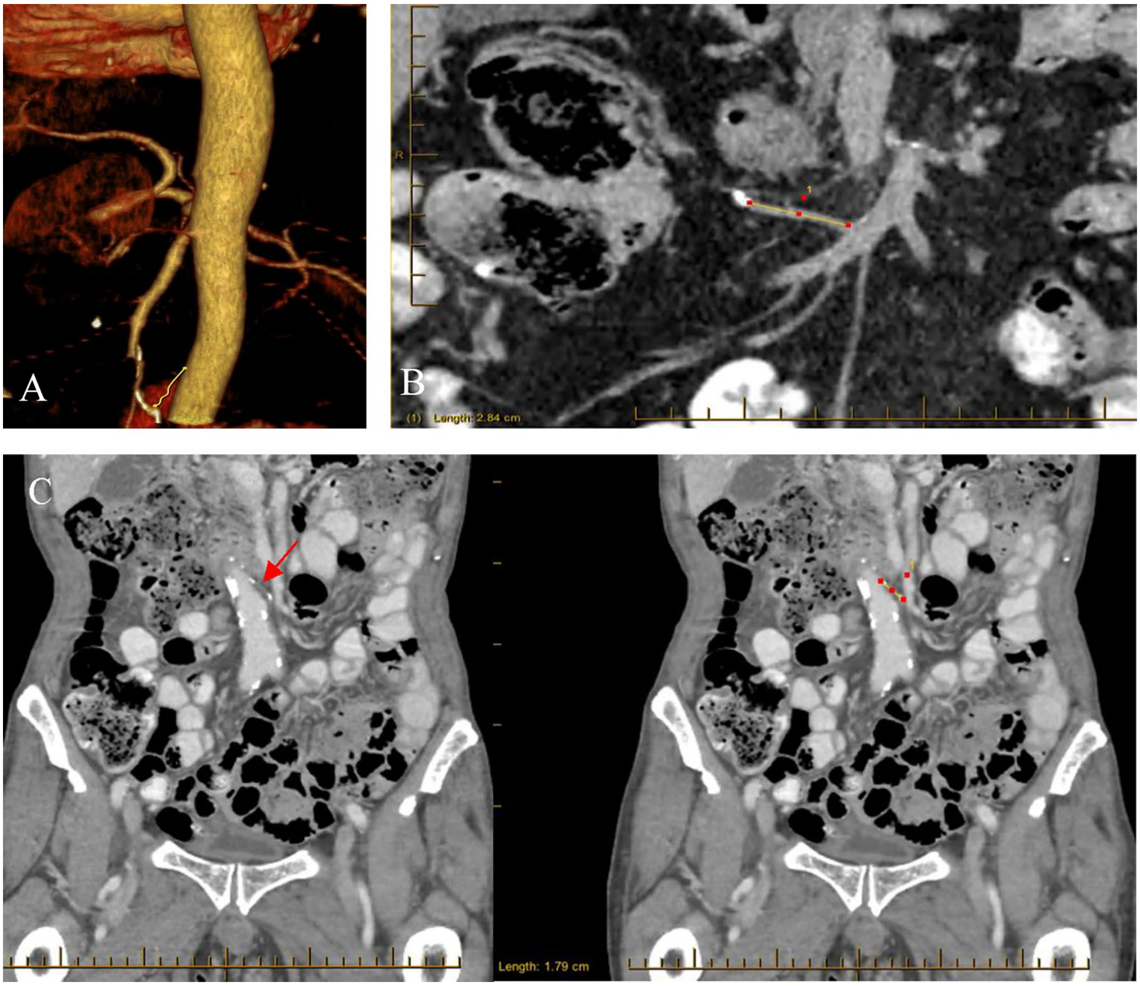

Fig. 2 **A** Coronal volume-rendered reformatted CT image of the IMA RAPL, defined by its termination at a surgical clip; **B** Multi-planar reconstructed (MPR) CT image with ICA RAPL defined and measured in the coronal plane; **C** MPR CT image with IMA RAPL defined (red arrow) and measured in the coronal plane. Surgical clip/ in (**B**) and granuloma in (**C**) are denoted by hyper-density at maximal limits of the measurement

Standard clinicopathological variables

Clinical information, operative details, tumour pathology, and follow-up data were obtained from the database for analysis [13, 14]. Specifically, details concerning the method of pathology reporting and staging have been previously described [15, 16].

Clinico-pathological variable of interest—CT measurement of IMA and ICA RAPL (Fig. 2)

The RAPL of the post-AR IMA or post-RH ICA were prospectively measured and recorded in patients identified from the prospective database. The RAPL was positively identified if the location of a visible named vessel correlated to its expected anatomical position, or terminated at a surgical staple, surgical clip, or “radiological granuloma”. Patients whose RAPLs measured ≤ 10 mm were regarded as having had a CVL [5].

The IMA and ICA were chosen as the pedicles to measure as they correlated to the main feeding arteries in our two most commonly performed operations. On the left side, the IMA reliably originates from the abdominal aorta and assumes a subtle leftward course, following its origin at the level of the third lumbar vertebral body [10, 17]. Contralaterally, the ICA is the most constant collateral of the SMA, emerging usually approximately at the level of the fifth or sixth jejunal artery and assumes an antero-inferior trajectory [10].

Surveillance CT imaging up to 3 years post-surgery was used to standardize the timeline for RAPL measurement. Helical imaging acquisition of the abdomen and pelvis obtained during either the venous or arterial (if available) phase was used for multiplanar reconstruction or volume-rendered reformat of the mesenteric vasculature, using the syngo®.via (Siemens Healthcare, Germany) platform. The CTs were reviewed by KN, and once best displayed, a calliper tool was used to measure the post-resection arterial stump lengths. Patients were excluded from the study if their CT scans had missing or inconclusive results, specifically when the RAPL could not be easily traced because of slice thickness or the absence of intravenous contrast, as shown in Fig. 1. The RAPL measurements were repeated randomly in 10% of the cases by a (blinded) specialist gastrointestinal radiologist (JY) to determine the reproducibility of the measurements and overall inter-observer reliability.

Pathology reporting and staging

Specialist pathologists examined resected specimens using a standard synoptic protocol, with pathology data coded by CC. Adenocarcinomas including mucinous and signet ring types were analysed. Fat clearance techniques were not employed in node retrieval. Tumour staging followed the AJCC pTNM system.

Surveillance and follow-up

Patients were reviewed at least 6 monthly for the first 2 years after resection and followed up yearly thereafter until death or December 2021, unless lost to follow-up [18]. The approach to surveillance and indications for post-operative adjuvant chemotherapy have been previously detailed [16].

The date of resection was the start of the follow-up period. Follow-up times were censored at the last contact for patients who did not experience the terminal event up to December 2021, were lost to follow-up, or remained alive. The approach to identifying the date and cause of death have been described previously [16, 19].

Outcome measures

The primary outcome measure focused on assessing the reproducibility of CT-based RAPL measurements in terms of reliability and agreement. Secondary outcome measures included OS, DFS, and any locoregional recurrence (LR) or systemic recurrence (SR) within the peritoneal cavity or elsewhere, and biopsy proven whenever possible [20–22].

Statistical analyses

Continuous variables were reported as mean (standard deviation [SD]) for normally distributed variables and as median (interquartile range [IQR] or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages. The inter-observer reliability of RAPL measurements was tested using intra-class correlation coefficient (ICC) modelling. Univariate and multivariate linear regression models tested correlation between RAPLs and clinicopathological factors. Survival and recurrence estimates were modelled using the Kaplan-Meier function with log-rank test performed to determine difference in survival distributions. Cox regression modelling tested for associations between outcome measures and relevant clinicopathological variables, including RAPL measurements. A multivariate model was not performed because multiple variables violated the proportional hazards assumptions, and alternative stratification of these violating variables rendered too small a case load for analysis. The level for two-tailed statistical significance was $P < 0.05$ with confidence intervals at the 95% level. All the analyses were performed using SPSS® version 29 (IBM, New York, USA).

Sample size

Sample size calculations were made based on a previously reported 5-year DFS of 59.4% [16].

A relative improvement of 10% (attributable to CVL) in the 5-year DFS was deemed clinically relevant. Estimating that 5% of our patients had a CVL resection in our institution, and calculating for a two-sided significance of 0.05 (*alpha* of 0.05 and power of 0.85), a minimum of 415 patients were required for adequate power [23].

AJCC stage III sub-group analysis

It was expected that the greatest survival advantage for patients undergoing CVL surgery would be observed in the AJCC stage III population. To understand the survival association between RAPL and Stage III disease, a sub-group analysis was performed on this population, alongside a separate Cox regression survival analysis.

Results

Study population

A total of 1425 patients had a CRC resection during the study period. Of these, 827 were suitable for analysis. We reviewed post-operative CTs for 424 consecutive patients (Fig. 1) of which 422 (99.5%) had a recordable RAPL. Of these 422 patients, 250 (59.2%) and 172 (40.8%) patients had AR and RH surgery, respectively. Detailed clinico-pathological characteristics of AR and RH patients are shown in Table 1.

The temporal trend in RAPL measurements is presented in Fig. 3. The median RAPL of patients who underwent an AR or RH was 26.4 (IQR 19.2–34.3) mm and 29.8

Table 1 Comparison of clinicopathological factors between patients with AR and RH

Variables	Total (%) or mean (SD) or median (range/IQR) ($n=422$)	Anterior resection ($n=250$)	Right hemicolectomy ($n=172$)
RAPL (median [IQR]), mm*	27.2 (19.8–38.1)	26.4 (19.2–34.3)	29.8 (21.6–40.9)
CVL (RAPL < 10 mm)			
No	407 (96.4)	239 (95.6)	168 (97.7)
Yes	15 (3.6)	11 (4.4)	4 (2.3)
Gender			
Male	228 (54.0)	144 (57.6)	84 (48.8)
Female	194 (46.0)	106 (42.4)	88 (51.2)
Age (mean [SD]), years	69.0 (12.3)	66.3 (12.1)	72.8 (11.4)
BMI (mean [SD]), kg/m^2	27.5 (5.8)	27.3 (5.9)	27.9 (5.6)
ASA grade			
I	61 (14.4)	44 (17.6)	17 (9.9)
II	229 (54.3)	138 (55.2)	91 (52.9)
III/IV	132 (31.3)	68 (27.2)	64 (37.2)
Duration of operation (median [IQR]), min	221.0 (171.5–274.5)	252.0 (215.0–302.0)	169.0 (143.75–201.0)
Emergency operation			
No	396 (93.8)	235 (94.0)	161 (93.6)
Yes	26 (6.2)	15 (6.0)	11 (6.4)
Operation modality			
Open	81 (19.2)	57 (22.8)	24 (14.0)
Laparoscopy	341 (80.8)	193 (77.2)	148 (86.0)
Procedure conversion			
No	312 (91.5)	173 (89.6)	139 (93.9)
Yes	29 (8.5)	20 (10.4)	9 (6.1)
Blood loss (ml)			
≤ 500	408 (96.7)	239 (95.6)	169 (98.3)
> 500	14 (3.3)	11 (4.4)	3 (1.7)
Tumour stage (TNM AJCC)			
Node negative (stage 1 and 2)	184 (43.6)	104 (41.6)	80 (46.5)
Node positive (stage 3)	238 (56.4)	146 (58.4)	92 (53.5)
Tumour size (median [IQR]), mm	4.0 (3.0–5.0)	3.8 (2.9–5.0)	4.5 (3.1–6.4)
Tumour perforation			
No	417 (98.8)	247 (98.8)	170 (98.8)
Yes	5 (1.2)	3 (1.2)	2 (1.2)
Histological type			
Non-mucinous or signet ring	381 (90.3)	237 (94.8)	144 (83.7)
Mucinous or signet ring	41 (9.7)	13 (5.2)	28 (16.3)
Histological differentiation			
Well or moderate	365 (86.5)	235 (94.0)	130 (75.6)
Poor	57 (13.5)	15 (6.0)	42 (24.4)
Histological grade			
Low or average	361 (85.5)	234 (93.6)	127 (73.8)
High	61 (14.5)	16 (6.4)	45 (26.2)
Lympho-vascular invasion			
No	291 (69.0)	173 (69.2)	118 (68.6)
Yes	131 (31.0)	77 (30.8)	54 (31.4)
Peri-neural invasion			
No	310 (73.5)	170 (68.0)	140 (81.4)
Yes	112 (26.5)	80 (32.0)	32 (18.6)

Table 1 (continued)

Variables	Total (%) or mean (SD) or median (range/IQR) ($n=422$)	Anterior resection ($n=250$)	Right hemicolectomy ($n=172$)
Number of lymph nodes examined (median [range])	19 (4–65)	19 (4–65)	20 (6–50)
Lymph node harvest (< 12)			
No	388 (91.9)	225 (90.0)	163 (94.8)
Yes	34 (8.1)	25 (10)	9 (5.2)
Time to surveillance CT from index operation (median [IQR), months	11.3 (7.3–16.7)	11.4 (7.3–17.7)	11.0 (7.1–14.7)
LOS (median [range]), days	7 (2–77)	7 (2–66)	6 (3–77)

RAPL residual arterial pedicle length, *CVL* central vascular ligation, *ASA* American Society of Anesthesiology, *TNM* 8th edition tumour, nodes, and metastasis staging system, *AJCC* American Joint Committee on Cancer, *LOS* length of stay

*A longer RAPL was noted in those who underwent RH surgery ($P=0.02$)

(IQR 21.6–40.9) mm, respectively. Eleven AR (4.4%) and four RH patients (2.3%) had CVL (i.e., RAPL ≤ 10 mm) performed.

The absolute inter-observer agreement for AR and RH RAPLs was 94.6% and 78.9%, respectively. Inter-rater reliability assessed by ICC was 0.97 (95% CI 0.93–0.99; $P<0.001$) and 0.89 (95% CI 0.64–0.96; $P<0.001$) in AR and RH patients, respectively.

Comparison of survival outcomes between clinicopathological characteristics in AR and RH patients (Table 2)

Table 2 summarizes the associations between clinicopathological characteristics and survival outcomes in patients who underwent AR and RH resections. Figure 4 presents the Kaplan-Meier plot of OS and DFS for these patients stratified according to RAPL.

Anterior resection

For those who underwent an AR, death occurred in 66 patients (26.4%). The 5-year OS and DFS rates were 75.6% (95% CI 72.5–78.7) and 66.6% (95% CI 63.4–69.8), respectively. A LR was diagnosed in 11 patients (4.4%), and SR was diagnosed in 58 patients (23.2%). The mean time to LR and SR was 1.5 (95% CI 1.0–1.9) years and 1.9 (95% CI 1.5–2.6) years, respectively.

RAPL was not associated with OS ($P=0.14$) or DFS ($P=0.26$), nor was CVL (OS [$P=0.31$]; DFS [$P=0.18$]). A poorer OS was associated with increasing age ($P<0.001$), ASA > 2 ($P<0.001$), AJCC stage III tumours ($P=0.008$), poorly differentiated ($P=0.04$) and high grade ($P=0.004$) tumours, the presence of lympho-vascular invasion (LVI [$P=0.03$]), and perineural invasion ($P=0.001$). Poor prognosis characterised by increased DFS hazards mirrored all of the above factors in addition to large tumours ($P=0.02$).

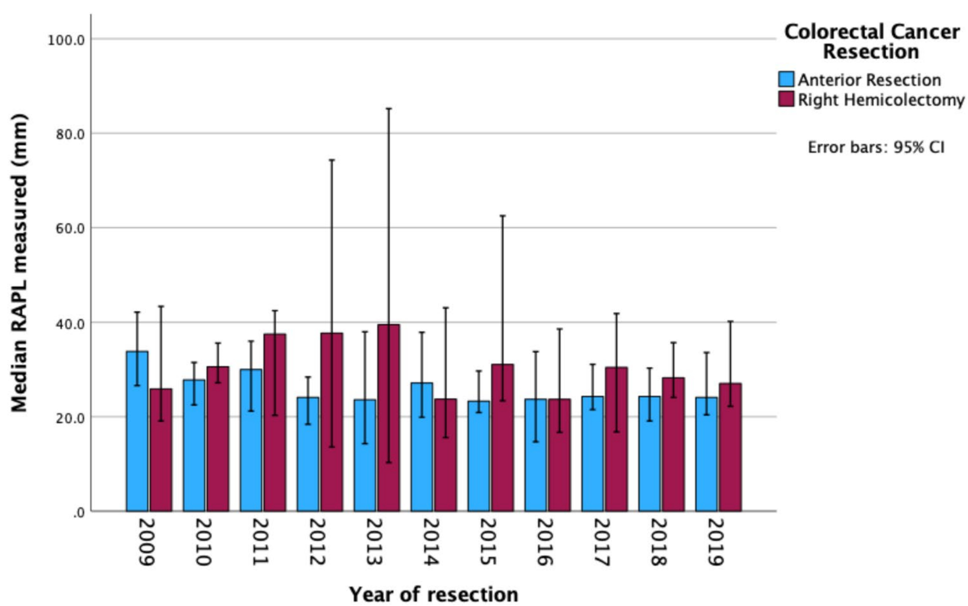

Fig. 3 Trend of RAPLs over the 11-year study period in patients undergoing an AR or RH for colorectal cancer

Table 2 Comparison of oncological outcomes between clinicopathological features of AR and RH patients

	Overall survival				Disease-free survival			
	Anterior resection		Right hemicolectomy		Anterior resection		Right hemicolectomy	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
RAPL (mm)	1.01 (0.99–1.02)	0.14	1.01 (0.99–1.03)	0.15	1.01 (0.99–1.02)	0.26	1.01 (0.99–1.03)	0.12
CVL								
No	Ref	–	Ref	–	Ref	–	Ref	–
Yes	1.70 (0.62–4.67)	0.31	1.96 (0.48–8.04)	0.35	1.87 (0.76–4.61)	0.18	1.56 (0.38–6.38)	0.54
Gender								
Male	Ref	–	Ref	–	Ref	–	Ref	–
Female	0.57 (0.34–0.95)	0.03	0.66 (0.39–1.12)	0.12	0.63 (0.41–0.99)	0.045	0.77 (0.48–1.24)	0.77
Age (years)	1.05 (1.03–1.08)	< 0.001	1.05 (1.02–1.08)	0.001	1.03 (1.01–1.05)	0.003	1.03 (1.01–1.06)	0.01
BMI (kg/m^2)	1.01 (0.97–1.05)	0.78	0.99 (0.94–1.04)	0.61	1.01 (0.98–1.04)	0.55	0.99 (0.95–1.04)	0.81
ASA grade								
I	Ref	–	Ref	–	Ref	–	Ref	–
II	2.15 (0.90–5.15)	0.09	0.99 (0.34–2.90)	0.98	1.99 (0.94–4.25)	0.07	1.43 (0.54–4.35)	0.43
III/IV	6.29 (2.59–15.28)	< 0.001	3.13 (1.11–8.86)	0.03	4.94 (2.27–10.73)	< 0.001	3.14 (1.11–8.86)	0.03
Emergency operation								
No	Ref	–	Ref	–	Ref	–	Ref	–
Yes	1.31 (0.47–3.60)	0.61	1.42 (0.51–3.92)	0.50	1.06 (0.43–2.62)	0.90	1.43 (0.58–3.57)	0.44
Operation modality								
Open	Ref	–	Ref	–	Ref	–	Ref	–
Laparoscopy	0.61 (0.37–1.01)	0.05	0.39 (0.22–0.71)	0.002	0.66 (0.42–1.03)	0.07	0.27 (0.16–0.46)	< 0.001
Intraoperative blood loss (ml)								
≤ 500	Ref	–	Ref	–	Ref	–	Ref	–
> 500	1.56 (0.62–3.91)	0.34	9.77 (2.23–42.85)	0.003	1.34 (0.54–3.32)	0.53	4.19 (1.00–18.48)	0.049
Tumour stage (TNM)								
Stage 1/2	Ref	–	Ref	–	Ref	–	Ref	–
Stage 3	2.08 (1.21–3.58)	0.008	1.57 (0.91–2.68)	0.10	1.80 (1.14–2.85)	0.01	1.89 (1.15–3.10)	0.01
Tumour size (cm)	1.13 (0.98–1.30)	0.09	1.08 (1.01–1.15)	0.02	1.16 (1.03–1.30)	0.02	1.11 (1.06–1.17)	< 0.001
Histological type								
Non-mucinous/signet ring	Ref	–	Ref	–	Ref	–	Ref	–
Mucinous/signet ring	1.21 (0.44–3.33)	0.71	1.91 (1.02–3.56)	0.04	2.04 (0.94–4.42)	0.07	1.83 (1.03–3.25)	0.04
Histological differentiation								
Well or moderate	Ref	–	Ref	–	Ref	–	Ref	–
Poor	2.31 (1.05–5.05)	0.04	1.21 (0.66–2.22)	0.54	2.13 (1.03–4.41)	0.04	1.37 (0.80–2.35)	0.26
Histological grade								
Low or average	Ref	–	Ref	–	Ref	–	Ref	–
High	3.00 (1.43–6.30)	0.004	1.18 (0.65–2.13)	0.58	3.07 (1.58–5.95)	< 0.001	1.32 (0.77–2.24)	0.31
Lympho-vascular invasion								
No	Ref	–	Ref	–	Ref	–	Ref	–
Yes	1.70 (1.04–2.78)	0.03	2.18 (1.27–3.74)	0.005	1.69 (1.10–2.60)	0.02	2.91 (1.78–4.75)	< 0.001
Peri-neural invasion								
No	Ref	–	Ref	–	Ref	–	Ref	–
Yes	2.21 (1.36–3.58)	0.001	1.18 (0.63–2.24)	0.61	2.51 (1.63–3.84)	< 0.001	1.59 (0.91–2.80)	0.10
Number of lymph nodes examined	0.98 (0.95–1.01)	0.15	0.97 (0.94–1.00)	0.08	0.99 (0.97–1.02)	0.57	0.98 (0.95–1.01)	0.20

