Naidu *et al.*
World Journal of Surgical Oncology (2024) 22:216
https://doi.org/10.1186/s12957-024-03496-1

World Journal of Surgical Oncology

RESEARCH

Open Access

Tissue morphometric measurements do not predict survival following colorectal cancer surgery

Krishanth Naidu[1,2,3], Pierre H. Chapuis[1,2,3], Charles Chan[3,4], Matthew J.F.X Rickard[1,2,3], Nicholas P. West[5], David G. Jayne[6,7] and Kheng-Seong Ng[1,2,3*]

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

*Correspondence:
Kheng-Seong Ng
k.s.ng@sydney.edu.au
Full list of author information is available at the end of the article

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 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.

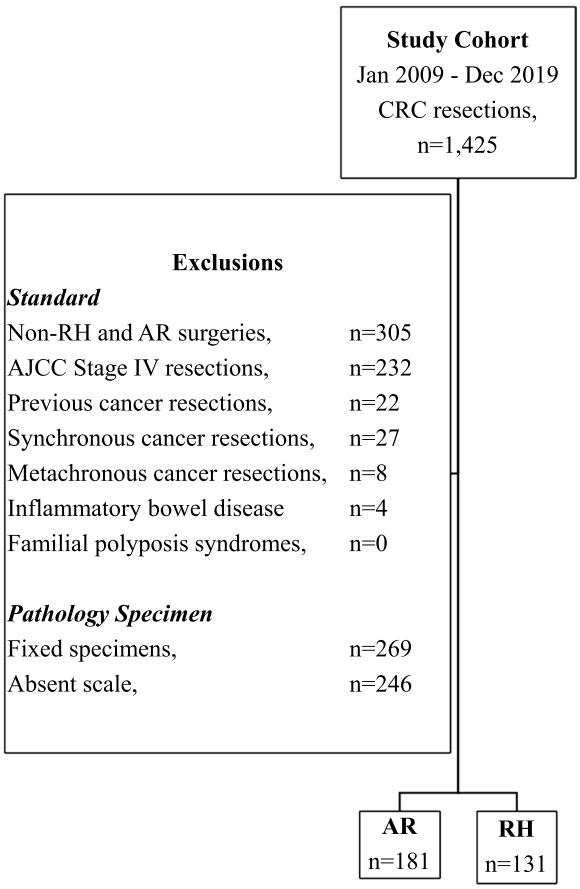

Fig. 1 Flow diagram of cohort definition

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

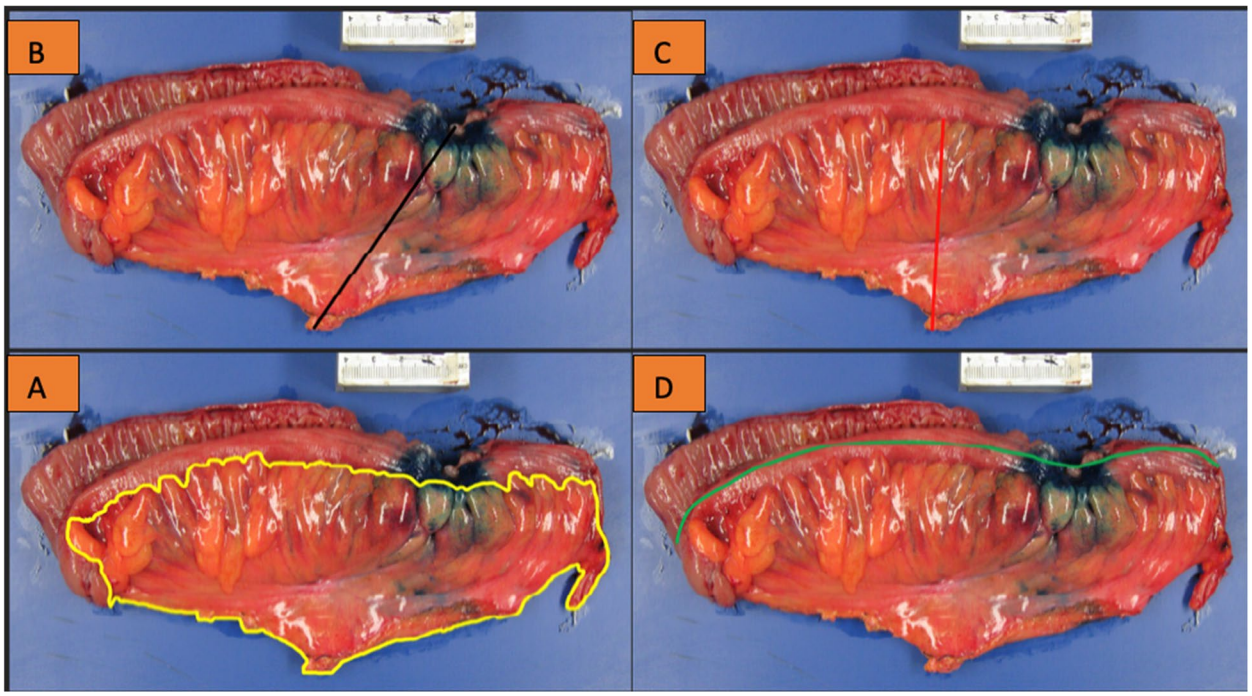

Fig. 2 TM measurements (**A**-**D**) of a fresh high anterior resection specimen includes—(**A**) the area of resected mesentery, (**B**) distance from the vascular tie to the tumour or (**C**) closest bowel wall and (**D**) length of bowel resected. The tumour is inked blue