HR hazard ratio, *RAPL* residual arterial pedicle length, *CVL* central vascular ligation, *ASA* American Society of Anesthesiology, *TNM* 8th edition tumour, nodes, and metastasis staging system, *AJCC* American Joint Committee on Cancer

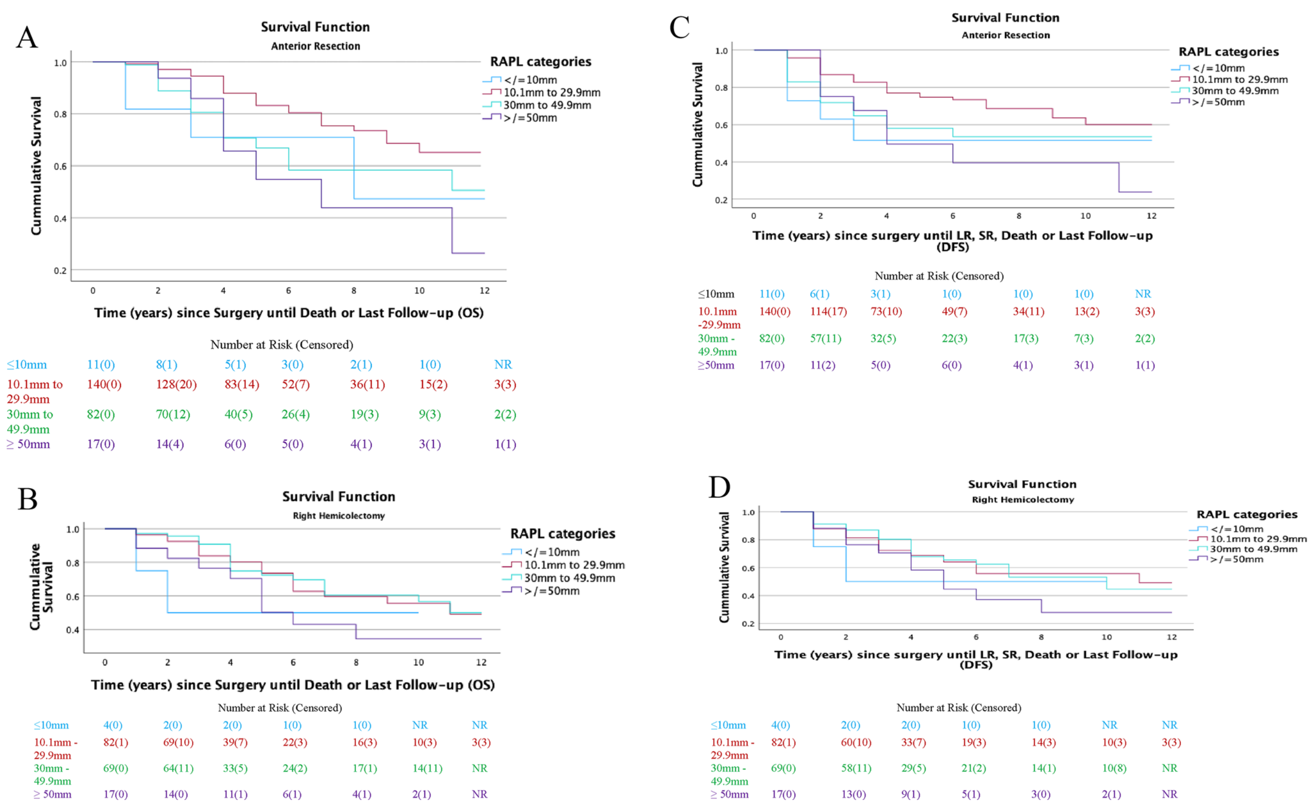

Fig. 4 Kaplan-Meier plots of OS and DFS of AR and RH patients stratified by RAPLs. **A** OS of AR patients; **B** DFS of AR patients; **C** OS of RH patients; **D** DFS of RH patients

Right hemicolectomy

Amongst the RH patients studied, death occurred in 57 patients (33.1%). The median DFS was 9.3 (95% CI 5.2–13.4) years. The 5-year OS and DFS rates were 69.6% (95% CI 65.5–73.7) and 61.8% (95% CI 57.6–66.0), respectively. A LR was diagnosed in three patients (1.7%) and SR diagnosed in 36 (20.9%). The median time to LR and SR was 2.8 years (95% CI 0.3–5.2) and 1.2 years (95% CI 0.7–1.6), respectively.

RAPL was not associated with OS ($P=0.15$) or DFS ($P=0.12$). Similarly, CVL was also not associated with OS ($P=0.35$) or DFS ($P=0.54$). Poorer OS was associated with increasing age ($P=0.001$), ASA score > 2 ($P=0.03$), intra-operative blood loss > 500 ml ($P=0.003$), larger tumours ($P=0.02$), mucinous or signet cancers ($P=0.04$), and LVI ($P=0.005$). Regarding DFS, a poorer prognosis mirrored all factors noted above.

Clinico-pathological characteristics and RAPL associations in AR and RH patients (Table 3A, B)

Anterior resection

On univariate analysis, a shorter RAPL was associated with female patients ($P=0.01$), laparoscopic operation ($P<0.001$), and more recent study years ($P=0.03$). A longer RAPL was associated with a higher BMI ($P=0.04$) and ASA grades ($P=0.01$). RAPL was not associated with the number of LNs examined ($P=0.54$). On multivariate analysis, only ASA grade ($P=0.04$) was significantly associated with a longer RAPL and laparoscopic operation ($P<0.001$) with a shorter RAPL.

Right hemicolectomy

RH patients had a longer RAPL than AR patients (Table 1). On univariate analysis, a shorter RAPL was observed in female patients ($P=0.002$) and laparoscopic operation ($P=0.02$). A longer RAPL was associated with increasing age ($P=0.008$) and a high ASA grade ($P=0.004$). Again, RAPL was not associated with the number of LNs examined

Table 3 Clinicopathological characteristics and RAPL associations in AR and RH patients (3A—univariate; 3B—multivariate analyses)

3A

	Residual arterial pedicle length (mm)			
	Anterior resection ($n = 250$)		Right hemicolectomy ($n = 172$)	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value
Year of operation (year)	− 0.59 (− 1.13 to − 0.06)	0.03	− 0.54 (− 1.20 to 0.13)	0.11
Gender	− 4.71 (− 8.46 to − 0.96)	0.01	− 6.87 (− 11.29 to − 2.45)	0.002
Male				
Female				
Age (years)	0.06 (− 0.09 to 0.22)	0.43	0.27 (0.07 to 0.47)	0.008
BMI (kg/m^2)	0.34 (0.14 to 0.66)	0.04	0.30 (− 0.12 to 0.71)	0.16
ASA grade	3.66 (0.87 to 6.46)	0.01	5.26 (1.75 to 8.78)	0.004
I				
II				
III/IV				
Emergency operation	3.43 (− 4.46 to 11.32)	0.39	3.90 (− 5.36 to 13.16)	0.41
No				
Yes				
Operation modality	− 9.45 (− 13.77 to − 5.14)	< 0.001	− 7.75 (− 14.20 to − 1.31)	0.02
Open				
Laparoscopy				
Intraoperative blood loss (ml)	3.43 (− 5.71 to 12.57)	0.46	10.95 (− 6.31 to 28.21)	0.21
≤ 500				
> 500				
Tumour stage (TNM AJCC)	− 0.06 (− 3.86 to 3.75)	0.98	− 0.26 (− 4.81 to 4.29)	0.91
Stage 1/2				
Stage 3				
Tumour size (cm)	0.29 (− 0.72 to 1.31)	0.57	0.04 (− 0.65 to 0.73)	0.91
Histological type	2.40 (− 6.08 to 10.81)	0.58	6.05 (− 0.03 to 12.13)	0.05
Mucinous or signet ring				
Non-mucinous or non-signet ring				
Histological differentiation	− 3.02 (− 10.92 to 4.87)	0.45	0.35 (− 4.93 to 5.63)	0.90
Well or moderate				
Poor				
Histological grade	− 0.61 (− 8.27 to 7.06)	0.88	1.88 (− 3.28 to 7.04)	0.47
High				
Low or average				
Lympho-vascular invasion	− 0.55 (− 4.62 to 3.51)	0.79	− 2.21 (− 7.09 to 2.67)	0.37
No				
Yes				
Peri-neural invasion	0.66 (− 3.37 to 4.68)	0.75	− 1.17 (− 7.00 to 4.66)	0.69
No				
Yes				
Number of lymph nodes examined	− 0.07 (− 0.28 to 0.15)	0.54	− 0.20 (− 0.48 to 0.08)	0.16
Time to surveillance CT from index operation	0.05 (− 0.16 to 0.25)	0.66	− 0.18 (− 0.46 to 0.09)	0.19
LOS (days)	0.11 (− 0.14 to 0.35)	0.38	0.07 (− 0.16 to 0.30)	0.56

Table 3 (continued)

3B

	Residual arterial pedicle length (mm)			
	Anterior resection ($n=250$)		Right hemicolectomy ($n=172$)	
	aβ (95% CI)	*P*-value	*aβ* (95% CI)	*P*-value
Year of operation (year)	− 0.23 (− 0.82 to 0.35)	0.44		
Gender	− 3.49 (− 7.23 to 0.24)	0.07	*− 5.67 (− 10.05 to − 1.29)*	*0.01*
Male				
Female				
Age (years)			0.16 (− 0.05 to 0.37)	0.14
BMI (kg/m^2)	0.22 (− 0.10 to 0.54)	0.17		
ASA grade	*3.08 (0.14 to 6.03)*	*0.04*	2.94 (− 0.89 to 6.77)	0.13
I				
II				
III/IV				
Operation modality	*− 8.70 (− 13.51 to − 3.89)*	*<0.001*	− 4.91 (− 11.30 to 1.48)	0.13
Open				
Laparoscopy				

RAPL residual arterial pedicle length, *CVL* central vascular ligation, *ASA* American Society of Anesthesiology, *TNM* 8th edition tumour, nodes, and metastasis staging system, *AJCC* American Joint Committee on Cancer, *CT* computed tomography, *LOS* length of stay

($P=0.16$). On multivariate analysis, only gender ($P=0.01$) was associated with a shorter RAPL.

AJCC stage III sub-group analysis

There were 238 AJCC Stage III patients with a recorded RAPL. Of these, 146 had an AR and 92 had a RH. The median RAPLs of patients who underwent an AR and RH were 26.7 (IQR 19.5–36.3) mm and 29.8 (IQR 21.2–40.5) mm, respectively. Within this cohort, six AR patients (4.1%) and three RH patients (3.3%) had a CVL performed.

The survival associations of the sub-cohort are summarised in Table 4. In patients who underwent an AR, RAPL was associated with poorer OS ($P=0.04$) but not DFS ($P=0.10$). In those who had a RH, RAPL was associated with neither OS ($P=0.27$) nor DFS ($P=0.64$).

Discussion

Over a 10-year period, this large and adequately powered prospective cohort study evaluated the role of RAPL measured on surveillance CT as a marker of surgical quality and EoL. This was achieved by investigating the association of RAPLs with survival outcomes and other clinicopathological variables in patients who had either an AR or RH for CRC. This study demonstrates the feasibility and reproducibility of measuring RAPLs in patients in whom CVL surgery was not routinely performed. Although some clinicopathological factors were individually associated with poorer survival outcomes, RAPLs showed no influence on these outcomes. Notably, RAPL was not associated with the extent of the LN harvest. These findings suggest that using RAPL as a quality marker for CRC when measured in a non-routine CVL population is of minimal value in predicting patient outcome.

Table 4 Oncological associations between AJCC Stage 3 patients and RAPL ($N=238$)

	Overall survival				Disease-free survival			
	Anterior resection		Right hemicolectomy		Anterior resection		Right hemicolectomy	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
RAPL (mm)	1.03 (1.00–1.05)	0.04	1.01 (0.99–1.03)	0.27	1.02 (0.99–1.04)	0.10	1.01 (0.99–1.02)	0.64

HR hazard ratio, *RAPL* residual arterial pedicle length

The current assessment of the extent of lymphadenectomy in CRC surgery relies on rudimentary measures, such as LN harvest, and a nodal count of 12 is widely accepted as an adequate lymphadenectomy for staging purposes [24, 25]. Measurements based on tissue morphometry, such as the area of mesentery excised, have been previously described and proposed as objective measures for lymphadenectomy extent [9]. Nevertheless, when considering lymphadenectomy for the purpose of ensuring all potentially involved nodes are excised, of which CVL is a possible 'gold standard', there should be a focus on quantifying the unresected nodal tissue remaining. Neither LN harvest nor tissue morphometry measurements of the pathology specimen can provide this assessment. Our study investigated whether in vivo measurement of RAPLs using routine surveillance CT could fill this role by providing an objective assessment of residual nodal tissue using RAPL as a surrogate marker.

This study confirmed the feasibility and reproducibility of measuring IMA and ICA pedicle length following AR and RH, respectively. IMA and ICA pedicles were consistently identified in > 400 postoperative scans, with identification aided through recognition of a surgical clip/staple, or "radiological granuloma". The reproducibility of these measurements was confirmed by a second independent observer demonstrating good inter-observer agreement. Notably, IMA pedicle lengths were, on average, shorter than ICA pedicle lengths, likely a reflection of the accepted and inherent complexity seen in the vascular anatomy of the right colon compared to the left. The difference in IMA and ICA pedicle lengths justifies our approach of considering AR and RH patients separately in a study designed to evaluate the association between pedicle length and survival.

The measurement of RAPL following CRC surgery has previously been described but with heterogenous study populations, study designs, and imaging protocols [10, 11, 26–28]. One previous study measured ICA stump length following RH surgery many months after the index operation [26] but did not investigate its relationship with survival outcomes. In another study, where radio-surgical correlation of IMA ligation was noted in only 41% of patients, no survival difference between the presence or absence of radio-surgical correlation of IMA ligation was observed [28]. In a separate registry-based study, Bostrom et al. highlighted no survival difference between the level of vascular tie when it was dichotomised to high and low ligation. These authors did not record individual RAPLs [29]. Only one study has previously investigated the relationship between RAPL and survival outcome, but in a cohort where CVL was routinely intended to be practiced. In that study, a pedicle length of < 1 cm (verifying CVL had been performed) was associated with survival benefit [7]. Our study aimed to determine whether a similar survival benefit was demonstrable in a cohort where CVL was not routinely practiced. Our original hypothesis that a shorter RAPL would confer some survival benefit was not substantiated, as there was no association between RAPL and OS or DFS in either AR or RH patients.

The result of our subgroup analysis warrants comment. When confined to Stage III patients alone, there was an apparent association between RAPL and OS, but not DFS, in AR patients. This association, however, was weak and is difficult to explain. It is challenging to resolve why a shorter RAPL would provide an OS benefit without improvement in DFS. Notably, this significant association was observed only on univariate analysis.

Our study was unable to demonstrate a clear association between RAPL and LN harvest. A more radical operation has been associated with a larger LN harvest in previous studies [8, 30, 31], so intuitively, an association between a shorter RAPL and a larger LN harvest should be expected. We suppose two reasons for this observation. The first relates to the surgical technique practiced within our unit, whereby some surgeons perform a full lymphadenectomy but preserve the vascular stump by sweeping central nodes from the pedicle root distally. This may result in a longer RAPL despite a complete lymphadenectomy being performed. Second, pursuing a higher vascular tie may not necessarily translate into acquiring a larger nodal harvest. In a previous study from our unit investigating the relationship between apical node positivity and survival outcome [32], over one-third of resected CRC specimens did not have an apical node present (defined as a node within 1 cm of the pedicle ligature), suggesting that extending pedicle transection proximally may not necessarily result in a greater (apical) LN yield.

Although this study was unable to demonstrate an association between RAPL and survival outcomes or LN yield, its clinical implications are important. Only one previous study used a methodology comparable to ours, but in a cohort of patients where CVL was routinely intended to be performed, and found a survival benefit with a shorter RAPL, specifically where CVL was verified [7]. The fact that our study was unable to replicate those results in a cohort of non-routine CVL implies the benefits of a shorter RAPL may only be reaped when a CVL is performed (RAPL ≤ 10 mm) and that probably CVL is an 'all or nothing phenomenon'. Furthermore, the lack of association between RAPL and LN yield serves to emphasise the continuing importance of comprehensive pathology assessment of resected specimens, of which accurate nodal count remains highly relevant to cancer staging and guiding the need for adjuvant therapy.

This prospective study has several limitations. In patients who underwent a RH for a hepatic flexure malignancy, the ICA was regarded as the principal vascular pedicle to standardise the identification of a single pedicle radiologically, despite other pedicles sharing lymphatic drainage in this region. As not all patients had their heights recorded in the database, we were unable to adjust RAPL measurements according to the patients' body mass indices. Additionally, in a unit where CVL was not routinely practiced, it is highly probable that aside from simply sweeping the central nodes up into the resection margin of the specimen without a high tie of the vessel, CVL was selectively performed in cases where central lymphadenopathy was suspected. This scenario presents a potential for introducing selection bias. However, the well-powered and large cohort size, long study duration, application of standardized surgery by specialist colorectal surgeons following anatomical planes, and routine detailed generic pathology reporting are strengths of our study. Additionally, the inclusion of urgent operations and minimally and maximally invasive surgical approaches improves the generalizability of our study.

Conclusion

This study is the first to demonstrate the feasibility of measuring RAPL using surveillance CTs following non-routine CVL surgery for CRC in AR and RH patients. The null association between RAPL and survival outcomes questions the role of RAPL as a quality marker for CRC surgery in patients in whom CVL is not intended. The role of RAPL measurement may be confined to post hoc verification that CVL surgery has been performed, as this appears to be where survival benefits have been documented. Otherwise, accurate and structured pathology assessment of resected specimens remains crucial for disease prognostication and guidance of the need for adjuvant therapy in patients who have had a potentially curative operation for CRC.

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Author contributions All authors contributed to the study conception and design. Material preparation was performed by all authors. Data collection and analysis were performed by Dr Krishanth Naidu. The first draft of the manuscript was written by Dr Krishanth Naidu. All the tables and figures were created by Dr Krishanth Naidu. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. A/Prof Charles Chan, A/Prof Matthew Rickard and Dr Kheng-Seong Ng supervised the undertaking of this research project.

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Data availability The data that support the findings of this study are available from the corresponding author, Dr Kheng-Seong Ng, upon reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sydney Local Health District Ethics Committee (2020/ETH03325 and CH62/62011–136-P Chapuis HREC/11/CRGH206).

Consent to participate and/or consent to publish Patients consented to having their data collected in a prospective colorectal database and for its use in future research. In this present study which deals with measurements of RAPLs in a specific cohort within the database, a waiver of consent was obtained from our Ethics committee.

Disclosure A published abstract was presented in the *Colorectal Disease* journal—Naidu K**,** Rickard MJFX, Ng, KS. Computed Tomography Assessment of Residual Arterial Pedicle Length Following Colon and Rectal Cancer Surgery: A Marker of Extent of Lymphadenectomy and Surgical Quality? Colorectal Disease. 2022; Colorectal Dis, 24: 48–170. https://doi.org/10.1111/codi.16050.

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APPENDIX II

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RESEARCH

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Tissue morphometric measurements do not predict survival following colorectal cancer surgery

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Abstract

Background Ex vivo tissue morphometric (TM) measurements have been proposed as a quality marker for colorectal cancer (CRC) surgery. However, their survival associations require clarification. This study aimed to evaluate the feasibility of capturing TM measurements based on ex vivo fresh specimen images and explore the association between these TM measurements and survival outcomes.

Methods A prospective cohort study at Concord Hospital, Sydney was conducted with Stage I to III CRC patients (2009–2019) who underwent an anterior resection (AR) or right hemicolectomy (RH). Using high-resolution digital photographs of fresh CRC specimens, ex vivo tissue morphometric (TM) measurements—resected mesentery area (TM A), distances from high vascular tie to tumour (TM B) and bowel wall (TM C), and bowel length (TM D)—were recorded using Image J. Overall survival (OS) and disease-free survival (DFS) estimates and their associations to clinicopathological variables were investigated with Kaplan–Meier and Cox regression analyses. Linear regression models tested association between TM measurements and lymph node (LN) yield.

Results Of the 1,425 patients who underwent CRC surgery, TM measurements were performed on 312 patients, with an average age of 69.4 years (SD 12.3), of whom 52.9% were male. The majority had an AR (57.8%). Among AR patients, a 5-year OS rate of 77.4% and a DFS rate of 70.1% were observed, with TM measurements bearing no relationship to survival outcomes. Similarly, RH patients exhibited a 5-year OS rate of 67.2% and a DFS rate of 63.1%, with TM measurements again showing no association with survival. Only TM D ($P=0.02$) measurements were associated with the number of LNs examined.

Conclusion This study successfully demonstrates the feasibility of measuring TM measurements on photographs of ex vivo fresh specimens following CRC surgery. The lack of association with survival outcomes questions the utility of TM measurements as a quality metric of CRC surgery.

Keywords Quality of surgery, CME, CVL and tissue morphometry

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Background

Colorectal cancer (CRC) surgery is based on the principles of completely excising the primary tumour with clear margins, preserving the integrity of the lympho-vascular package along an avascular embryological (i.e., mesorectal or mesocolic) plane, and performing an adequate lymphadenectomy [1, 2]. An array of metrics, described according to the structure, process and outcome framework, has been developed to assess the quality of CRC surgery holistically [3–6]. While substantial emphasis has been placed on surgical margins and plane of excision as process metrics, discussions regarding metrics that define the extent of lymphadenectomy (EoL) have been less comprehensive [5, 7]. Most studies have focused on assessment of lymph node yield (LNY) as a single marker of EoL [5].

More recently, studies have explored the impact of ex vivo tissue morphometric (TM) measurements (e.g., area of mesentery excised) on clinico-pathological outcomes following CRC surgery, raising the possibility of this being a novel histopathological metric by which quality of CRC surgery may be assessed [3, 4, 8]. Our group has recently published the feasibility of performing ex vivo TM measurements using routine formalin fixed pathology specimen images post-colon cancer surgery [8]. However, successful application of these measurements for rectal cancer resections have been inconsistently reported [9, 10]. Moreover, the prognostic significance of ex vivo TM measurements with respect to its influence on survival outcomes remains unknown, precluding its potential use as a reliable quality metric in CRC surgery [8–10].

Therefore, the aim of this study was (i) to validate feasibility of ex vivo TM measurements using fresh pathology specimen images of colon and rectal cancer resections; and (ii) investigate the association between ex vivo TM measurements and survival outcomes. We hypothesised that TM measurements would be independently associated with patient survival.

Methods

A prospective observational cohort study was performed of patients who underwent a resection for a solitary primary CRC. Patients included were those who had any form of an AR (i.e., high or low) or a RH operation performed between January 2009 and December 2019 at Concord Repatriation General Hospital (CRGH), Sydney. These patients were identified from a prospectively maintained institutional database that has been in continual existence since 1971 [11, 12]. Patients with American Joint Committee on Cancer (AJCC) Stage IV cancer, synchronous or metachronous cancer, inflammatory

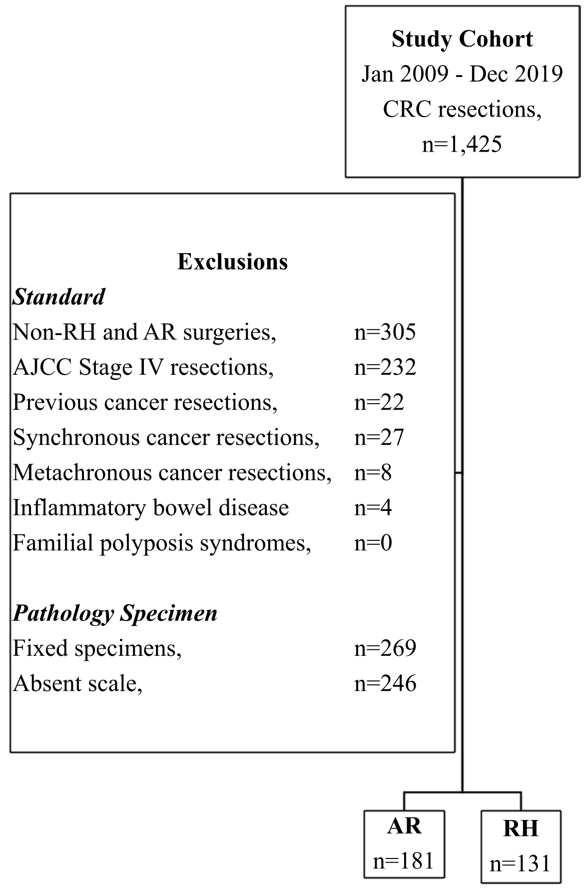

Fig. 1 Flow diagram of cohort definition

bowel disease, or polyposis coli, were excluded (Fig. 1). Ethical approval (2020/ETH03325 and CH62/62011–136-P Chapuis HREC/11/CRGH206) was granted by the Sydney Local Health District Ethics Committee, with included patients consenting for the use of their data and tumour specimens for research.

Surgical procedures

The standard approach of our unit in performing an AR and RH operation has been previously described [13]. In our unit, routine exposure of the superior mesenteric artery (SMA) or vein (SMV) and abdominal aorta is not performed.

Clinicopathological variables of interest – ex vivo TM measurements from fresh specimen images (Fig. 2)

Four new variables were prospectively measured and recorded. These were based on routine photographs of fresh resected specimens, stored as high-resolution digital images. Each specimen was photographed from both anterior and posterior viewpoints, with the mesentery presented flat, without stretching. Each image

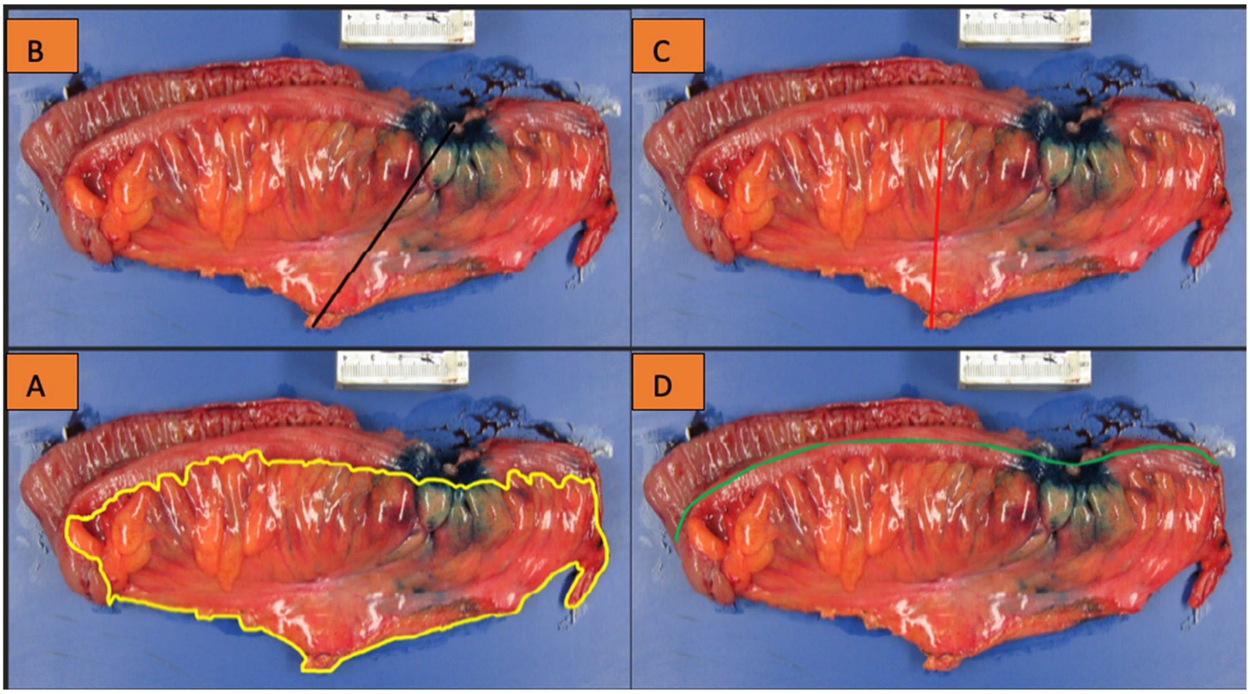

Fig. 2 TM measurements (**A**-**D**) of a fresh high anterior resection specimen includes—(**A**) the area of resected mesentery, (**B**) distance from the vascular tie to the tumour or (**C**) closest bowel wall and (**D**) length of bowel resected. The tumour is inked blue

was carefully calibrated with an included metric scale. As previously described, these calibrated images were then used to accurately determine several key measurements: the area of the resected mesentery (TM A, cm^2), the distance from the high vascular tie (HVT) to the tumour's centre (TM B, cm) and its nearest bowel wall (TM C, cm), and the length of the bowel segment removed (TM D, cm) [8]. If there was more than one artery supplying the tumour, the shortest was defined as TM B. These measurements were performed by KN using *Image J* (NIH, Maryland, USA) [8], blinded to patient outcomes. Importantly, patients whose specimens were photographed post formalin-fixation were excluded from analysis (Fig. 1). *Image J* is a Java-based image processing program that can display, edit, and analyse a wide range of image types [14]. It features tools and supports simultaneous processing of multiple images for statistical analysis and measurements [14].

To understand our TM measurement data in the context of other published studies, we compared our measurements with those from resections performed at St. James's University Hospital (SJUH) and University Hospital of Erlangen (UHE), which utilized an identical protocol for TM measurements [3].

Standard clinicopathological variables

The extraction of standard clinico-pathological data—including clinical information, operative details, tumour pathology, and follow-up data—as well as the details of pathology reporting and staging for adenocarcinomas (such as mucinous and signet ring variants), have been outlined in earlier publications [11, 12]. These data included the LNY from each specimen; fat clearance techniques were not employed in node retrieval.

Surveillance and follow-up

Patients underwent reviews at a minimum of every six months for the initial two years post-resection and were subsequently followed up on an annual basis either until their death or December 2021, barring instances of lost follow-up [15]. The surveillance protocol combined clinical examinations, laboratory tests, and advanced imaging, with periodic colonoscopies and multidisciplinary reviews for high-risk patients to evaluate adjuvant chemotherapy options [16]. The indications for post-operative adjuvant chemotherapy were routinely considered in a multidisciplinary setting for all patients, considering factors such as age, patient preferences, presence of comorbidities, adverse tumour pathological features, social circumstances, and best practice guidelines [15, 16].

The follow-up period commenced from the date of resection. Follow-up times were censored at last contact for patients who did not experience the terminal event up to December 2021, who were lost to follow-up, or who remained alive. Death dates were primarily determined from the records of the patient's surgeon, family physician, or hospital. In a limited number of cases, this information was sourced from the national death registration system [17]. The primary cause of death was classified per the International Classification of Diseases-10. All clinical and surgical data were recorded by one of our team members (PC).

Outcome measures

The primary outcome measures included:

(i) overall survival (OS), defined as the time span from the date of resection to the date of death from any cause, with data censored at the last known contact for patients still alive [18]; and
(ii) disease free survival (DFS), which refers to the time period following CRC resection during which a patient remains alive and shows no signs of disease recurrence [18–20].

Statistical analyses

The study population for this study was defined based on the period where photographs of fresh pathology specimens were routinely available. From January 2009 onwards, pathology specimens in our unit have been exclusively photographed in their fresh state. In the absence of pilot data, a sample size calculation was not performed. Continuous variables were reported as mean (standard deviation [SD]) for normally distributed variables and as median (interquartile range [IQR] or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages.

Survival estimates were modelled using the Kaplan–Meier function with log-rank tests performed to determine differences in survival distributions. Cox-regression modelling tested for associations between outcome measures and relevant clinicopathological variables, including ex vivo TM measurements. Linear regression models tested associations between TM measurements and LNY. The level for 2-tailed statistical significance was $P < 0.05$ with confidence intervals at the 95% level. All the analyses were carried out using SPSS® v.29 (IBM, New York, USA).

AJCC stage III Sub-group analysis

It was anticipated that an increased EoL would provide the most significant survival advantage for patients undergoing CRC surgery for AJCC stage III disease. A sub-group analysis was therefore conducted on this population, along with a separate Cox regression survival analysis.

Results

Study population

Some 1,425 patients underwent a resection for a CRC during the study period of which 1,113 patients were sequentially excluded (Fig. 1) leaving 312 patients suitable for analysis. In these patients, 165 (52.9%) were male, the mean age was 69.4 years (SD 12.3), the mean BMI was 27.6kg/m^2 (SD5.4), 179 patients had an ASA grade of II (57.4%), and the median hospital length of stay (LOS) was 7 days (range 2–66). An urgent operation was performed in nine patients (2.9%). Of those operated urgently, eight patients (88.9%) were obstructed. An open operation was performed in 47 patients (15.1%). Of the 265 patients (84.9%) managed with a laparoscopic operation, conversion to open surgery was required in 19 (7.2%).

Of the patients studied, 181 (57.8%) underwent an AR procedure and 131 (42.2%) had a RH operation. In those who had an AR procedure, the following mean TM measurements were recorded: TM A – 164.4cm^2 (SD 69.1), TM B – 14.1 cm (SD 4.9), TM C – 9.9 cm (SD 3.9), and TM D – 27.7 cm (SD 9.2). Comparatively, in patients who had RH surgery the mean TM measurements were: TM A – 108.4cm^2 (SD 49.0), TM B – 10.7 cm (SD 3.0), TM C – 7.3 cm (SD 2.4), and TM D – 24.7 cm (SD 9.4). The comparisons of these measurements with those previously documented at SJUH and UHE are presented in Table 1. Detailed clinical characteristics, histopathological descriptions, and ex vivo TM measurements for both the AR and RH cohorts are presented in Table 2.

Comparison of survival outcomes between clinico-pathological characteristics in AR and RH patients (Table 3)

Table 3 summarises the associations between clinico-pathological characteristics, including ex vivo TM measurements, and survival outcomes in patients who underwent either an AR or RH resection.

Anterior resection

Amongst those who underwent an AR, death occurred in 42 patients (23.2%). The 5-year OS and DFS rates were 77.4% (95%CI 73.7–81.1) and 70.1% (95%CI 66.3–73.9) respectively. A LR was diagnosed in nine patients (5.0%), while SR was identified in 34 patients (18.8%). The median time to LR and SR was 1.3 years (95%CI 1.2–1.4) and 1.5 years (95%CI 1.2–1.8), respectively.

None of the ex vivo TM measurements were associated with OS or DFS. With respect to other

Table 1 Median tissue morphometry (TM) measurements based upon fresh resection specimens, comparing Concord Hospital ($n=312$), St. James's University Hospital (Leeds [$n=40$])[3], and University Hospital of Erlangen (Erlangen [$n=49$])[3]

Variables	Concord Hospital		St. James's University Hospital[a]		University Hospital of Erlangen[a]	
	Anterior resection	Right hemicolectomy	Left sided resection	Right sided resection[b]	Left sided resection	Right sided resection[b]
TM A, cm^2	149.6	99.8	131.7	88.8	241.3	167.7
TM B, cm	13.6	10.4	9.7	8.1	14.5	12.9
TM C, cm	9.2	7.2	8.5	7.2	10.8	10.2
TM D, cm	26.4	23.0	26.0	24.3[†]	39.2	34.8[†]

TM A Area of mesentery resected, *TM B* Distance from high vascular tie to tumour, *TM C* Distance from high vascular tie to nearest bowel wall*; and TM D* Length of bowel resected

[a] Interquartile ranges were not reported

[b] Right sided resections at Leeds and Erlangen recorded small bowel and large bowel lengths separately. These were combined to reflect the total bowel resected

clinico-pathological characteristics, poorer OS was associated with increasing age ($P=0.004$), ASA grade greater than II ($P=0.001$), AJCC stage III tumours ($P=0.03$) and those with perineural invasion (PNI[$P<0.001$]). Similarly, poor prognosis characterised by increased DFS hazards mirrored all the factors above.

Right hemicolectomy

Amongst the RH patients studied, death occurred in 47 patients (35.9%). The median OS and DFS was 7.7 years (95% CI 4.0–11.3) and 6.8 years (95% CI 3.2–10.4), respectively. The 5-year OS and DFS rates were 67.2% (95%CI 62.3–72.1) and 63.1% (95%CI 58.3–67.9) respectively. A LR was diagnosed in three patients (2.3%), while SR was seen in 24 patients (18.3%). The median time to LR and SR was 2.8 years (95%CI 0.3–5.2) and 1.7 years (95%CI 0.6–2.7), respectively.

Similar to the AR cohort, none of the ex vivo TM measurements for RH surgery patients were associated with OS or DFS. Clinically, poorer OS was associated with an increasing age ($P=0.002$) and patients with ASA grade greater than II ($P=0.02$). Pathologically, poorer OS was noted in tumours that were AJCC stage III ($P=0.02$), harbouring a mucinous or signet-ring pathology ($P=0.03$), had lymphovascular invasion (LVI[$P<0.001$]) or PNI ($P=0.004$). Conversely, a longer OS was associated with patients who underwent laparoscopic surgery ($P<0.001$).

Regarding DFS, factors associated with a poorer prognosis included an increasing age ($P=0.006$), an ASA grade greater than II ($P=0.02$), AJCC stage III tumours ($P=0.02$), the presence of mucinous or signet-ring pathology ($P=0.03$), and tumours harbouring LVI ($P<0.001$) or PNI ($P<0.001$). Meanwhile, patients who underwent laparoscopic surgery were associated with a reduced DFS hazard ($P<0.001$).

AJCC stage III sub-group analysis (Table 4)

There were 133 AJCC Stage III patients who had TM measurements recorded. Of these, 80 had an AR and 53 had a RH. In those who had an AR procedure, the following mean TM measurements were recorded: TM A – 163.5cm^2 (SD 67.3), TM B – 13.8 cm (SD 5.1), TM C – 9.8 cm (SD 4.0), and TM D – 27.5 cm (SD 9.3). Comparatively, in patients who had RH surgery the mean TM measurements were: TM A – 106.4cm^2 (SD 55.8), TM B – 10.6 cm (SD 3.1), TM C – 6.9 cm (SD 2.5), and TM D – 25.9 cm (SD 10.7).

The survival associations of the sub-cohort are summarised in Table 4. In both AR and RH sub-cohorts, none of the ex vivo TM measurements were associated with OS or DFS.

Associations between ex vivo TM measurements and Lymph Node Yield (LNY) following AR and RH operations (Table 5)

Table 5 summarises the associations between ex vivo TM measurements and the LNY of all study patients.

With each additional unit of bowel resection length, there was a corresponding increase in the number of lymph nodes (LNs) examined, as indicated by the TM D (length of resected bowel) index (β 0.11 [95% CI 0.02–0.20; $P=0.02$]). No significant associations were observed between LNY and other TM indices.

Discussion

In a previous study, we demonstrated the feasibility of performing ex vivo TM measurements using routine photographs of fixed CRC specimens, and explored surgical, patient, and disease factors associated with these measurements. Our present prospective study builds on that work by investigating the association between such TM measurements and survival outcomes, applied to a cohort of colon and rectal cancer resections spanning an

Table 2 Comparison of clinicopathological factors between anterior resection and right hemicolectomy surgery patients

Variables	Total (%) or Mean (SD) or Median (Range/IQR) ($n=312$)	Anterior Resection ($n=181$)	Right Hemicolectomy ($n=131$)
TM A (Mean [SD]), cm^2	140.9(67.3)	164.4(69.1)	108.4(49.0)
TM B (Mean [SD]), cm	12.6(4.5)	14.1(4.9)	10.7(3.0)
TM C (Mean [SD]), cm	8.8(3.6)	9.9(3.9)	7.3(2.4)
TM D (Mean [SD]), cm	26.5(9.4)	27.7(9.2)	24.7(9.4)
Gender			
Male	165(52.9)	107(59.1)	58(44.3)
Female	147(47.1)	74(40.9)	73(55.7)
Age (Mean [SD]), years	69.4(12.3)	66.8(12.3)	72.9(11.5)
BMI (Mean [SD]), kg/m^2	27.6(5.4)	27.2(5.2)	28.1(5.7)
ASA Grade			
I	41(13.1)	29(16.1)	12(9.2)
II	179(57.4)	110(60.8)	69(52.7)
III/IV	92(29.5)	42(23.2)	50(38.2)
Emergency Operation			
No	303(97.1)	175(96.7)	128(97.7)
Yes	9(2.9)	6(3.3)	3(2.3)
Emergency Operation (Reason)			
Non-emergency	303(97.1)	175(96.7)	128(97.7)
Obstruction	8(2.6)	5(2.8)	3(2.3)
Perforation	1(0.3)	1(0.6)	-
Operation Modality			
Laparoscopy	265(84.9)	149(82.3)	116(88.5)
Open	47(15.1)	32(17.7)	15(11.5)
Procedure Conversion			
No	246(92.8)	137(92.6)	109(94.0)
Yes	19(7.2)	12(8.1)	7(6.0)
Blood Loss (mls)			
≤ 500	302(96.8)	172(95.0)	130(99.2)
>500	10(3.2)	9(5.0)	1(0.8)
Tumour Stage (TNM AJCC)			
Stage 1	68(21.8)	44(24.4)	24(18.3)
Stage 2	111(35.6)	57(31.5)	54(41.2)
Stage 3	133(42.6)	80(44.4)	53(40.5)
Tumour Size (cm) (Median [Range])	4.0(0.8–34.0)	3.5(0.8–9.0)	4.5(0.8–34.0)
Tumour Perforation			
No	308(98.7)	179(98.9)	129(98.5)
Yes	4(1.3)	2(1.1)	2(1.5)
Histological Type			
Non-Mucinous or Signet ring	282(90.4)	175(96.7)	107(81.7)
Mucinous or Signet ring	30(9.6)	6(3.3)	24(18.3)
Histological Differentiation			
Well or Moderate	272(87.2)	171(94.5)	101(77.1)
Poor	40(12.8)	10(5.5)	30(22.9)
Histological Grade			
Low or Average	270(86.5)	170(93.9)	100(76.3)
High	42(13.5)	11(6.1)	31(23.7)
Lympho-Vascular Invasion			
No	226(72.4)	127(70.2)	99(75.6)
Yes	86(27.6)	54(29.8)	32(24.4)

Table 2 (continued)

Variables	Total (%) or Mean (SD) or Median (Range/IQR) (*n* = 312)	Anterior Resection (*n* = 181)	Right Hemicolectomy (*n* = 131)
Peri-Neural Invasion			
No	247(79.2)	136(75.1)	111(84.7)
Yes	65(20.8)	45(25.0)	20(15.3)
Number of Lymph Nodes Examined (Median [Range])	19(4–47)	19(4–45)	20(6–47)
Lymph Node Harvest (< 12)			
No	286(91.7)	161(89.0)	125(95.4)
Yes	26(8.3)	20(11.0)	6(4.6)
LOS (Median [Range]), days	7(2–66)	7(2–66)	6(3–30)

TM Tissue Morphometry A-D, *BMI* Body Mass Index, *ASA* American Society of Anesthesiology, *TNM 8th* edition tumour, nodes, and metastasis staging system; and *AJCC* American Joint Committee on Cancer

eleven-year period. By determining their prognostic significance, this study sought to confirm or deny the utility of ex vivo TM measurements as a quality metric of 'good CRC surgery'.