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 clinicopathological 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

Acknowledgements
The authors acknowledge all surgeons (A Keshava, P Stewart, M Suen, H Cheung, C Young and M Solomon) and histopathologists who have contributed and assessed specimens included in this study. We also acknowledge P Quirke and A Quyn from St. James's University Hospital, Leeds for reviewing the analysed data.

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.

Funding
KN was supported by the Eric Bishop Research Scholarship (Royal Australasian College of Surgeons) and Medtronic Colorectal Research Fellowship (Colorectal Surgical Society of Australia and New Zealand). KSN was supported by the University of Sydney and RACS Senior Lecturer Fellowship. This project is supported by grant funding.

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.

Author details
[1]Colorectal Surgery Unit, Concord Hospital, Concord, NSW 2139, Australia. [2]Concord Institute of Academic Surgery, Concord Hospital, Building 20, Level 1, Hospital Road, Concord, NSW 2139, Australia. [3]Concord Clinical School, Clinical Sciences Building, University of Sydney, Concord Hospital, Concord, NSW 2139, Australia. [4]Department of Anatomical Pathology, Concord Hospital, Concord, NSW 2139, Australia. [5]Pathology and Data Analytics, Leeds Institute of Medical Research, University of Leeds, Leeds, UK. [6]John Goligher Colorectal Unit, St. James's University Hospital, Leeds, UK. [7]Academic Surgery, Leeds Institute of Medical Research at St. James's, University of Leeds, Leeds, UK.

Received: 7 July 2024 Accepted: 10 August 2024
Published online: 22 August 2024

References

1. Sondenaa K, Quirke P, Hohenberger W, et al. The rationale behind complete mesocolic excision (CME) and a central vascular ligation for colon cancer in open and laparoscopic surgery : proceedings of a consensus conference. Int J Colorectal Dis. 2014;29(4):419–28.
2. Brown KGM, Ng K-S, Solomon MJ, Chapuis PH, Koh CE, Ahmadi N, Austin KKS. Complete mesocolic excision for colon cancer: current status and