Practically, assessment of the quality of CRC surgery encompasses two key considerations: (i) the plane of excision, and (ii) the EoL. While both elements are presumed to have equal significance for patient outcomes, much of the initial research in assessing surgical quality concentrated on the correct plane of excision to ensure intactness of the enveloping mesorectal or mesocolic fascia and avoidance of tumour transection. For instance, the MRC CR07 trial revealed that 3-year local recurrence rates were estimated at 4%, 7%, and 13% for mesorectal, intramesorectal, and muscularis propria dissections, respectively [7]. Also, when in the mesocolic plane, a 15% survival advantage at 5 years is conferred, compared to operating in the muscularis propria plane, with the benefit peaking to 27% in patients diagnosed with Stage III disease [4].

The twin consideration for surgical quality—EoL—has received relatively less attention. The evaluation of the EoL in CRC surgery is traditionally based on rudimentary metrics such as LN harvest, with a nodal count of at least 12 generally considered adequate for staging purposes [21, 22]. However, relying solely on LNY as a marker of EoL has its limitations. Firstly, the assessment of LNY depends on the thoroughness of the pathologist [3]. Furthermore, the increasing use of neoadjuvant therapy may lead to a reduction in the number of nodes excised [23, 24]. Despite these challenges, the growing focus on central vascular ligation (CVL) signifies a shift in assessing the EoL aspect of CRC surgery quality given the emphasis on high tie of the parent pedicle [1, 8, 9]. Therefore, when ex vivo analysis of CVL specimens are performed, TM measurements of resected specimens have revealed greater area of mesentery resected, longer amounts of bowel divided and longer distances from the tumour to the ligation of tumour-supplying vessels or closest bowel wall than non-CVL cases [3, 25, 26]. A natural inference of this is to expect a significant association between ex vivo TM measurements and survival outcomes in CRC surgery, which if proven, would provide argument for its role as a quality metric of CRC (in particular, CVL) surgery.

However, contrary to our initial hypothesis, we found no association between ex vivo TM measurements and either OS or DFS in the AR or RH surgery cohorts. This lack of association was also observed specifically in the Stage III group where it could be expected that an increased EoL would provide the most significant survival advantage. The absence of survival association was consistent for both: (i) 'oncologically relevant' TM measurements (TM A, TM B, and TM C), which would be expected to reflect mesenteric excision of lymph nodes along the central tumour-draining pedicle, and (ii) the arguably 'less oncologically relevant' TM measurement (TM D), which would explain excision of longitudinal peri-colic lymph nodes not necessarily draining the primary tumour. Consequently, while feasible and reproducible, the utility of ex vivo TM measurements as quality metric for CRC surgery was not demonstrated.

Notably, few studies have previously investigated a survival relationship with ex vivo TM measurements, and of these, focus has been on populations predominantly undergoing non-CVL surgery for colonic malignancies [4, 27, 28]. Specifically, West et al. [4], confined their investigation of area and lengths to *cross-sectional measurements* and conducted these measurements on fixed tissue specimens. Storli et al. [27], solely focussed on the length of the resected bowel measured from fixed colon cancer specimens, while Galizia et al. [28], documented the length from the vascular tie to the bowel wall in addition to the length of the bowel resected in those who had

Table 3 Comparison of Survival Outcomes between Clinicopathological Characteristics in AR and RH patients

Variable	Overall Survival				Disease-Free Survival			
	AR		RH		AR		RH	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
TM A (cm^2)	1.00(1.00–1.01)	0.38	1.00(1.00–1.00)	0.30	1.00(1.00–1.01)	0.40	1.00(0.99–1.00)	0.57
TM B (cm)	0.95(0.86–1.06)	0.78	0.95(0.86–1.06)	0.36	1.01(0.96–1.07)	0.60	0.97(0.88–1.06)	0.50
TM C (cm)	0.99(0.92–1.04)	0.79	0.92(0.82–1.04)	0.17	0.98(0.92–1.05)	0.56	0.93(0.83–1.04)	0.19
TM D (cm)	1.02(0.99–1.04)	0.19	1.00(0.97–1.03)	0.83	1.01(0.99–1.04)	0.35	1.01(0.99–1.04)	0.36
Gender								
Male	ref	-	ref	-	ref	-	ref	-
Female	0.53(0.27–1.04)	0.07	0.63(0.36–1.12)	0.12	0.64(0.37–1.13)	0.12	0.85(0.49–1.46)	0.55
Age (years)	1.05(1.02–1.08)	0.003	1.05(1.02–1.08)	0.002	1.02(1.00–1.05)	0.049	1.04(1.01–1.07)	0.006
BMI (kg/m^2)	1.02(0.96–1.07)	0.55	0.99(0.94–1.05)	0.84	1.02(0.98–1.07)	0.35	1.01(0.96–1.06)	0.68
ASA Grade								
I	ref	-	ref	-	ref	-	ref	-
II	2.66(0.79–8.86)	0.11	1.47(0.34–6.42)	0.61	1.91(0.74–4.92)	0.18	1.85(0.43–7.94)	0.41
III/IV	8.08(2.34–27.9)	< 0.001	5.36(1.27–22.54)	0.02	5.57(2.07–14.97)	< 0.001	5.85(1.39–24.53)	0.02
Emergency Operation								
No	ref	-	ref	-	ref	-	ref	-
Yes	0.05(0.00–513.03)	0.52	2.02(0.28–14.83)	0.49	0.63(0.09–4.58)	0.65	1.49(0.20–10.82)	0.70
Operation Modality								
Open	ref	-	ref	-	ref	-	ref	-
Laparoscopy	0.69(0.36–1.1.35)	0.29	0.33(0.17–0.63)	< 0.001	0.70(0.39–1.28)	0.25	0.28(0.15–0.53)	< 0.001
Intraoperative Blood Loss (mls)								
≤ 500	ref	-	ref	-	ref	-	ref	-
> 500	1.60(0.57–4.52)	0.38	NA	0.84	1.33(0.48–3.71)	0.59	NA	0.77
Tumour Stage (TNM)								
Stage 1	ref	-	ref	-	ref	-	ref	-
Stage 2	1.53(0.56–4.13)	0.41	1.44(0.52–3.98)	0.48	1.91(0.83–4.40)	0.13	1.23(0.48–3.14)	0.70
Stage 3	2.66(1.09–6.48)	0.03	3.00(1.15–7.80)	0.02	2.51(1.15–5.49)	0.02	2.96(1.23–7.12)	0.02
Histological Type								
Non-Mucinous or Non-Signet Ring	Ref	-	Ref	-	Ref	-	Ref	-
Mucinous or Signet Ring	0.05(0.00–52.51)	0.39	2.10(1.08–4.09)	0.03	0.53(0.07–3.84)	0.53	2.05(1.09–3.86)	0.03
Lympho-Vascular Invasion								
No	ref	-	ref	-	ref	-	ref	-
Yes	1.82(0.98–3.38)	0.06	3.57(1.87–6.83)	< 0.001	1.64(0.95–2.82)	0.07	3.74(2.03–6.91)	< 0.001
Peri-Neural Invasion								
No	ref	-	ref	-	ref	-	ref	-
Yes	3.08(1.67–5.68)	< 0.001	2.62(1.35–5.08)	0.004	3.07(1.80–5.25)	< 0.001	2.92(1.55–5.50)	< 0.001
Number of Lymph Nodes Examined	0.97(0.93–1.02)	0.20	0.98(0.94–1.02)	0.23	0.99(0.96–1.03)	0.62	0.98(0.94–1.02)	0.23

HR Hazard Ratio, *TM* Tissue Morphometry A-D, *BMI* Body Mass Index, *ASA* American Society of Anesthesiology and *TNM 8th* edition tumour, nodes, and metastasis staging system

a right sided colon cancer resection but applied median cut-offs when examining survival association. In keeping with our findings, none of these studies observed significant differences in patient survival according to TM measurement(s).

Identifying the specific reasons for the lack of association between ex vivo TM measurements and survival outcomes is challenging. It is plausible that any increase in TM measurements was attributed to excision of mesenteric fat without concomitant increase in LN harvest. This is supported by our finding that TM measurements

Table 4 Comparison of Survival Outcomes between TM measurements in AJCC Stage III AR and RH patients ($N = 133$)

Variable	Overall Survival				Disease-Free Survival			
	AR		RH		AR		RH	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
TM A (cm^2)	1.00(1.00–1.01)	0.46	1.00(1.00–1.01)	0.63	1.00(1.00–1.01)	0.30	1.00(1.00–1.01)	0.46
TM B (cm)	1.00(0.93–1.08)	0.96	0.96(0.84–1.10)	0.54	1.01(0.94–1.08)	0.87	1.00(0.89–1.14)	0.95
TM C (cm)	0.99(0.89–1.09)	0.82	0.92(0.80–1.07)	0.29	0.97(0.89–1.07)	0.56	0.94(0.82–1.08)	0.36
TM D (cm)	1.01(0.98–1.05)	0.50	1.00(0.97–1.04)	0.86	1.01(0.98–1.04)	0.66	1.01(0.98–1.04)	0.55

HR Hazard Ratio, *TM* Tissue Morphometry A-D

Table 5 Differences in the association of TM measurements and LNY in all CRC patients

	Number of LN examined	
	β (95% CI)	*P*-value
TM A (cm^2)	0.01(-0.004 to 0.02)	0.20
TM B (cm)	-0.07(-0.26 to 0.12)	0.47
TM C (cm)	0.07(-0.18 to 0.31)	0.59
TM D (cm)	*0.11(0.02 to 0.20)*	*0.02*

TM Tissue Morphometry A-D, *LNY* Lymph Node Yield and *LN* Lymph node(s)

were not associated with LNY (except for a positive association observed with length of bowel resected [i.e., TM D], owing to an increased harvest of longitudinal peri-colic LNs with increased TM D). Clearly, increased TM measurements without increase in 'oncologically relevant' LN harvest, would not be expected to confer survival benefit. It is also noteworthy that ex vivo TM measurements record parameters of the excised specimen but give no understanding of the residual in vivo vascular pedicle measurement, which in many ways is more oncologically relevant as the residual pedicle contains draining LNs left in situ. Measurements of in vivo residual pedicles post resection have previously been described [9], and may be a more sensitive predictor of survival post CRC surgery.

This study validates the feasibility of measuring ex vivo TM parameters using high-definition images of non-formalin fixed specimens. To ensure the standardised measurements on these fresh specimens, we excluded 269 fixed specimen records, mitigating the risk of shrinkage artifact. Moreover, the variation in ex vivo TM measurements between the AR and RH groups underscores our rationale for analysing AR and RH patients separately. Our standardised approach to TM measurements also allows comparison of data with other groups which have employed similar methodology. The ex vivo TM measurements in our study (at CRGH) exhibit comparability to those recorded at SJUH in Leeds (Table 1). However, when compared to the measurements from UHE, both CRGH and SJUH demonstrate smaller measurements globally [3]. Notably, the standard practice at CRGH and SJUH involves non-routine CVL surgery, in contrast to UHE where CVL surgery is routinely performed [3]. This discrepancy in surgical practices is presumed to contribute to the observed differences, with routine CVL surgery at UHE likely being a contributing factor to the larger TM A and the extended lengths in TM B, TM C and TM D [25, 26].

The comparison between the resected specimens from a Japanese D3 resection and our non-routine CVL surgery reveals both distinct contrasts and similarities [29–31]. Specifically, the excised mesentery area (i.e., TM A) and the length of the resected bowel (i.e., TM D) were observed to be smaller and shorter in the cohort undergoing D3 dissection [29]. The observed discrepancies in TM measurements between the Japanese cohort and our study could reflect divergent surgical techniques, particularly the adherence of Japanese surgeons to the stringent '10cm rule' and their routine practice of central vascular ligation [30]. Furthermore, phenotypic variations in body structure and large bowel anatomy between the populations of the two regions might also have influenced the differences in TM measurements [29, 32]. Despite these variances, the oncological outcomes after D3 resection is similar to that reported by us [33, 34]. Additionally, central radicality, as indicated by the lengths of TM B and TM C, remained comparable across the Japanese literature and our data [29, 30].

The association (or the apparent lack thereof) between survival outcomes and either LN positivity or LNY merits discussion. Expectedly, stage 3 disease was associated with poorer survival. Interestingly though, the removal of additional LNs seemed to have no impact on OS and DFS. These observations suggest that once LN metastatic disease is established, survival may be influenced by other clinicopathological factors, including the progression to systemic disease. Therefore, although our practice is to perform a comprehensive lymphadenectomy that incorporates central nodes from the vessel root into the specimen's resection margin, the advantage of removing additional nodes (i.e., more than 12 nodes) in Stage 3 CRC patients is unclear based on our study's data. It is important to note that this conclusion might not apply to LR rate, which was low in our series.

This prospective study has several limitations. Notably, the absence of height records for some patients in the database prevented us from adjusting ex vivo TM measurements based on the patients' body mass indices. Furthermore, we could not account for the plane of mesocolic excision due to missing data for some patients. Given the lack of association between TM measurements and survival on univariate analysis, though, it is unlikely that any significant association would have been confounded by plane of excision status. Finally, this study may be underpowered as there was an absence of pilot data to guide a robust sample size calculation. However, our use of fresh specimens over an eleven-year period to record TM measurements addresses concerns about specimen shrinkage artifact. Moreover, incorporating both urgent operations and a range of surgical approaches—from minimally invasive to open—improves the generalizability of our findings.

This study focused on evaluating the feasibility of using ex vivo fresh specimen images for recording TM measurements, with the expectation that it could be useful as a quality metric of 'good CRC surgery'. However, the absence of significant association between TM measurements and survival outcomes does not support its use as a quality metric. This finding serves to further highlight the inherent challenges in assessing quality of CRC surgery, but also underscores the need to further explore aspects of EoL, particularly as ex vivo TM measurements do not offer insight.

Abbreviations

AJCC American Joint Committee on Cancer
AR Anterior Resection
ASA American Society of Anaesthesiologist
CRC Colorectal Cancer
CRGH Concord Repatriation General Hospital
CVL Central Vascular Ligation
DFS Disease Free Survival
EoL Extent of Lymphadenectomy
IQR Interquartile Range
LN Lymph Node
LNY Lymph Node Yield
LOS Length of Stay
LR Locoregional Recurrence
LVI Lympho Vascular Invasion
OS Overall Survival
PNI Perineural Invasion
RH Right Hemicolectomy
SD Standard Deviation
SJUH St James's University Hospital
SMA Superior Mesenteric Artery
SMV Superior Mesenteric Vein
SR Systemic Recurrence
TM Tissue Morphometry
UHE University Hospital of Erlangen

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Authors' contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by KN. The first draft of the manuscript was written by KN. All the tables and figures were created by KN. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. MJFXR and KSN supervised the undertaking of this research project.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval (2020/ETH03325 and CH62/62011–136-P Chapuis HREC/11/CRGH206) was granted by the Sydney Local Health District Ethics Committee, with included patients consenting for the use of their data and tumour specimens for research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Publisher's Note

APPENDIX III

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ORIGINAL ARTICLE

Lymph node ratio prognosticates overall survival in patients with stage IV colorectal cancer

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Abstract

Background Lymph node ratio (LNR) is suggested to address the shortcomings of using only lymph node yield (LNY) or status in colorectal cancer (CRC) prognosis. This study explores how LNR affects survival in patients with metastatic colorectal cancer (mCRC), seeking to provide clearer insights into its application.

Methods This observational cohort study investigated stage IV patients with CRC (1995–2021) who underwent an upfront resection of their primary tumour at Concord Hospital, Sydney. Clinicopathological data were extracted from a prospective database, and LNR was calculated both continuously and dichotomously (LNR of 0 and LNR > 0). The primary endpoint was overall survival (OS). The associations between LNR and various clinicopathological variables were tested using regression analyses. Kaplan–Meier and Cox regression analyses estimated OS in univariate and multivariate survival models.

Results A total of 464 patients who underwent a primary CRC resection with clear margins (mean age 68.1 years [SD 13.4]; 58.0% M; colon cancer [$n = 339$,73.1%]) had AJCC stage IV disease. The median LNR was 0.18 (IQR 0.05–0.42) for colon cancer (CC) resections and 0.21 (IQR 0.09–0.47) for rectal cancer (RC) resections. A total of 84 patients had an LNR = 0 (CC = 66 patients; RC = 18 patients). The 5-year OS for the CC cohort was 10.5% (95% CI 8.7–12.3) and 11.5% (95% CI 8.4–14.6) for RC. Increasing LNR demonstrated a decline in OS in both CC ($P < 0.001$) and RC ($P < 0.001$). In patients with non-lymphatic dissemination only (LNR = 0 or N0 status), there was better survival compared with those with lymphatic spread (CC aHR1.50 [1.08–2.07;$P = 0.02$], RC aHR 2.21 [1.16–4.24;$P = 0.02$]).

Conclusions LNR is worthy of consideration in patients with mCRC. An LNR of 0 indicates patients have a better prognosis, underscoring the need for adequate lymphadenectomy to facilitate precise mCRC staging.

Keywords Lymph node ratio · LNR · Lymphadenectomy and metastatic colorectal cancer

Introduction

In patients with metastatic colorectal cancer (mCRC) who undergo a primary tumour resection with clear margins, a distinct observation is noted in that the lymph node yield (LNY) from resected specimens is frequently substandard (i.e. fewer than 12 nodes are retrieved), when compared with their non-metastatic counterparts [1–4]. This observation may be embedded in the perception that once distant metastatic disease has occurred, addressing LN evaluation is futile [5].

While it is true that the presence of distant metastases represents the most advanced stage of the disease, it may not negate the importance of LN evaluation in mCRC management. Understanding the extent of regional LN involvement may still be relevant, as LN status (even in the presence of distant metastases) may potentially reflect differences in

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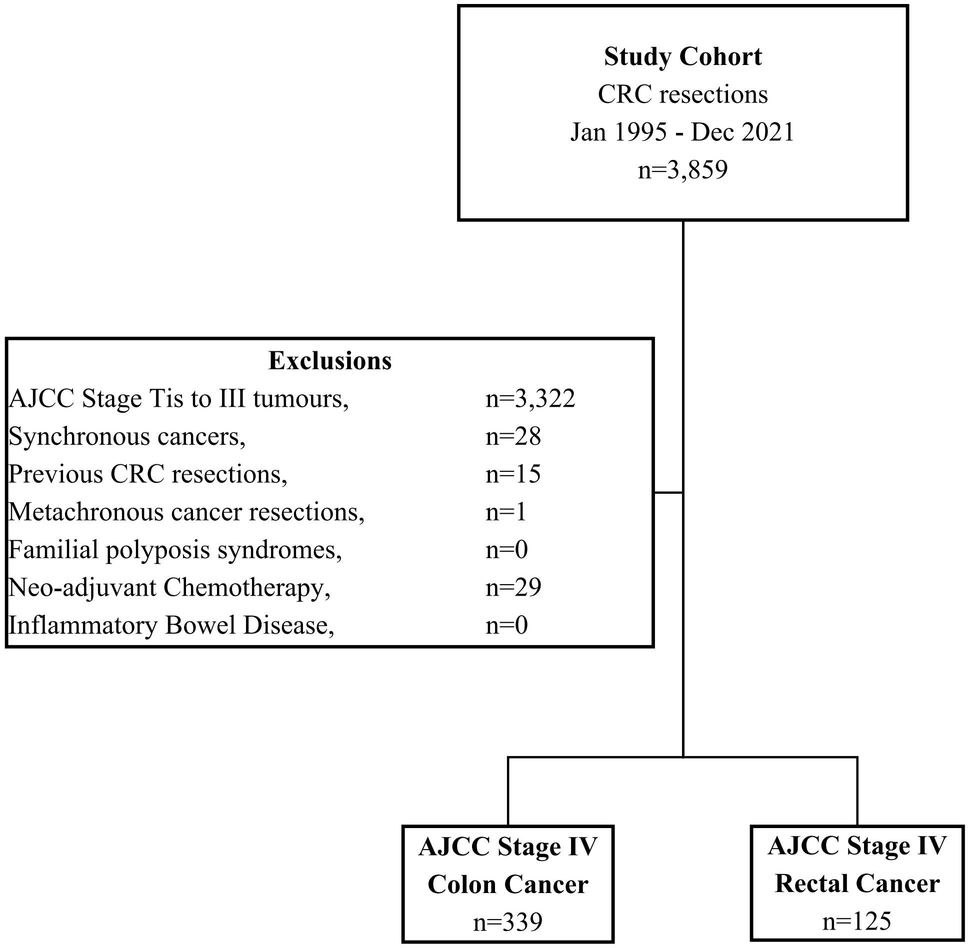

Fig. 1 Flow diagram of cohort definition

disease behaviour, tumour aggressiveness and overall survival. Similarly, precise LN evaluation might help inform decisions on the use of post-operative therapy, targeted treatments and the frequency of follow-up care [3].

Lymph node ratio (LNR), calculated as the number of metastatic LNs divided by the total number of examined nodes [6], may be a useful parameter that incorporates information relating to both LN status and LNY. The prognostic significance of LNR in stage III CRC has been well documented, with previous studies demonstrating that those with higher LNR values tend to have poorer survival [6–8]. Notably, LNR offers a more distinct prognostic differentiation when compared with the sole reliance on a simple count of positive nodes [7, 8]. However, the impact of LNR on mCRC patients' survival remains unclear. In particular, patients' survival with an LNR of 0 (i.e. node negative, or N0, metastatic disease) have not been sufficiently explored in literature.

In mCRC patients with an LNR of 0 (i.e. N0 status), the absence of metastatic LNs coexists with metastatic spread of disease through other routes such as haematogenous or transcoelomic spread. Intuitively, these patients are presumed to have a better prognosis due to a potentially decreased tumour burden. Investigating the survival implications of dichotomizing LNR (LNR of 0 and LNR > 0) may refine our understanding of patients with an LNR of 0. Additionally, such a dichotomization may provide insight into the need to perform an adequate lymphadenectomy in patients with mCRC undergoing primary tumour resection (i.e. upfront surgery).

This study aims to examine if LNR is an independent predictor of overall survival (OS) in patients with mCRC following resection of the primary tumour. We analysed LNR both as a continuous variable and dichotomously (LNR of 0, LNR > 0), hypothesising that the rising LNR correlates with poorer survival, while LNR = 0 would be associated with better outcome.

Materials and methods

A cohort study of consecutive patients who had an upfront resection for a newly diagnosed AJCC stage IV colorectal adenocarcinoma at Concord Hospital, Sydney, between January 1995 and December 2021, was performed. The study population was drawn from a prospectively maintained institutional database, which has been in continual existence since January 1971. Patients with in situ neoplasia, AJCC stage I to III CRC, polyposis coli, inflammatory bowel disease and/or synchronous or metachronous CRC were excluded (Fig. 1). The study received ethical approval from the Sydney Local Health District Ethics Committee (2019/ETH07841).

Pre-operative assessment

For patients planned for an elective operation, pre-operative tumour status was assessed in a multidisciplinary setting based on clinical examination, colonoscopy, computed tomography, ultrasonography, MRI and, more recently, selective positron emission tomography.

Neo-adjuvant therapy

In more recent study periods, systemic neo-adjuvant chemotherapy (nCT) was introduced for patients with mCRCs. These patients were excluded from this study for several reasons. First, nCT has been shown to artefactually influence LNY [9], potentially confounding LNR's impact on outcomes. Moreover, an upfront resection followed by post-operative treatment of synchronous metastases formed the mainstay of management during much of the study period and today remains a recognised strategy in the treatment of mCRC [2, 4, 10–13].

Neo-adjuvant therapy in rectal cancer

For non-urgent rectal cancers located in the lower two-thirds of the rectum, a patient's suitability for neo-adjuvant radiotherapy (short versus long-course with or without chemotherapy) was decided by multidisciplinary consensus based on: the patient's individual needs, tumour location, clinical and radiological features, biopsy findings, metastasis extent, history of previous pelvic irradiation and the patient's fitness for operation.

Surgical management

Primary CRC

During the majority of the study period, the predominant approach to managing mCRC entailed upfront surgical resection of the primary CRC followed by post-operative chemotherapy. This strategy continues to be a recognized method in mCRC treatment [2, 4, 10–15]. For patients who underwent emergency primary CRC resection, the surgical indications were typically large bowel obstruction, perforation or bleeding.

A resection for a primary CRC was classified into three categories: standard, extended or a segmental operation. This classification was based on the extent of the vascular ligation of the tumour which consequently determined the extent of lymphadenectomy performed [16]. Standard resections included right hemicolectomy, left hemicolectomy, transverse colectomy, sigmoid colectomy, a Hartmann's procedure, anterior resection and abdominoperineal excision (APER), wherein the operation involved the ligation of strategic vessels, such as the ileocolic artery, right colic artery (if present), middle colic vessels, ascending left colic artery and inferior mesenteric artery. An extended resection comprised either an extended right hemicolectomy or a subtotal colectomy, involving the ligation of a combination of the aforementioned vessels. For instance, in the case of an extended right hemicolectomy, the ligation includes the ileocolic artery, right colic artery (if present) and middle colic vessels. Lastly, segmental resections entailed the removal of the primary tumour without a dedicated vascular pedicle ligation, resulting in varying LNY [16]. Tumours between (and including) the caecum and rectosigmoid junction were defined as colonic. Rectal cancers were defined as those whose inferior edge was within 15 cm from the anal verge, measured by rigid sigmoidoscopy [17].

Metastasectomy

For patients who underwent a metastasectomy, this was performed during the index operation or as a separate staged operation. The decision and timing hinged on factors including the clinical presentation of the primary cancer, the extent of metastatic spread, the patient's fitness for surgery and response to post-operative chemotherapy.

In selected cases where metastasectomy was deemed unsuitable but the patient was symptomatic and had a good performance status, a palliative resection of the primary tumour and regional lymphadenectomy was performed followed by palliative chemotherapy, if appropriate.

Pathology reporting and staging

Specialist pathologists examined resected specimens using a standard synoptic protocol, with pathology data coded by CC [16]. Adenocarcinomas including mucinous and signet ring types were analysed. Tumour staging followed the AJCC pTNM system [18].

Standard clinicopathological variables

Clinical information, operative details, tumour pathology, treatment information and follow-up data were extracted from the database for analysis, as previously described [16]. Groupings for year of resection was also performed to acknowledge the potential changes in treatments approaches over time.

Clinicopathological variable of interest—LNR

The LNR, which represents the ratio of involved LNs to the total number of LNs harvested, calculated, and documented as an additional variable. It was also dichotomised as LNR of 0 and LNR > 0. This dichotomisation aimed to identify patients with mCRC with metastatic spread exclusively through non-lymphatic pathways (i.e. LNR of 0).

Surveillance

In patients with mCRC who underwent a synchronous metastasectomy and primary tumour resection, their follow-up mostly resembled that of individuals with non-metastatic disease [16, 19]. Generally, this involved clinical examinations and liver function tests every 3 months, along with serial carcinoembryonic antigen measurements for the first 2 years post-operation, followed by these evaluations every 6 months for an additional three years, if clinically appropriate. Imaging typically included annual computed tomography scans for the first 2 years, with subsequent imaging guided by clinical and biochemical findings. More recently, selective positron emission tomography imaging has also been utilized. Patients were monitored annually until death or December 2021, with less than 5% of patients being lost to follow-up. Colonoscopy was generally conducted at 1-year post-resection.

Patients with unresectable metastatic disease following the resection of the primary tumour were referred to medical oncology and palliative care services for consideration of palliative chemotherapy. In these patients, a personalised and less demanding follow-up protocol was often employed.

Post-operative chemotherapy

A multidisciplinary team routinely considered post-operative chemotherapy for all patients with mCRC who underwent primary tumour resection. Factors such as age, patient preferences, comorbidities, adverse pathological features, social circumstances, and best practice guidelines were considered.

The chemotherapy regimens utilised varied but were in accordance with best practice at the time and for the most part were: bolus injections of 5-fluorouracil (5-FU) and leucovorin administered daily in 5-day blocks and repeated every month for 6 months, as per the Mayo Clinic regimen [20]; 5-FU and leucovorin were repeated weekly for six doses with a 2-week rest between, as per the Roswell Park regimen [21]. An alternate option includes semi-monthly 22 h 5-FU infusion with leucovorin [22] or modified oxaliplatin, leucovorin and 5-FU (FOLFOX) was administered every 2 weeks [23]. In some, oral capecitabine and intravenous oxaliplatin (CAPOX or XELOX) was considered for six cycles. In some palliative settings, duplet (i.e. leucovorin, 5-FU with either oxaliplatin [FOLFOX] or irinotecan [FOLFIRI]) or triplet (i.e. leucovorin, 5-FU, oxaliplatin and irinotecan [FOLFOXIRI]) chemotherapy was considered.

Outcome measures

The primary outcome measure was OS. This was considered the most pragmatic measure of survival in this patient cohort.

Patient follow-up commenced from the date of resection. Follow-up times were censored at the last contact for patients who did not experience a terminal event up to December 2021 or were lost to follow-up or remained alive. The date of death was primarily obtained from the patient's surgeon, family physician or hospital records, with occasional use of the national death registration system [16]. The cause of death was coded by PC based on the International Classification of Diseases-10.

Statistical analysis

Continuous variables were reported as mean (standard deviation [SD]) for normally distributed variables and as median (interquartile range [IQR] or range) for non-normal distributions. Categorical variables were reported as frequencies and percentages. Linear regression tested association between LNR (as a continuous variable) and other clinico-pathological variables. Logistic regression tested association between dichotomised LNRs (LNR of 0 or LNR > 0) and other clinico-pathological variables. These regression

Table 1 Comparison of clinicopathological factors between patients with colon and rectal cancer ($n = 464$)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	Colon cancer (*n* [%], total = 339)	Rectal cancer (*n* [%], total = 125)
Gender			
Male	269(58.0)	191(56.3)	78(62.4)
Female	195(42.0)	148(43.7)	47(37.6)
Age (mean [SD]), years	68.1(13.4)	69.1(13.6)	65.4(12.6)
BMI (mean [SD]), kg/m^2	27.1(5.5)	27.1(5.7)	27.0(5.0)
ASA grade			
I	68(14.7)	40(11.8)	28(22.4)
II	232(50.0)	164(48.4)	68(54.4)
III/IV	164(35.3)	135(39.8)	29(23.2)
Year of resection			
1995–1999	95(20.5)	61(18.0)	34(27.2)
2000–2004	95(20.5)	59(17.4)	36(28.8)
2005–2009	94(20.3)	68(20.1)	26(20.8)
2010–2014	82(17.7)	71(20.9)	11(8.8)
2015–2019	74(15.9)	64(18.9)	10(8.0)
2020 onwards	24(5.2)	16(4.7)	8(6.4)
Emergency operation			
No	387(83.4)	265(78.2)	122(97.6)
Yes	77(16.6)	74(21.8)	3(2.4)
Type of operation			
Segmental resection	3(0.6)	3(0.9)	–
Standard resection	431(92.9)	306(90.3)	125(100.0)
Extended resection	30(6.5)	30(8.8)	–
Operation modality			
Open	321(69.2)	229(67.6)	92(73.6)
Laparoscopy	143(30.8)	110(32.4)	33(26.4)
Blood loss (millilitres)			
≤ 500	428(92.2)	317(93.5)	111(88.8)
> 500	36(7.8)	22(6.5)	14(11.2)
Tumour location (colon)			
Right	145(42.8)	145(42.8)	
Left	194(57.2)	194(57.2)	
Tumour location (rectum)			
Upper	47(37.6)		47(37.6)
Mid	43(34.4)		43(34.4)
Lower	35(28.0)		35(28.0)
Neoadjuvant RT (rectal)			
No	108(86.4)	NA	108(86.4)
Yes	17(13.6)	NA	17(13.6)
Distant metastatic sites			
Liver or other(s)	352(82.3)	279(82.3)	103(82.4)
Liver and other(s)	75(16.3)	54(15.9)	21(16.8)
Distant metastasectomy			
No	374(80.6)	275(81.1)	99(79.2)
Yes	83(17.9)	58(17.1)	25(20.0)
Tumour size (median [range]), cm	5.0(0.6–15.0)	5.0(0.6–15.0)	5.0(0.6–10.0)
Tumour perforation			
No	413(89.0)	307(90.6)	106(84.8)
Yes	51(11.0)	32(9.4)	19(15.2)
Obstructing tumour			

Table 1 (continued)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	Colon cancer (*n* [%], total = 339)	Rectal cancer (*n* [%], total = 125)
No	402(86.6)	280(82.6)	122(97.6)
Yes	62(13.4)	59(17.4)	3(2.4)
T stage			
T1 and T2	13(2.8)	6(1.8)	7(5.6)
T3	262(56.5)	173(51.0)	89(71.2)
T4	189(40.7)	160(47.2)	29(23.2)
Histological type			
Mucinous/signet ring	47(10.1)	39(11.5)	8(6.4)
Non-mucinous/signet ring	417(89.9)	300(88.5)	117(93.6)
Histological differentiation			
Well or moderate	297(64.0)	212(62.5)	85(68.0)
Poor	167(36.0)	127(37.5)	40(32.0)
Histological grade			
Low or average	251(54.1)	184(54.3)	67(53.6)
High	213(45.9)	155(45.7)	58(46.4)
Lympho-vascular invasion			
No	160(34.5)	120(35.4)	40(32.0)
Yes	304(65.5)	219(64.6)	85(68.0)
Peri-neural invasion			
No	254(54.7)	188(55.5)	66(52.8)
Yes	210(45.3)	151(44.5)	59(47.2)
Lymph nodes – examined (median [range])	17(2–87)	17(2–87)	17(3–49)
Lymph node yield			
< 12	103(22.2)	72(21.2)	31(24.8)
≥ 12	361(77.8)	267(78.8)	94(75.2)
Lymph node ratio—positive nodes/ total nodes examined (median [IQR])	0.20(0.06–0.43)	0.18(0.05–0.42)	0.21(0.09–0.47)
Lymph node ratio (LNR [dichotomised])			
LNR of 0	84(18.1)	66(19.5)	18(14.4)
LNR > 0	380(81.9)	273(80.5)	107(85.6)

BMI body mass index, *ASA* American Society of Anesthesiology, *RT* radiotherapy, *NA* not applicable, *T stage*, tumour stage (part of AJCC TNM system)

Table 2 Comparison of LNY between patients with colon and rectal cancer with LNR of 0 and LNR > 0 (*n* = 464)

Variables	*N* (%) or mean (SD) or median (range/IQR)	Colon cancer (total, 339)		Rectal cancer (total, 125)	
		LNR of 0 (*n* [%], total, 66)	LNR > 0 (*n* [%], total, 273)	LNR of 0 (*n* [%], total, 18)	LNR > 0 (*n* [%], total, 107)
Lymph node yield					
< 12	103(22.2)	16(24.2)	56(20.5)	9(50.0)	22(20.6)
≥ 12	361(77.8)	50(75.8)	217(79.5)	9(50.0)	85(79.4)

LNR lymph node ratio

models were performed for colon and rectal cancer separately, due to perceived differences in their outcomes, biological behaviour and overall surgical treatment approach. Survival estimates were modelled using the Kaplan–Meier function with log-rank test performed to determine difference in survival distributions.

The association between LNR and OS was assessed by Cox regression, performed for colon and rectal cancers separately. To adjust for confounding, all clinico-pathological variables that showed associations ($P < 0.05$) with LNR on their respective linear (for continuous LNR) and logistic (for dichotomised LNR) regression analyses were also included

Table 3 Comparison of clinicopathological factors and LNR (stage IV)

	Lymph node ratio			
	Colon ($n=339$)		Rectum ($n=125$)	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value
Gender				
Male	Reference	–	Reference	–
Female	0.004(−0.05 to 0.06)	0.89	–0.004(−0.10 to 0.09)	0.93
Age (mean [SD]), years	−0.001(−0.003 to 0.001)	0.39	0.0001(−0.004 to 0.003)	0.87
BMI (mean [SD]), kg/m^2	0.0001(−0.01 to 0.01)	0.88	0.004(−0.01 to 0.02)	0.61
ASA grade	−0.03(-0.07 to 0.01)	0.15	−0.02(−0.09 to 0.05)	0.61
I				
II				
III/IV				
Year of surgery	*−0.02(−0.04 to −0.003)*	*0.02**	−0.02(−0.05 to 0.01)	0.19
1995–1999 2000–2004 2005 to 2009 2010–2014 2015–2019 2020 onwards				
Emergency operation				
No	Reference	–	Reference	–
Yes	−0.03(−0.10 to 0.03)	0.32	0.07(−0.25 to 0.38)	0.68
Tumour perforation				
No	Reference	–	Reference	–
Yes	−0.05(−0.14 to 0.05)	0.30	0.11(−0.02 to 0.24)	0.09
Obstructive presentation				
No	Reference	–	Reference	–
Yes	−0.04(−0.11 to 0.04)	0.35	0.07(−0.25 to 0.38)	0.68
Operation modality		0.27		
Open	Reference		Reference	–
Laparoscopy	−0.03(−0.09 to 0.03)		−0.09(−0.19 to 0.02)	
Operation type		0.05	†	0.12
Segmental	−0.09(−0.18 to 0.002)			
Standard				
Extended				
Colon cancer location				
Right	*Reference*	–		
Left	*−0.07(−0.12 to −0.01)*	*0.02**		
Rectal cancer location			−0.04(−0.10 to 0.02)	0.17
Upper				
Mid				
Lower				
Neoadjuvant RT (rectal)				
No			Reference	–
Yes			−0.06(−0.19 to 0.08)	0.44
Distant metastatic sites				
Liver or other	*Reference*	–	*Reference*	–
Liver and other(s)	*0.33(0.24 to 0.42)*	*< 0.001**	*0.41(0.26 to 0.57)*	*< 0.001**
Distant mets resected				
No	*Reference*	–	*Reference*	–
Yes	*−0.09(−0.16 to −0.02)*	*0.02**	*0.13(−0.25 to −0.02)*	*0.03**

Table 3 (continued)

	Lymph node ratio			
	Colon (*n* = 339)		Rectum (*n* = 125)	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value
Tumour size (median [IQR]), cm	0.003(–0.01 to 0.02)	0.67	0.01(–0.02 to 0.04)	0.40
T stage	*0.16(0.07 to 0.17)*	*< 0.001**	0.09(−0.01 to 0.18)	0.07
T1 and T2				
T3				
T4				
Histological type				
Mucinous/signet ring	Reference	–	*Reference*	–
Non-mucinous/signet ring	−0.05(–0.14 to 0.03)	0.22	*−0.26(–0.45 to –0.07)*	*0.008**

BMI body mass index, *ASA* American Society of Anesthesiology, *RT* radiotherapy *T stage* tumour stage (part of AJCC TNM system); † All stage IV rectal cancers were managed with a standard resection*;* * $P < 0.05$

in multivariable models. To avoid collinearity, LNR considered as continuous and dichotomised variables, were entered into two separate models. $P < 0.05$ was considered significant. All analyses were performed using SPSS® v.29 (IBM, New York, USA).

Results

Study population

A total of 3859 patients underwent primary CRC resection, including 464 with AJCC stage IV disease. Among these, 269 (58.0%) were male and the mean age was 68.1 years (SD 13.4). Of the total, 339 patients (73.1%) had colon cancer. A standard operation was performed in 306 patients with colon cancer and 125 patients with rectal cancer. Specifically, among the patients with colon cancer, there were 122 (39.9%) cases of right hemicolectomy, 4 (1.3%) cases of transverse colectomy, 13 (4.2%) cases of left hemicolectomy, 11 (3.6%) cases of sigmoid colectomy, 50 (16.3%) cases of Hartmann's procedure, and 106 (34.6%) cases of anterior resection. In the rectal cancer group, there were 28 (22.4%) cases of Hartmann's procedure, 70 (56.0%) cases of anterior resection and 27 (21.6%) cases of APER. The study population's characteristics are summarized in Table 1.

Of the 464 stage IV patients, 286 (61.6%) had isolated liver metastases, while 96 (20.7%) had metastases to other organs (lung and/or brain) exclusively. In 75 patients (16.3%), metastatic disease affected both the liver and other organs. A metastasectomy was performed in 83 patients (17.9%). A total of 65 patients (78.3%) had a staged metastasectomy; of these, 50 (76.9%) were performed for synchronous metastatic disease and 15 (23.1%) for metachronous disease.

LNR

Both colon and rectal cancer resection specimens showed a median LN count of 17 nodes (colon range: 2–87, rectal range: 3–49). Most resections yielded 12 or more nodes (Table 2). In colon cancer resections, a median of three positive nodes (range: 0–28) were found, while rectal cancer resections had a median of four involved nodes (range 0–44). This resulted in a median LNR of 0.18 (IQR 0.05–0.42) for colon cancer resections and 0.21 (IQR 0.09–0.47) for rectal cancer resections. Notably, 66 (19.5%) patients with colon cancer and 18 (14.4%) rectal cancer patients had an LNR of 0.

Clinico-pathological characteristics and LNR associations in metastatic colon and patients rectal cancer (Tables 3, 4 and 5)

The associations between standard clinico-pathological factors and LNR are summarised in Table 3 (LNR as a continuous variable), Table 4 and 5 (LNR as a dichotomous variable).

Colon cancer

(1) LNR (continuous): On linear regression, LNR was significantly associated with the year of resection (β −0.02 [95% CI −0.04 to −0.003]; P=0.02), left-sided colon cancers (β −0.07 [95% CI −0.12 to −0.01]; P=0.02), number of metastatic sites (β 0.33[95% CI 0.24–0.42]; P<0.001), distant metastasectomy (β −0.09 [95% CI −0.16 to −0.02]; P=0.02) and depth of tumour invasion (β 0.16 [95% CI 0.07–0.17]; P<0.001)

Table 4 Comparison of clinicopathological factors between patients with LNR of 0 and LNR > 0 in stage IV colon cancer ($n=339$)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	LNR of 0 *n* (%) (Total of 66)	LNR > 0 *n* (%) (Total of 273)	*P* value	Odds ratio (95% CI)
Gender					
Male	191(56.3)	41(62.1)	150(54.9)	–	Reference
Female	148(43.7)	25(37.9)	123(45.1)	0.29	1.35(0.78–2.33)
Age (mean [SD]), years	69.1(13.6)	74.0(11.1)	67.9(13.9)	*0.001**	*0.96(0.94–0.99)*
BMI (mean [SD]), kg/m^2	27.1(5.7)	26.5(4.9)	27.3(5.8)	0.50	1.03(0.95–1.11)
ASA grade				0.12	0.72(0.47–1.09)
I	40(11.8)	7(10.6)	33(12.1)		
II	164(48.4)	26(39.4)	138(50.5)		
III/IV	135(39.8)	33(50.0)	102(37.4)		
Year of surgery				0.47	1.07(0.89–1.28)
1995–1999	61(18.0)	14(21.2)	47(17.2)		
2000–2004	59(17.4)	9(13.6)	50(`8.3)		
2005–2009	68(20.1)	19(28.8)	49(17.9)		
2010–2014	71(20.9)	10(15.2)	61(22.3)		
2015–2019	64(18.9)	10(15.2)	54(19.8)		
2020 onwards	16(4.7)	4(6.1)	12(4.4)		
Emergency operation			219(80.2)	–	Reference
No	265(78.2)	46(69.7)	54(19.8)	0.07	0.57(0.31
Yes	74(21.8)	20(30.3)			–1.04)
Tumour perforation					
No	307(90.6)	58(87.9)	249(91.2)	–	Reference
Yes	31(9.1)	8(12.1)	23(8.8)	0.41	0.70(0.30–1.63)
Obstructive presentation					
No	280(82.6)	52(78.8)	228(83.5)	–	Reference
Yes	59(17.4)	14(21.2)	45(16.5)	0.37	0.73(0.38–1.43)
Operation modality					
Open	229(67.6)	47(71.2)	182(66.7)		Reference
Laparoscopy	110(32.4)	19(28.8)	91(33.3)	0.48	1.24(0.69–2.23)
Operation type	3(0.9)	1(1.5)	2(0.7)	0.31	1.68(0.62–4.55)
Segmental	306(90.	61(92.4)	245(89.7)		
Standard	3)	4(6.1)	26(9.5)		
Extended	30(8.8)				
Colon cancer location					
Right	145(42.8)	22(33.3)	123(45.1)	–	Reference
Left	194(57.2)	44(66.7)	150(54.9)	0.09	0.61(0.35–1.07)
Distant metastatic sites					
Liver or other	279(82.3)	59(89.4)	220(80.5)	–	*Reference*
Liver and other(s)	54(15.9)	5(7.6)	49(17.9)	*0.049**	*2.63(1.002–6.89)*
Distant mets resected					
No	275(81.1)	57(86.4)	218(79.9)		Reference
Yes	58(17.1)	7(10.6)	51(18.7)	0.13	1.91(0.82–4.42)
Tumour size (median [IQR]), cm	5.0(4.0–6.0)	5.5(4.1–7.3)	5.0(4.0–6.0)	*0.02**	*0.87(0.78–0.98)*
T stage				*0.02**	*2.24(1.33–3.77)*
T1 and T2	6(1.8)	1(1.5)	5(1.8)		
T3	173(51.0)	46(69.7)	127(46.5)		
T4	160(47.2)	19(28.8)	141(51.6)		
Histological type				0.86	1.08(0.47–2.47)
Mucinous/sig net ring	39(11.5)	8(12.1)	31(11.4)		

Table 4 (continued)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	LNR of 0 *n* (%) (Total of 66)	LNR > 0 *n* (%) (Total of 273)	*P* value	Odds ratio (95% CI)
Non-mucinous/signet ring	300(88.5)	58(87.9)	242(88.6)		

BMI body mass index, *ASA* American Society of Anesthesiology, *T stage* tumour stage (part of AJCC TNM system); * $P < 0.05$

(2) LNR (dichotomised): On logistic regression, LNR>0 was associated with age (OR 0.96 [95% CI 0.94, 0.99]; *P*=0.001), number of metastatic sites (OR 2.63 [95% CI 1.002, 6.89]; *P*=0.049), tumour size (OR 0.87 [95% CI 0.78, 0.98]; *P*=0.02) and depth of tumour invasion (OR 2.24 [95% CI 1.33, 3.77];*P*=0.002).

Rectal cancer

(1) LNR (continuous): On linear regression, LNR was significantly associated with number of metastatic sites (β 0.41[95% CI 0.26, 0.57]; *P*<0.001), distant metastasectomy (β −0.13[95% CI −0.25, −0.02]; *P*=0.03) and histology type (β −0.26[95% CI -0.45, −0.07]; *P*=0.008).

(2) LNR (dichotomised): On logistic regression, LNR>0 was associated with decreasing rectal tumour height (OR 0.45 [95% CI 0.23, 0.87]; *P*=0.02), neoadjuvant (chemo)radiotherapy (OR 0.23 [95% CI 0.07, 0.73]; *P*=0.01), and depth of tumour invasion (OR 6.55 [95% CI 1.86, 23.09]; *P*=0.003).

Comparison of survival outcomes between clinico-pathological characteristics including LNR in patients with metastatic colon and rectal cancer (Table 6 and Table 7)

The associations between LNR and OS for both colon and rectal cancer patients, are presented in Table 6 and 7. Additionally, Fig. 2 and Fig. 3 present the Kaplan–Meier plots illustrating the OS for these patients.

Colon cancer

Death occurred in 289 colon cancer patients (85.3%), primarily due to CRC, with only 34 patients (11.8%) succumbing to non-cancer causes. The 5 year OS was 10.5% (95% CI 8.7–12.3).

(1) LNR (continuous): on multivariate analysis with LNR as a continuous variable, increasing LNR (*P*=0.02) and T stage (*P* = 0.006) correlated with poorer survival. Conversely, improved OS was associated with later years of the study (*P*<0.001), left-sided tumours (*P* = 0.002) and distant metastasectomy (*P*<0.001).

(2) LNR (dichotomised): when analysed as a categorical variable in a multivariate model, LNR>0 was associated with poorer survival (*P* = 0.02), as was increasing age (*P*<0.001), number of metastatic sites (*P* = 0.03) and increasing depth of tumour invasion (*P*=0.003).

Rectal cancer

In patients with rectal cancer, 109 individuals (87.2%) died, with 103 (94.5%) deaths attributed to rectal cancer and six to other causes. The 5 year OS rate was 11.5% (95% CI 8.4–14.6).

(1) LNR (continuous): In a multivariate model, poorer survival was noted with increasing LNR (*P* < 0.001), while improved survival was observed in those undergoing a distant metastasectomy (*P* < 0.001).

(2) LNR (dichotomised): On multivariate analysis, only LNR > 0 was associated with poorer survival (*P*=0.02).

Discussion

This extensive 27 year cohort study aimed to investigate the prognostic significance of LNR in patients with stage IV CRC who underwent upfront resection of their primary tumour. The relationship between LNR and OS was tested (adjusting for confounders of LNR), treating LNR as both a continuous and dichotomous variable. In both colon and rectal cancer patients, an increasing LNR was found to worsen OS. Moreover, patients with mCRC who exclusively had non-lymphatic routes of dissemination (i.e. LNR of 0 or N0 status) displayed a more favourable prognosis than those with lymphatic spread (i.e. LNR > 0).

The significance of LNR as a prognostic factor in mCRC has been previously investigated but with varying outcome measures and study populations [1, 2, 4, 24–30]. Most studies, similar to ours, have confirmed an association between increasing LNR and poorer prognosis [1, 2, 4, 24–32]. Some, however, included patients with stage I/II CRC in

Table 5 Comparison of clinicopathological factors between patients with LNR = 0 and LNR > 0 in stage IV rectal cancer ($n = 125$)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	LNR = 0 *n* (%) (Total of 18)	LNR > 0 *n* (%) (Total of 107)	*P* value	Odds ratio (95% CI)
Gender					
Male	78(62.4)	9(50.0)	69(64.5)	–	Reference
Female	47(37.6)	9(50.0)	38(35.5)	0.89	0.92(0.30–2.82)
Age (mean [SD]), years	65.4(12.6)	66.8(12.8)	65.1(12.6)	0.61	0.99(0.95–1.03)
BMI (mean [SD]), kg/m^2	27.0(5.0)	28.0(6.8)	26.9(4.7)	0.61	0.96(0.81–1.14)
ASA grade				0.49	0.77(0.36–1.62)
I	28(22.4)	2(11.1)	26(24.3)		
II	68(54.4)	12(66.7)	56(52.3)		
III/IV	29(23.2)	4(22.2)	25(23.4)		
Year of surgery				0.50	1.13(0.79–1.62)
1995–1999	37(29.6)	8(44.4)	26(24.3)		
2000–2004	36(28.8)	4(22.2)	32(29.9)		
2005–2009	26(20.8)	1(5.6)	25(23.4)		
2010–2014	11(8.8)	2(11.1)	9(8.4)		
2015–2019	10(8.0)	2(11.1)	8(7.5)		
2020 onwards	8(6.4)	1(5.6)	7(6.5)		
Emergency operation					
No	122(97.6)	18(100.0)	104(97.2)		
Yes	3(2.4)	–	3(2.8)		
Tumour perforation					
No	106(84.8)	17(94.4)	89(83.N2)	–	Reference
Yes	19(5.2)	1(5.6)	18(16.8)	0.24	3.44(0.43–27.51)
Obstructive presentation					
No	122(97.6)	18(100.0)	104(97.2)	–	Reference
Yes	3(2.4)	–	3(2.8)	1.00	NA
Operation modality					
Open	92(73.6)	13(72.2)	79(73.8)	–	Reference
Laparoscopy	33(26.4)	5(27.8)	28(26.2)	0.89	0.92(0.30–2.82)
Operation type		–	–		
Segmental	–	–	–		
Standard	125(100.0)	18(100.0)	107(100.0)		
Extended	–	–	–	–	
Rectal cancer location				*0.02**	*0.45(0.23–0.87)*
Upper	47(37.6)	3(16.7)	44(41.1)		
Mid	43(34.4)	6(33.3)	37(34.6)		
Lower	35(28.0)	9(50.0)	26(24.3)		
Neoadjuvant RT (rectal)					
No	108(86.4)	12(66.7)	96(89.7)	–	*Reference*
Yes	17(3.6)	6(33.3)	11(10.3)	*0.01**	*0.23(0.07–0.73)*
Distant metastatic sites					
Liver or other	103(82.4)	18(100.0)	85(79.4)		Reference
Liver and other(s)	21(16.8)	–	21(19.6)	NA	NA
Distant mets resected					
No	99(79.2)	12(66.7)	87(81.3)	–	Reference
Yes	25(20.0)	6(33.3)	19(17.8)	0.14	0.44(0.15–1.31)
Tumour size (median [IQR]), cm	5.0(4.0–6.0)	5.3(4.5–7.1)	5.0(4.0–6.0)	0.35	0.88(0.66–1.16)
T stage				*0.03**	*6.55(1.8 6–23.09)*
T1 and T2	7(5.6)	3(16.7)	4(3.7)		

Table 5 (continued)

Variables	*N* (%) or mean (SD) or median (range/ IQR)	LNR = 0 *n* (%) (Total of 18)	LNR > 0 *n* (%) (Total of 107)	*P* value	Odds ratio (95% CI)
T3	89(71.2)	15(83.3)	74(69.2)		
T4	29(23.2)	–	29(27.1)		
Histological type					
Mucinous/signet ring	8(6.4)	–	8(7.5)	–	Reference
Non-mucinous/signet ring	117(93.6)	18(100.0)	99(92.5)	NA	NA

BMI body mass index, *ASA* American Society of Anesthesiology, *RT* radiotherapy, *T stage* tumour stage (part of AJCC TNM system); * $P < 0.05$

their study cohorts, wherein defining an LNR would seem inappropriate (given these patients all have an LNR of 0, by definition). This approach may skew the nodal metastasis and survival outcome data [28, 30]. Additionally, a few studies investigated only colon cancer patients [2, 27], focussed solely on either palliative resection [1] or curative resection [4, 29] and violated principles of collinearity [24, 32]. Notwithstanding this, our study adds to a growing body of literature that promotes the prognostic importance of LNR and its valuable contribution to patient survival following resection of the primary tumour, even in patients with stage IV CRC.

This study uniquely dichotomised patients into those with an LNR of 0, and those with LNR > 0. To date, limited studies have explored survival differences based on this LNR dichotomization [3, 33]. In the study by Zhang et al. [3], LNR was stratified into five groups, including LNR of 0, to validate the discriminatory performance of previously published LNR cut-offs by Rosenberg et al., [33]. However, in both studies, the large number of LNR of 0 patients were confounded by the inclusion of patients with early-stage CRC. Dichotomisation of patients into those with an LNR of 0 and LNR > 0 is more meaningful, as it groups tumours based on their observed routes of spread. Specifically, patients with an LNR of 0 represent those with tumours that have spread exclusively via haematogenous routes and/or transcoelomic spread but not by lymphatic spread. By contrast, tumours with an LNR > 0 are those demonstrating potentially all three routes of dissemination. Intuitively, the absence of LN spread in the presence of haematogenous or transcoelomic spread, as observed in LNR of 0 patients, may identify patients whose tumours have potentially favourable biological behaviour and decreased tumour burden. Indeed, in our study, LNR of 0 served as an independent predictor of improved survival in patients with mCRC.

Notably, the majority of patients in our cohort had at least twelve LNs harvested during surgery. This probably reflects a standardised surgical approach to performing a CRC resection in our unit, irrespective of the presence of distant metastases. This approach is not universally embraced, though, as some surgeons argue that the lymphadenectomy holds little prognostic value for a mCRC resection as the presence of distant metastases overrides prognostication in these circumstances [5, 34]. Nevertheless, a separate study by Jiao et al. demonstrated improved survival in patients with mCRC with at least twelve LNs harvested, highlighting the role of an oncologically adequate lymphadenectomy as an element of good quality mCRC surgery [11]. Building on this, the demonstrable prognostic importance of LNR (specifically LNR of 0 or N0 status) as demonstrated in our study can only be reliably ascertained in the presence of an adequate lymphadenectomy (i.e., LNY of at least twelve nodes).

The finding of a relatively small proportion of patients (16.6%) in our series who underwent an urgent operation warrants discussion. Published literature suggests that patients requiring urgent surgery represent a population with poorer outcomes compared with those with non-urgently resected CRCs [16]. This disparity in outcomes might stem from complex clinical presentations, such as perforation, obstruction or bleeding, and potentially insufficient LNY, which could lead to imprecise staging [16]. However, contrary to the perception that emergency surgery may be responsible for a substandard oncological resection, this study revealed no significant difference between urgent surgery and LNR, irrespective of its assessment as a continuous or dichotomous variable. In our study, the majority of operations were elective. This approach of elective upfront primary tumour surgery for mCRC remains widely recognised and practised today, underlining the generalisability of our findings [2–4, 11, 13, 14, 25].

The optimal extent of resection and lymphadenectomy for patients with mCRC remains controversial. While segmental resection with limited lymphadenectomy may be considered in some cases, our study does not support its routine adoption. As already mentioned, the significant difference in survival observed between those with exclusively

Table 6 Clinicopathological Features and Oncological Associations stage IV (*n* = 464) – LNR CONTINUOUS

	Overall survival							
	Colon				Rectum			
	HR (95% CI)	*P* value	aHR (95% CI)	*P* value	HR (95% CI)	*P* value	aHR (95% CI)	*P* value
Year of surgery	*0.84(0.77–0.91)*	*< 0.001**	*0.84(0.77–0.92)*	*< 0.001**				
1995–1999								
2000–2004								
2005–2009								
2010–2014								
2015–2019								
2020 onwards								
Colon cancer location								
Right	*Reference*		*Reference*	–				
Left	*0.68(0.54–0.86)*	*–0.001**	*0.68(0.54–0.87)*	*0.002**				
Distant metastatic sites								
Liver or other	*Reference*	-	Reference	–	Reference	–	Reference	–
Liver and other(s)	*1.52(1.12–2.06)*	*0.007*	1.24(0.90–1.71)	0.18	1.34(0.81–2.22)	0.26	0.82(0.48–1.40)	0.46
Distant mets resected								
No	*Referencere*	–	*Reference*	–	*Reference*	–	*Reference*	–
Yes	*0.36(0.25–0.50)*	*< 0.001**	*0.43(0.30–0.62)*	*< 0.001**	*0.36(0.22–0.60)*	*< 0.001**	*0.39(0.23–0.65)*	*< 0.001**
T Stage	*1.42(1.14–1.76)*	*0.02**	*1.41(1.11–2.68)*	*0.006**				
T1 and T2								
T3								
T4								
Histological type								
Mucinous/signet ring					*Reference*	–	*Reference*	–
Non-mucinous/signet ring					0.80(0.39–1.64)	0.54	0.95(0.46–1.98)	0.90
LNR (continuous)	*2.72(1.78–4.16)*	*< 0.001**	*1.72(1.11–2.68)*	*0.02**	*3.83(1.98–7.40)*	*< 0.001**	*3.48(1.76–6.88)*	*< 0.001**

HR hazard ratio, *aHR* adjusted hazard ratio, *RT* radiotherapy, *LNR* lymph node ratio; * $P < 0.05$

haematogenous or transcoelemic spread (i.e. LNR of 0 or N0 status) and LNR > 0 (where lymphatic routes are also implicated), emphasizes the importance of conducting an adequate lymphadenectomy to accurately ascertain the nodal status of mCRCs. Achieving this may be more feasible with a standard or extended resection compared with segmental resections. While our study was not primarily focused on evaluating the extent of resection in patients with mCRC, it highlights the benefits of performing a standardised approach to CRC resection whenever possible in all patients, regardless of whether the operation is executed laparoscopically or by an open approach. Furthermore, one could argue that standardising surgical techniques across all stages of CRC, regardless of metastatic status, contributes to a safer surgical approach by eliminating the risk of taking 'shortcuts'.

This study has limitations. The retrospective design introduces inherent biases. The limited rate of distant metastasectomy and the omission of patients who underwent nCT reduces our study population and narrows the generalizability of our findings. Likewise, the use of neoadjuvant (chemo) radiotherapy in patients with rectal cancer may have influenced LNY and nodal positivity rates. Whilst only 13% of patients received neo-adjuvant (chemo) radiotherapy, selection was primarily based on a patient's individual needs in accordance with contemporary practice guidelines at the time. Moreover, while our study primarily focused on patients with mCRC who received upfront primary tumour resection – a recognized treatment strategy [13, 14] – the uptake of nCT and liver-first surgery in managing mCRC may limit this study's generalizability to other treatment paradigms [35]. Finally, the limited availability of tumour biomarkers (e.g. BRAF, NRAS) restricts our analysis and comprehensive understanding of the biological behaviour of the tumour in patients with mCRC.

Table 7 Clinicopathological Features and Oncological Associations stage IV ($n = 464$) – LNR CATEGORISED

	Overall survival							
	Colon				Rectum			
	HR (95% CI)	*P* value	aHR (95% CI)	*P* value	HR (95% CI)	*P* value	aHR (95% CI)	*P* value
Age, years	*1.02(1.01–1.03)*	*< 0.001**	*1.02(1.02–1.03)*	*< 0.001**				
Rectal cancer location					1.00(0.79–1.26)	0.99	1.09(0.84–1.43)	0.52
Upper								
Mid								
Lower								
Neoadjuvant RT (rectal)								
No					*Reference*	–	*Reference*	–
Yes					0.64(0.34–1.20)	−0.17	0.77(0.40–1.49)	−0.43
Distant metastatic sites								
Liver or other	*Reference*	–	*Reference*	–				
Liver and other(s)	*1.52(1.12–2.06)*	*−0.0*	*1.42(1.04–1.94)*	*−0.03*				
Tumour size, cm	1.01(0.96–1.07)	*07**	1.00(0.95–1.06)	0.91				
T stage T1 and T2 T3 T4	*1.42(1.14–1.76)*	*0.002**	*1.41(1.12–1.77)*	*0.003**	1.08(0.73–1.58)	0.71	0.94(0.60–1.48)	0.80
LNR (Categorised)								
LNR > 0	*Reference*	–	*Reference*	–	*Reference*	–	*Reference*	
LNR of 0	*1.44(1.06–1.96)*	*0.02**	*1.50(1.08–2.07)*	*0.02**	*2.21(1.21–4.06)*	*0.01**	*2.21(1.16–4.24)*	*0.02**

HR hazard ratio, *aHR* adjusted hazard ratio, *RT* radiotherapy, *LNR* lymph node ratio; * $P < 0.05$

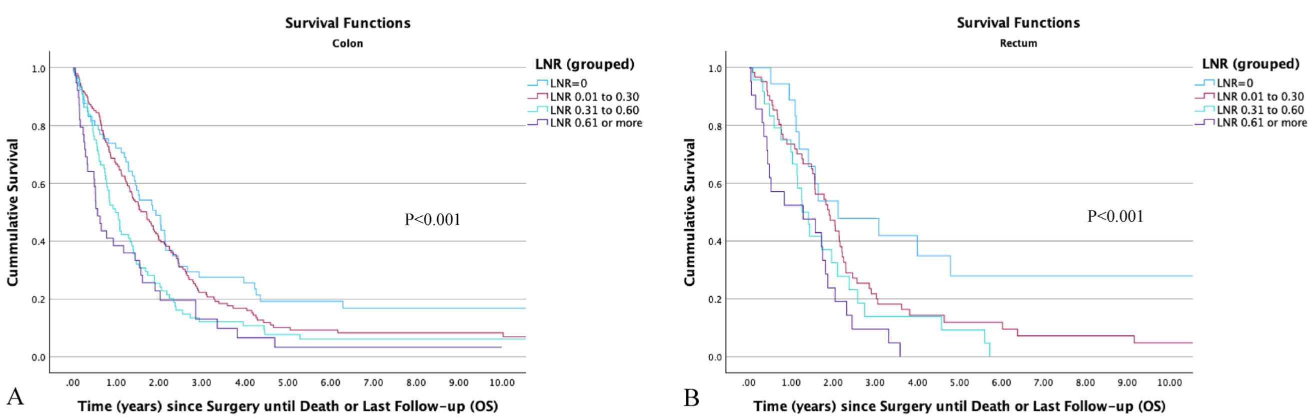

Fig. 2 Kaplan–Meier plots of OS of patients with stage IV colon (**A**) and rectal (**B**) cancer stratified by LNRs

This study enhances our understanding of mCRC management by investigating the prognostic value of the LNR in patients who underwent upfront surgery for the primary tumour. Notably, this study stands out as the only cohort study to date that has specifically examined the survival outcomes using dichotomization of LNR into LNR of 0 and LNR > 0. Our results highlight that this dichotomization holds useful prognostic information, with LNR of 0 (i.e. N0 status) patients identified as a unique subgroup with a better prognosis. Thus, rather than dismissing it as futile, we emphasize the importance of conducting an adequate lymphadenectomy (i.e. harvesting at least 12 LNs) to accurately stage patients with mCRC. Furthermore, identifying LNR of 0 patients as a distinct subgroup calls for further research into their molecular and genetic characteristics, paving the way for refined, individualized treatment approaches and targeted therapies in the management of mCRC.

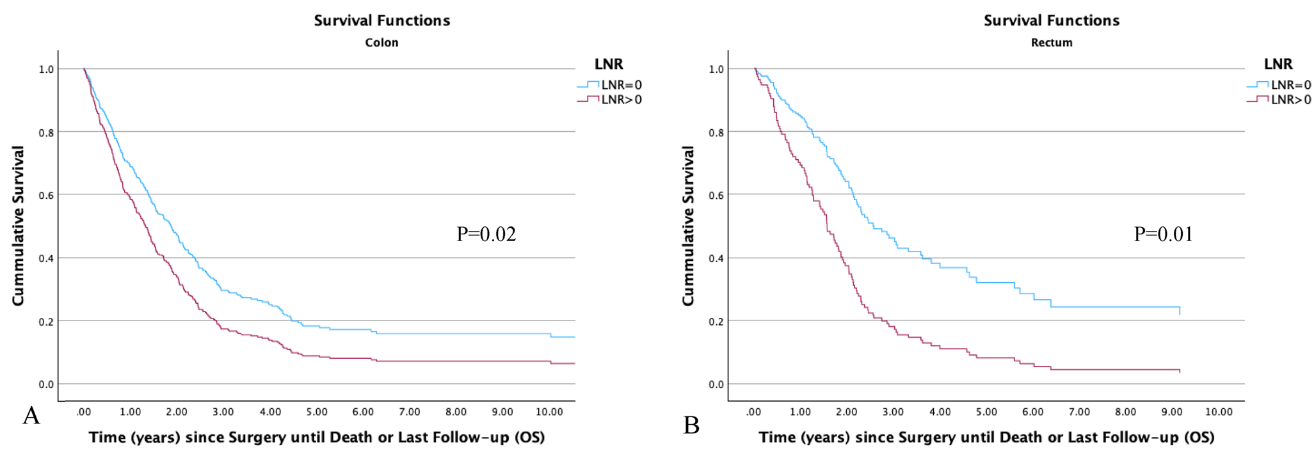

Fig. 3 Kaplan–Meier plots of OS of patients with stage IV colon (**A**) and rectal (**B**) cancer stratified by LNR of 0 and LNR > 0

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Author's contributions All authors contributed to the study conception and design. Material preparation was performed by all authors. Data collection and analysis were performed by K.N. The first draft of the manuscript was written by K.N. All the tables and figures were created by K.N. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. M.R., C.C. and K.-S.N. supervised the undertaking of this research project.

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Data availability The data that support the findings of this study are available from the corresponding author, Dr Kheng-Seong Ng, upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Sydney Local Health District Ethics Committee (2019/ETH07841).

Consent to Participate and/or Consent to Publish Patients consented to having their data collected in a prospective colorectal database and for its use in future research.

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APPENDIX IV

ORIGINAL ARTICLE

Splenic flexure cancer survival: a 25-year experience and implications for complete mesocolic excision (CME) and central vascular ligation (CVL)

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Key words
CME, CVL and splenic flexure cancer.

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Abstract

Background: The management of splenic flexure cancers (SFCs) in the era of complete mesocolic excision (CME) and central vascular ligation (CVL) is challenging because of its variable lymphatic drainage. This study aimed to compare survival outcomes for SFCs and non-SFCs, and better understand the clinicopathological characteristics which may define a distinct SFC phenotype.
Methods: An observational cohort study at Concord Hospital, Sydney was conducted with patients who underwent resection for colon adenocarcinoma (1995–2019). Clinicopathological data were extracted from a prospective database. Overall survival (OS) and disease-free survival (DFS) estimates and their associations to clinicopathological variables were investigated with Kaplan–Meier and Cox regression analyses.
Results: Of 2149 patients with colon cancer, 129 (6%) had an SFC. The overall 5-year OS and DFS rates were 63.6% (95% CI 62.5–64.7) and 59.4% (95% CI 58.3–60.5), respectively. SFCs were not associated with OS ($P = 0.6$) or DFS ($P = 0.5$). SFCs were more likely to present urgently ($P < 0.001$) with obstruction ($P < 0.001$) or perforation ($P = 0.03$), and more likely to require an open operation ($P < 0.001$). These characteristics were associated with poorer survival outcomes. No differences were noted between SFCs and non-SFCs with respect to tumour stage ($P = 0.3$).
Conclusion: SFCs have a distinct phenotype, the individual characteristics of which are associated with poorer survival. However, the survivals of SFCs and non-SFCs are similar, possibly because the most important determinant of outcome, tumour stage, is no different between the groups. This may have implications for the surgical approach to SFCs with respect to standardization of CME and CVL surgery for these cancers.

Introduction

In the era of complete mesocolic excision (CME) and central vascular ligation (CVL), dissection along bloodless embryonic tissue planes with a 'high' vascular tie (HVT) theoretically ensures removal of a pre-defined lympho-neuro-vascular package enveloped between intact parietal peritoneum. This approach has been demonstrated to result in superior specimen quality[1,2] and convey a survival advantage in several cohort studies.[1,3–6] However, it is difficult to apply the principles of CME to areas of watershed blood supply such as the splenic flexure (SF) owing to the lack of clarity as to whether SF lymphatic drainage preferences the middle colic artery (MCA) or the inferior mesenteric artery (IMA) nodal basins, or both.[7–9]

From early cadaveric studies to more recent *in-vivo* non-physiological experimentation, metastatic routes stretching from the paracolic nodes to the superior mesenteric artery, the MCA, the accessory MCA, the IMA and left colic artery (LCA) nodal basins have been described.[7,9–14] These random anatomical incongruences, together with the lack of dedicated CME and CVL

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guidelines for splenic flexure cancers (SFC)[4] have invariably led to different surgical approaches, and crucially, a failure to target a dominant lymphovascular drainage pedicle.[4] Intuitively, it could be argued that SFCs, unlike other colonic cancer (CC) subsites are at risk of a substandard and potentially non-curative resection.

We hypothesize that SFCs are associated with poorer survival outcomes compared to other CC subsites. Therefore, the primary aim of this study was to analyse a single centre's 25-year experience with colectomy for CC, comparing long-term survival outcomes of SFCs and non-SFCs. A subsidiary aim was to explore whether clinicopathological characteristics and macroscopic features might indicate a unique phenotype of SFCs which would account for any differences in their long-term survival outcomes compared to other subsites.

Materials and methods

Consecutive patients who underwent a potentially curative colectomy for a solitary CC between January 1995 and December 2019 at Concord Hospital, Sydney, Australia, were included in this observational retrospective cohort study. Patients were identified from a prospectively maintained institutional database, where clinical, operative details, tumour pathology, and follow-up data were extracted for analysis, comparing SFC and non-SFC resections.[15] Patients excluded were those with a rectal cancer, *in-situ* neoplasia, polyposis coli, a previous CC, and those who developed a metachronous cancer (Fig. 1).

Ethical approval was granted by the Sydney Local Health District Ethics Committee (CH62/62011-136-P Chapuis HREC/11/CRGH206).

Pathology reporting and staging

The precise tumour location was marked by the surgeon on a diagram at the completion of the operation, with SFCs defined as tumours located between the distal 10 cm of the transverse colon to the proximal 10 cm of the descending colon.[8] All resected specimens were examined by pathologists with a special interest in colorectal cancer (CRC) using a standard synoptic protocol, and all pathology data were coded by one of us (ChC).[16] Only adenocarcinomas (including mucinous and signet ring types) were included in the analyses. Tumour morphology was defined as pedunculated, where a tumour was attached to the colonic mucosa by a stalk or pedicle, regardless of base size; sessile, where a tumour was flat and lacked a pedicle; ulcerated, where a tumour had a crater-like appearance; and stenosing, where a tumour had an occluding and narrowed lumen. These morphological characteristics were not mutually exclusive. Tumours were staged according to the American Joint Committee on Cancer (AJCC) pTNM system.[17] All pathology variables included in the data set were recorded for every specimen and their presence or absence noted explicitly.

Surgical management of SFCs

At our institution, SFC patients were managed with either an extended right hemicolectomy (ERH), a left hemicolectomy (LH), or a segmental resection (SgR). An ERH was defined as resection of the distal terminal ileum through to the proximal descending colon with ligation of the ileocolic artery, right colic artery (if present), MCA and LCA. In a LH, the distal third of the transverse colon, SF and proximal descending colon is resected with ligation of the left branch of the MCA and LCA. In the SgR technique, the cancer is resected with 5 cm of colon either side of it, with no dedicated pedicle ligation. All resections were performed by colorectal surgeons with CVL not routinely practised.[18] An urgent operation was defined as an unscheduled operation.

Surveillance and follow-up

Patients were reviewed at least six-monthly for the first 2 years after resection and followed up yearly thereafter until death or December 2021, unless lost to follow-up.[19] Surveillance included clinical examination, liver function tests, serial carcinoembryonic antigen measurements and, more recently, selective computed tomography and positron emission tomography imaging. Colonoscopy was generally performed at 1 year and repeated between 3 and 5 years after resection. Patients with lymph node positive tumours and associated poor prognostic features such as age, comorbidities, post-operative complications, and adverse prognostic features (e.g., venous or perineural invasion) were routinely discussed at a multi-disciplinary meeting, generally with a view for offering adjuvant chemotherapy.

The date of resection was the starting point for follow-up times. Follow-up times were censored at last contact for patients who did not experience the terminal event up to December 2021, who were lost to follow-up, or who remained alive. The underlying cause of death was coded according to the International Classification of Diseases-10. All clinical and operative data were recorded by one of us (PC) in addition to information provided by the GP, operating surgeon, and close family members.

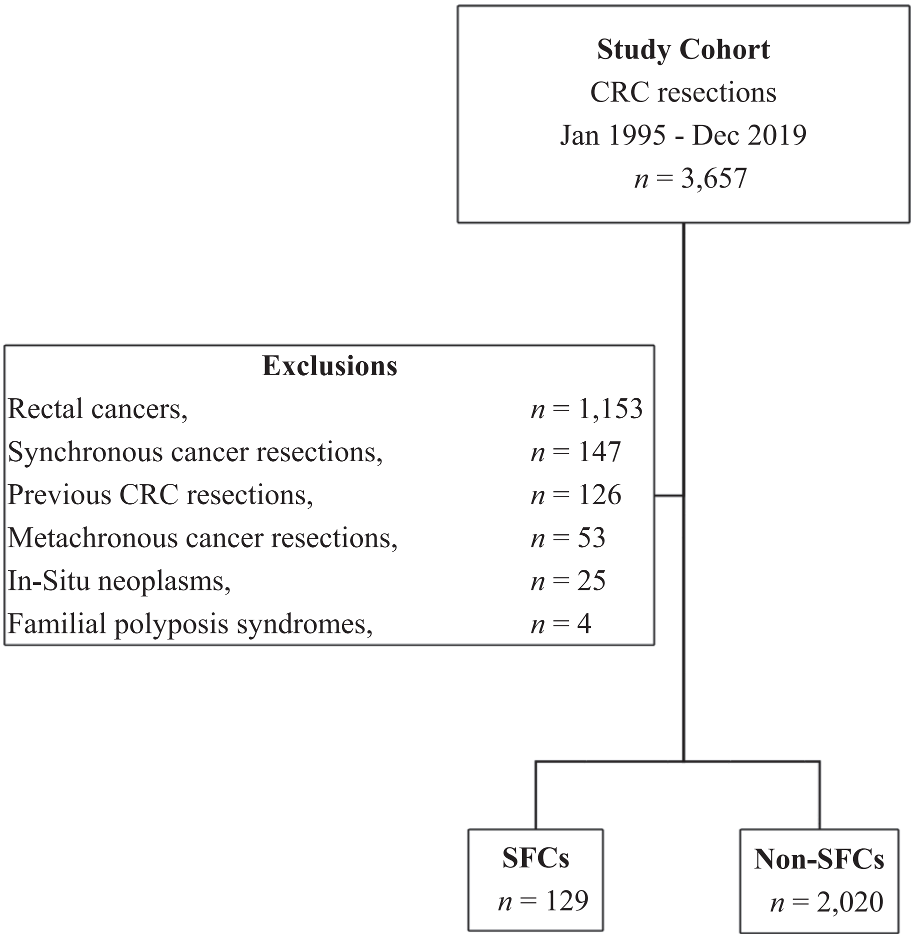
Outcome measures

The primary outcome measures were overall survival (OS) and disease-free survival (DFS).[8,20] Secondary outcome measures included locoregional recurrence (LR) and systemic recurrence (SR), where recurrence was defined as clinically or radiologically suspected or biopsy-proven tumour in the peritoneal cavity, or newly diagnosed distant metastasis.[20]

Statistical analyses

Continuous variables were reported as mean (standard deviation [SD]) for normally distributed variables and as median (inter-quartile range [IQR] or range [minimum to maximum values]) for non-normal distributions. Categorical variables were reported as frequencies and percentages. Logistic regression analyses were performed to determine any differences in continuous or categorical data between the SFC and non-SFC cohorts. Survival and recurrence estimates were modelled using the Kaplan–Meier function with log-rank test performed to determine difference in survival distributions. Cox-regression modelling tested associations between outcome measures and relevant clinicopathological variables. The

Fig. 1. Flow diagram of cohort definition.



level for 2-tailed statistical significance was $P < 0.05$ with confidence intervals at the 95% level. All analyses were completed using SPSS v.28 (IBM, New York, USA).

Results

Study population

A total of 3657 patients underwent a resection for a primary CRC during the study period of which 1508 patients were sequentially excluded (Fig. 1), leaving 2149 suitable patients for analysis. Of these, 129 patients (6%) had a SFC, with 45 (34.9%), 64 (49.6%), 20 (15.5%) patients operated on in the first decade, second decade and last 5 years of the study period, respectively. The features of all these patients are summarized in Table 1.

Amongst the SFC patients, 67 (51.9%) were male, their mean age was 69.4 years (SD 14.7), and the median hospital length of stay (LOS) was 10 days (range 8–15). An ERH was performed in 59 patients (45.7%); this was regardless of the mode of presentation. However, in 101 SFC patients operated electively, 45 patients (53.6%) had a LH performed, 35 patients (41.7%) had an ERH, and four (4.7%) had a SgR. When grouped according to exact tumour location, 66 patients (51.2%) had a cancer located at the 'true' splenic flexure (i.e., corresponding to the colonic angulation relating to the splenocolic ligament), 41 (31.8%) had a cancer at the distal transverse colon and 22 (17.0%) had a cancer situated at the proximal descending colon. Of the 66 patients with a 'true' SFC, 28 (42.4%) underwent an ERH, and 26 (39.4%) had a LH performed. Of the 41 patients with a distal transverse colon cancer, 27 (65.9%) underwent an ERH, and 9 (22.0%) underwent a LH. Of the 22 patients with a proximal descending colon cancer, 4 (18.2%) underwent an ERH, and 11 (50.0%) had a LH. An urgent operation was performed in 28 SFC patients (21.7%). Of those operated urgently, 23 patients (82.1%) were obstructed. An open operation was performed in 99 patients (76.7%).

Detailed clinical characteristics, the predominant macroscopic tumour morphology, and histopathological descriptions of SFCs and non-SFCs and comparisons of clinicopathological factors between SFC and other subsites are shown in Table 1. The comparison of survival outcomes between clinicopathological characteristics of all cases are detailed in Table 2.

Follow-up and survival outcomes

For the entire study population (SFCs and non-SFCs), death occurred in 1195 patients (55.6%). Of the 954 surviving patients, the median follow-up time was 10 years. The median OS and DFS was 9.4 years (95% CI 8.6–10.3) and 8.4 years (95% CI 7.4–9.3), respectively. The 5-year OS and DFS rates were 63.6% (95% CI 62.5–64.7) and 59.4% (95% CI 58.3–60.5), respectively. A LR was

Table 1 Comparison of clinicopathological factors between patients with SFC and non-SFC

Variables	*N* (%) or Mean (SD) or Median (Range/ IQR)	Non-SFC *n* (%) (Total = 2020)	SFC *n* (%) (Total = 129)	*P*-value	Odds ratio (95% CI)
Gender					
Male	1150 (53.5)	1083 (53.6)	67 (51.9)	–	(reference)
Female	999 (46.5)	937 (46.4)	62 (48.1)	0.7	1.1 (0.7–1.5)
Age (Mean [SD]), years	70.5 (12.4)	70.6 (12.3)	69.4 (14.7)	0.3	0.99 (0.98–1.00)
ASA grade					
I	351 (15.3)	311 (15.4)	24 (18.6)	–	(reference)
II	1200 (52.3)	1057 (52.3)	61 (47.3)	0.2	0.7 (0.5–1.2)
III/IV	745 (32.4)	652 (32.3)	44 (34.1)	0.6	0.9 (0.5–1.5)
Emergency operation					
No	1939(90.2)	1838(91.0)	101(78.3)	–	(reference)
Yes	210(9.8)	182(9.0)	28(21.7)	<0.001	2.8 (1.8–4.4)
Emergency operation (reason)					
Non-emergency	1.939 (90.2)	1838 (91.0)	101 (78.3)	–	(reference)
Bleeding	6 (0.3)	6 (0.3)	–	1.0	NA
Obstruction	165 (7.7)	142 (7.0)	23 (17.8)	<0.001	2.9 (1.8–4.8)
Perforation	35 (1.6)	30 (1.5)	5 (3.9)	0.03	3.0 (1.2–8.0)
Other	4 (0.2)	4 (0.2)	–	1.0	NA
Operation modality					
Laparoscopy	871 (40.5)	841 (41.6)	30 (23.3)	–	(reference)
Open	1278 (59.5)	1179 (58.4)	99 (76.7)	<0.001	2.3 (1.6–3.6)
Procedure conversion					
No	738 (90.0)	713 (90.3)	25 (83.3)	–	(reference)
Yes	82 (10.0)	77 (9.7)	5 (16.7)	0.2	1.9 (0.7–5.0)
Splenic injury					
No	2125 (98.9)	2003 (99.2)	122 (94.6)	–	(reference)
Yes	24 (1.1)	17 (0.8)	7 (5.4)	<0.001	6.8 (2.8–16.6)
Splenectomy					
No	2138(99.5)	2013(99.7)	125 (96.9)	–	(reference)
Yes	11 (0.5)	7 (0.3)	4 (3.1)*	<0.001	9.2 (2.7–31.9)
Anastomotic leak					
No	1960 (98.2)	1844 (98.3)	116 (95.9)	–	(reference)
Yes	36 (1.8)	31 (1.7)	5 (4.1)	0.06	2.56 (0.98–6.72)
Blood loss (mL)					
<500	2063 (96.0)	1949(94.5)	114 (88.4)	–	(reference)
>500	86 (4.0)	71(3.5)	15 (11.6)	<0.001	3.6 (2.0–6.5)
Tumour stage (TNM AJCC)					
Stage 1/2	1219 (56.7)	1142 (56.5)	77 (59.7)	–	(reference)
Stage 3	603 (28.1)	572 (28.3)	31 (24.0)	0.3	0.8 (0.5–1.2)
Stage 4	327 (15.2)	306 (15.1)	21 (16.3)	0.9	1.1 (0.6–1.7)
Tumour Size (Mean [SD]), cm	4.8 (2.4)	4.8 (2.4)	4.7 (2.5)	1.0	1.0 (0.9–1.1)
Macroscopic characteristic					
Pedunculated					
No	1657 (77.1)	1544 (93.2)	113 (87.6)	–	(reference)
Yes	492 (22.9)	476 (23.6)	16 (12.4)	0.004	0.5 (0.3–0.8)
Sessile					
No	1715 (79.8)	1625 (80.4)	90 (69.8)	–	(reference)
Yes	434 (20.2)	395 (19.6)	39 (30.2)	0.004	1.8 (1.2–2.6)
Stenosing					
No	1820 (84.7)	1728 (85.5)	92 (71.3)	–	(reference)
Yes	329 (15.3)	292 (14.5)	37 (28.7)	<0.001	2.4 (1.6–3.6)
Ulcerating					
No	1249 (58.1)	1173 (58.1)	76 (58.9)	–	(reference)
Yes	900 (41.9)	847 (41.9)	53 (41.1)	0.9	0.9 (0.7–1.4)
Tumour perforation					
No	2054 (95.6)	1930 (95.5)	124 (96.1)	–	(reference)
Yes	95 (4.4)	90 (4.5)	5 (3.9)	0.8	0.9 (0.3–2.2)
Histological type					
Non-mucinous/signet ring	1902 (88.5)	1790 (88.6)	112 (86.8)	–	(reference)
Mucinous	225 (10.5)	209 (10.3)	16 (12.4)	0.5	1.2 (0.7–2.1)
Signet ring	22 (1.0)	21 (1.1)	1 (0.8)	0.8	0.8 (0.1–5.7)
Histological differentiation					
Well or moderate	1764 (82.1)	1657 (82.0)	107 (82.9)	–	(reference)
Poor	385 (17.9)	363 (18.0)	22 (17.1)	0.8	0.9 (0.6–1.5)
Histological grade					
Low/Average	1715 (79.8)	1612 (79.8)	103 (79.8)	–	(reference)
High	434 (20.2)	408 (20.2)	26 (20.2)	1.0	1.0 (0.6–1.6)
Lympho-vascular invasion					
No	1511 (70.3)	1423 (70.4)	88 (68.2)	–	(reference)
Yes	638 (29.7)	597 (29.6)	41 (31.8)	0.6	1.1 (0.8–1.6)

Table 1 Continued

Variables	N (%) or Mean (SD) or Median (Range/ IQR)	Non-SFC n (%) (Total = 2020)	SFC n (%) (Total = 129)	P-value	Odds ratio (95% CI)
Peri-neural invasion					
No	1785 (83.1)	1680 (83.2)	105 (81.4)	–	(reference)
Yes	364 (16.9)	340 (16.8)	24 (18.6)	0.6	1.1 (0.7–1.8)
Lymph nodes—examined (median [range])	17 (0–119)	17 (0–119)	18 (3–79)	<0.001	1.02 (1.01–1.04)
Lymph node harvest (<12)					
No	1692 (78.7)	1598 (79.1)	94 (72.9)	–	(reference)
Yes	457 (21.3)	422 (20.9)	35 (27.1)	0.1	1.4 (0.9–2.1)
LOS (median [range]), days	9.0 (7.0–12.0)	9.0 (7.0–12.0)	10.0 (8.0–15.0)	0.003	1.02 (1.01–1.04)

Abbreviations: AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiology; LOS, length of stay; SFC, splenic flexure cancers; TNM, 8th edition tumour, nodes, and metastasis staging system.

*All four splenectomies were performed for non-oncological reasons (i.e., splenic capsular tear or haemorrhage).

diagnosed in 69 patients (3.2%), and SR was diagnosed in 361 patients (16.8%). The median time to LR and SR of the study population was 1.2 years (95% CI 0.9–1.5) and 1.5 years (95% CI 1.2–1.7), respectively.

Comparison of survival outcomes between SFCs and non-SFCs (Table 2)

The median OS and DFS of patients with SFC were 9.9 years (95% CI 6.1–13.7) and 9.5 years (95% CI 5.5–13.5), respectively. The 5-year OS and DFS rates of patients with SFC were 65.1% (95% CI 60.8–69.4) and 61.4% (95% CI 57.1–65.7), respectively. SFCs were not associated with OS (P = 0.6), DFS ($P = 0.5$), LR ($P = 0.9$) or SR ($P = 0.5$). These findings persisted with adjustment for age at surgery, with no association seen with OS [aHR = 1.0 (95% CI 0.8–1.3), $P = 1.0$] or DFS [aHR = 1.0 (95% CI 0.8–1.2), $P = 0.8$]. Further, on a subgroup analysis of patients undergoing elective surgery, SFCs were not associated with OS ($P = 0.2$) or DFS ($P = 0.2$).

With respect to clinicopathological characteristics within the entire cohort, a poorer OS was associated with being male ($P = 0.01$), increasing age ($P < 0.001$), ASA >1 ($P < 0.001$), having an urgent resection ($P < 0.001$) performed for either obstruction ($P < 0.001$) or perforation ($P < 0.001$), having an open surgery ($P < 0.001$), and an intraoperative blood loss of more than 500 mL ($P = 0.01$). For pathological features, a poorer OS was associated with AJCC stage III ($P < 0.001$) and IV ($P < 0.001$) tumours, and those that were predominantly stenosed ($P < 0.001$) or ulcerated ($P < 0.001$). A lymphadenectomy with a lymph node harvest of less than 12 nodes ($P = 0.002$) and an increased LOS were also associated with poorer OS ($P < 0.001$). OS was longer in the presence of a pedunculated lesion ($P < 0.001$). Poor prognosis characterized by increased DFS hazards mirrored all the factors associated with OS above and are displayed in Table 2.

Clinicopathological variables and SFC associations

Table 1 summarizes the associations between SFCs and other clinicopathological variables. When compared with non-SFCs, SFCs were associated with an urgent operation ($P < 0.001$), and an obstructed ($P < 0.001$) or a perforated ($P = 0.03$) presentation. SFCs were more likely to be managed by an open procedure ($P < 0.001$) and when begun laparoscopically, SFCs showed no difference in rates of requiring an open conversion ($P = 0.2$). Surgery for SFCs were more likely to be associated with a splenic injury ($P < 0.001$) or requiring a splenectomy ($P < 0.001$).

No differences were noted between SFCs and non-SFCs with regards to AJCC tumour stage (Stage III [$P = 0.3$] and Stage IV [$P = 0.9$]) or tumour size ($P = 1.0$). Macroscopically, SFC patients were more likely to harbour a stenosing tumour ($P < 0.001$). SFC tumours also favoured a sessile morphology ($P = 0.004$). The median lymph node harvest for SFC resections was (18 nodes [range 3–79]; $P < 0.001$). The lymph node harvest (median [range]) for each specific tumour subsite are as follows: 'true' splenic flexure—17 (3–79); distal transverse colon—20 (6–79); proximal descending colon—14 (6–35). The median length of hospitalization for SFC patients was longer than for non-SFC patients (10 days [8–15] versus 9 days [7–12]; $P = 0.003$). There was no difference between SFCs and non-SFCs with regards to gender, age, ASA grade, anastomotic leak rates, tumour perforation status, histological type, degree of tumour differentiation, overall tumour grade and the presence of lymphovascular or perineural invasion.

Discussion and conclusion

This large cohort study of SFC resections, over a long historical period (25 years), highlights key findings that may re-define the approach to SFC management. It appears that SFCs may behave differently and have distinct macroscopic characteristics, some of which are individually associated with poorer survival outcomes when applied to the entire study population. Despite description of such a distinct phenotype, survival outcomes between SFCs and non-SFCs are similar, and tumour stage remains a critical determinant of oncological outcome. The question of which surgery should be performed for SFCs is difficult to answer from the results of this study alone, but the similar propensity of SFCs to metastasise when compared with non-SFCs (reflected by their similar tumour stages at presentation) suggests standardized oncological principles with respect to adequate lymphadenectomy (as is recognized for non-SFC resections) should still apply, despite challenges in performing an appropriate lymphadenectomy for this watershed colonic region.

Table 2 Comparison of survival outcomes between clinicopathological factors within the entire cohort

	OS		DFS	
	P-value	Hazards ratio (95% CI)	*P*-value	Hazards ratio (95% CI)
Splenic flexure cancer				
No	–	(reference)	–	(reference)
Yes	0.6	0.9 (0.7–1.2)	0.5	0.9 (0.7–1.2)
Gender				
Male	–	(reference)	–	(reference)
Female	0.01	0.86 (0.77–0.97)	0.01	0.87 (0.78–0.97)
Age(years)	<0.001	1.05 (1.04–1.05)	<0.001	1.04 (1.03–1.05)
ASA Grade				
I	–	(reference)	–	(reference)
II	<0.001	1.8 (1.5–2.2)	<0.001	1.8 (1.5–2.2)
III/IV	<0.001	4.3 (3.6–5.3)	<0.001	4.1 (3.3–4.9)
Emergency operation				
No	–	(reference)	–	(reference)
Yes	<0.001	2.3 (1.9–2.7)	<0.001	2.3 (2.0–2.7)
Emergency operation (reason)				
Non-emergency	–	(reference)	–	(reference)
Bleeding	0.9	1.0 (0.2–4.0)	0.6	1.4 (0.4–4.2)
Obstruction	<0.001	2.3 (1.9–2.8)	<0.001	2.3 (1.9–2.8)
Perforation	<0.001	2.4 (1.6–3.6)	<0.001	2.5 (1.7–3.8)
Operation modality				
Laparoscopy	–	(reference)	–	(reference)
Open	<0.001	1.3 (1.2–1.5)	<0.001	1.3 (1.1–1.4)
Splenic injury				
No	–	(reference)	–	(reference)
Yes	0.6	1.1 (0.7–1.8)	0.8	1.1 (0.7–1.7)
Splenectomy				
No	–	(reference)	–	(reference)
Yes	0.1	1.7 (0.9–3.3)	0.1	1.8 (0.9–3.4)
Blood loss (mL)				
<500	–	(reference)	–	(reference)
>500	0.01	1.4 (1.1–1.8)	0.01	1.4 (1.1–1.8)
Tumour stage (TNM AJCC)				
Stage 1/2	–	(reference)	–	(reference)
Stage 3	<0.001	1.5 (1.3–1.7)	<0.001	1.6 (1.4–1.8)
Stage 4	<0.001	7.2 (6.2–8.4)	<0.001	7.1 (6.1–8.3)
Macroscopic characteristic				
Pedunculated				
No	–	(reference)	–	(reference)
Yes	<0.001	0.7 (0.6–0.8)	<0.001	0.7 (0.6–0.8)
Sessile				
No	–	(reference)	–	(reference)
Yes	0.3	0.9 (0.8–1.1)	0.2	0.9 (0.8–1.1)
Stenosing				
No	–	(reference)	–	(reference)
Yes	<0.001	1.4 (1.2–1.6)	<0.001	1.5 (1.3–1.7)
Ulcerating				
No	–	(reference)	–	(reference)
Yes	<0.001	1.3 (1.1–1.4)	<0.001	1.3 (1.1–1.4)
Lymph nodes—examined	<0.001	0.989 (0.983–0.995)	0.002	0.991 (0.985–0.997)
Lymph node harvest (<12)				
No	–	(reference)	–	(reference)
Yes	0.002	1.2 (1.1–1.4)	0.007	1.2 (1.0–1.4)
LOS (days)	<0.001	1.026 (1.022–1.031)	<0.001	1.02 (1.01–1.03)

Abbreviations: AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiology; LOS, Length of Stay; SFC, splenic flexure cancers; TNM, 8th edition tumour, nodes, and metastasis staging system.

To the best of our knowledge, this study is the first to characterize a distinct phenotype of SFCs using a constellation of macroscopic tumour characteristics and clinicopathological features. In our study, SFCs were more likely to present urgently with obstruction (due to their stenosing nature) or perforation and more likely to require an open operation. These factors are associated with poorer survival outcomes. SFCs were also less likely to have a pedunculated morphology which was associated with better survival outcomes. Despite this over-representation of adverse prognostic features amongst SFCs, no differences in survival outcomes were observed between SFCs and non-SFCs. This is likely because the most important determinant of oncological outcomes viz. their propensity to metastasise (reflected by tumour stage) were similar between the two groups; correspondingly, no difference in their OS and DFS were noted.

Studies principally comparing clinicopathological factors between SFCs and non-SFCs with a view to understanding their survival association are limited. As such, no studies have previously reported macroscopic features of SFCs and their survival associations.[8,11,21–32] Furthermore, interpretation of previous studies investigating SFC outcomes are confounded by inconsistencies in the definition of the SF[21,24,25,27,29]; reporting only short-term non-oncological outcomes[31]; failure to distinguish elective and emergency SFC surgeries[21,23]; comparing the survival outcomes of combined colonic flexure cancers to other colonic sub-sites[26]; considering patients who underwent a conversion from minimally-invasive to an open operation to be classified as part of the minimally-invasive cohort[32] and excluding AJCC Stage IV cancers.[11,28,30,32] Indeed, the overwhelming majority of studies on SFCs have focussed on describing feasibility of specific surgical approaches[11,28,30,31,33] and a comparison of the operation modality (i.e., minimally-invasive versus open)[32] rather than understanding the relationship between their tumour characteristics and survival outcomes when compared with non-SFCs.

Our finding of similar survival outcomes between SFCs and non-SFCs is supported by several studies in the literature.[22,23,25,27] However, reported 5-year OS rates range widely between 28% and 84%.[27,34] Two studies have reported poorer survival outcomes between SFCs and non-SFCs.[21,35] Specifically, Aldridge *et al*. noted a higher rate of LR (4%) and lower 5-year OS rate (50%)[21]; this may be a consequence of the increased rates of obstruction (49%) and perforation (10%) reported in that study compared with ours. Lykke *et al*. reported that SFCs (5-year OS rate of 56.6%) were associated with poorer prognosis compared to sigmoid cancers, but did not define the limits of the SF and limited their analysis to AJCC Stage I–III CC OS (i.e., recurrence data had not been registered).[35] They speculated that a poorer prognosis was likely due to operational difficulties with central ligation of the branches of the MCA. That said, heterogeneity of SF blood supply with shared lymphatic drainage patterns between IMA and MCA pedicles suggests that central ligation of the IMA should receive an equivalent focus. Indeed, a recent study using lymphatic scintigraphy mapping in patients with non-pathological SFs demonstrated preferential lymphatic drainage to LCA lymph nodes in the vast majority of cases.[9] Thus, failure to recognize these disparate drainage routes may contribute to performing a non-radical potentially curative resection.

Despite these inconsistencies, tumour stage of a CC at presentation consistently remains the most important indicator of survival outcome following surgery.[8,17,36] This is reaffirmed in our study with poorer OS and DFS noted in those with AJCC stage III and IV disease. Importantly, there was no difference between SFCs and non-SFCs in AJCC stage at presentation. Only one other study could support this but failed to surmise the reason.[22] Nonetheless, our observation that tumour stage at time of diagnosis was similar between SFCs and non-SFCs is relevant to the explanation of equivalent oncological outcomes between the two groups.

Our study was not intended to establish the appropriate operation for SFCs (i.e., ERH, SgR, or LH). While it would be intuitive that operative approach could be guided by the precise location of the cancer (i.e., distal transverse, 'true' splenic flexure, or proximal descending colon), our available data was insufficient to suitably answer this. This is because separate survival analyses for each operative approach sub-analysed according to tumour location would need to be performed, and the numbers for these subgroup analyses would be too small for any meaningful interpretation. Further, it is difficult to predict the oncological outcome of any of these operations as none automatically translate to having a HVT. Nevertheless, even when limiting consideration to elective SFC resections, we confirm variation in practice within one surgical unit, with the choice of procedure likely contingent on the surgeon's experience and preference. That said, given their similar propensity to metastasise (reflected by the similar tumour stages at diagnosis), any push towards a limited resection and lymphadenectomy for SFCs[31,33,37–39] is not supported by the results of this study, especially considering the increasing move towards CME and CVL as standard practice for other CCs. Further, in recognition of technical challenges to applying CME and CVL principles to the watershed area of the SF, one key to better understanding the surgical approach and in turn standardizing a SFC resection may lie in *in vivo* techniques designed to map SF lymphatics to better anatomically characterize a dominant lymphatic pedicle.[9,12] Such a tailored approach, applied to individual patients (in recognition of the heterogenous and disparate drainage patterns between different patients), may facilitate a targeted lymphadenectomy that conforms to CME and CVL principles without incurring excessive colonic devascularisation that may ultimately prove oncologically unnecessary.

This study was limited by the retrospective nature of analysis and the impact of bias thereof. Comparison of SFCs and non-SFCs is confounded by the relatively small number of patients in the SFC group, but this is a recognized biological phenomenon. Any desire to perform a randomized-controlled trial comparing CME and CVL for SFC would be challenging owing to the low incidence of this disease. However, the large cohort size; long study duration; application of standardized surgery by specialist colorectal surgeons following anatomical planes; detailed pathology reporting; and a minimum loss of patient follow-up, are strengths of our study. Also, this study emphasizes the importance of a standardized SF definition as inconsistencies in previous literature with regards to its distal limit and together with the description of SFC location has led to controversy.[21,23,40]

This study is the first to report a distinct phenotype of SFCs, the individual characteristics of which are associated with poorer outcomes. While the over-representation of these adverse clinicopathological features would intuitively suggest poorer survival amongst SFC patients, this was not observed in our study. The similar survival outcomes between SFCs and non-SFCs is likely because the most critical determinant of oncological outcome viz. tumour stage was similar between the two groups. Recognizing that the propensity of SFCs to metastasise is no different to non-SFCs, oncological principles to their resection should not differ, and contemporary practices of CME and CVL to obtain adequate lymphadenectomy should be equivalently considered for SFCs, just as in non-SFCs. Applying these principles to a watershed colonic region such as the SF, though, remains challenging, and future research should be directed at better understanding the heterogenous lymphatic drainage of the SF and identifying a dominant lymphatic pedicle to facilitate a targeted lymphadenectomy in individual patients.

Author contributions

Krishanth Naidu: Conceptualization; data curation; formal analysis; investigation; methodology; software; writing – original draft.

Pierre Chapuis: Data curation; project administration; resources; supervision; writing – review and editing. **Kilian Brown:** Data curation; resources; writing – review and editing. **Charles Chan:** Data curation; resources; writing – review and editing. **Matthew Rickard:** Resources; supervision; writing – review and editing. **Kheng-Seong Ng:** Conceptualization; project administration; resources; supervision; writing – review and editing.

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Conflict of interest statement

None declared.

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CHAPTER X: REFERENCES

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