controversies. ANZ J Surg. 2024;94:309–19. https://doi.org/10.1111/ans.18741.
3. West NP, Hohenberger W, Weber K, Perrakis A, Finan PJ, Quirke P. Complete mesocolic excision with central vascular ligation produces an oncologically superior specimen compared with standard surgery for carcinoma of the colon. J Clin Oncol. 2010;28(2):272–8.
4. West NP, Morris EJ, Rotimi O, Cairns A, Finan PJ, Quirke P. Pathology grading of colon cancer surgical resection and its association with survival: a retrospective observational study. Lancet Oncol. 2008;9(9):857–65.
5. Almoudaris AM, Clark S, Vincent C, Faiz O. Establishing quality in colorectal surgery. Colorectal Dis. 2011;13(9):961–73.
6. Donabedian A. Evaluating the quality of medical care. 1966. Milbank Q 2005; 83(4): 691–729.
7. Quirke P, Steele R, Monson J, et al. Effect of the plane of surgery achieved on local recurrence in patients with operable rectal cancer: a prospective study using data from the MRC CR07 and NCIC-CTG CO16 randomised clinical trial. Lancet. 2009;373(9666):821–8.
8. Ng KS, West NP, Scott N, Holzgang M, Quirke P, Jayne DG. What factors determine specimen quality in colon cancer surgery? A cohort study. Int J Colorectal Dis. 2020;35(5):869–80.
9. Vogelsang RP, Gogenur M, Dencker D, Bjorn Bennedsen AL, Levin Pedersen D, Gogenur I. Routine CT evaluation of central vascular ligation in patients undergoing complete mesocolic excision for sigmoid colon cancer. Colorectal Dis. 2021;23(8):2030–40.
10. Munkedal DL, West NP, Iversen LH, Hagemann-Madsen R, Quirke P, Laurberg S. Implementation of complete mesocolic excision at a university hospital in Denmark: An audit of consecutive, prospectively collected colon cancer specimens. Eur J Surg Oncol. 2014;40(11):1494–501.
11. Newland RC, Chapuis PH, Pheils MT, MacPherson JG. The relationship of survival to staging and grading of colorectal carcinoma: a prospective study of 503 cases. Cancer. 1981;47(6):1424–9.
12. Newland RC, Chapuis PH, Smyth EJ. The prognostic value of substaging colorectal carcinoma. A prospective study of 1117 cases with standardized pathology. Cancer. 1987;60(4):852–7.
13. Bokey EL, Chapuis PH, Dent OF, Mander BJ, Bissett IP, Newland RC. Surgical technique and survival in patients having a curative resection for colon cancer. Dis Colon Rectum. 2003;46(7):860–6.
14. contributors W. ImageJ. https://en.wikipedia.org/w/index.php?title=ImageJ&oldid=1217807379. (accessed July 30, 2024.
15. Dent OF, Newland RC, Chan C, Bokey L, Chapuis PH. Trends in pathology and long-term outcomes after resection of colorectal cancer: 1971–2013. ANZ J Surg. 2017;87(1–2):34–8.
16. Naidu K, Chapuis PH, Brown KGM, Chan C, Rickard M, 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;93(7–8):1861–9.
17. Adams K, Chapuis PH, Keshava A, et al. Recurrence and colon cancer-specific death in patients with large bowel obstruction requiring urgent operation: a competing risks analysis. Colorectal Dis. 2021;23(10):2604–18.
18. Bokey L, Chapuis PH, Chan C, et al. Long-term results following an anatomically based surgical technique for resection of colon cancer: a comparison with results from complete mesocolic excision. Colorectal Dis. 2016;18(7):676–83.
19. Steffen C, Bokey EL, Chapuis PH. Carcinoma of the splenic flexure. Dis Colon Rectum. 1987;30(11):872–4.
20. Chan CL, Bokey EL, Chapuis PH, Renwick AA, Dent OF. Local recurrence after curative resection for rectal cancer is associated with anterior position of the tumour. Br J Surg. 2006;93(1):105–12.
21. Fielding LP, Arsenault PA, Chapuis PH, et al. Clinicopathological staging for colorectal cancer: an International Documentation System (IDS) and an International Comprehensive Anatomical Terminology (ICAT). J Gastroenterol Hepatol. 1991;6(4):325–44.
22. Haboubi N, Berho M. Lymph node harvest (LNH) in colorectal cancer; a critical appraisal. Pol Przegl Chir. 2015;86(10):497–504.
23. Miller ED, Robb BW, Cummings OW, Johnstone PA. The effects of preoperative chemoradiotherapy on lymph node sampling in rectal cancer. Dis Colon Rectum. 2012;55(9):1002–7.
24. Mechera R, Schuster T, Rosenberg R, Speich B. Lymph node yield after rectal resection in patients treated with neoadjuvant radiation for rectal cancer: A systematic review and meta-analysis. Eur J Cancer. 2017;72:84–94.
25. Gouvas N, Agalianos C, Papaparaskeva K, Perrakis A, Hohenberger W, Xynos E. Surgery along the embryological planes for colon cancer: a systematic review of complete mesocolic excision. Int J Colorectal Dis. 2016;31(9):1577–94.
26. Siani LM, Pulica C. Laparoscopic complete mesocolic excision with central vascular ligation in right colon cancer: Long-term oncologic outcome between mesocolic and non-mesocolic planes of surgery. Scand J Surg. 2015;104(4):219–26.
27. Storli KE, Sondenaa K, Furnes B, et al. Short term results of complete (D3) vs. standard (D2) mesenteric excision in colon cancer shows improved outcome of complete mesenteric excision in patients with TNM stages I-II. Tech Coloproctol. 2014;18(6):557–64.
28. Galizia G, Lieto E, De Vita F, et al. Is complete mesocolic excision with central vascular ligation safe and effective in the surgical treatment of right-sided colon cancers? A prospective study. Int J Colorectal Dis. 2014;29(1):89–97.
29. West NP, Kobayashi H, Takahashi K, et al. Understanding optimal colonic cancer surgery: comparison of Japanese D3 resection and European complete mesocolic excision with central vascular ligation. J Clin Oncol. 2012;30(15):1763–9.
30. Kobayashi H, West NP. CME versus D3 Dissection for Colon Cancer. Clin Colon Rectal Surg. 2020;33(6):344–8.
31. Kobayashi H, West NP, Takahashi K, et al. Quality of surgery for stage III colon cancer: comparison between England, Germany, and Japan. Ann Surg Oncol. 2014;21(Suppl 3):S398-404.
32. Sadahiro S, Ohmura T, Yamada Y, Saito T, Taki Y. Analysis of length and surface area of each segment of the large intestine according to age, sex and physique. Surg Radiol Anat. 1992;14(3):251–7.
33. Yoshida M, Ishiguro M, Ikejiri K, et al. S-1 as adjuvant chemotherapy for stage III colon cancer: a randomized phase III study (ACTS-CC trial). Ann Oncol. 2014;25(9):1743–9.
34. Shimada Y, Hamaguchi T, Mizusawa J, et al. Randomised phase III trial of adjuvant chemotherapy with oral uracil and tegafur plus leucovorin versus intravenous fluorouracil and levofolinate in patients with stage III colorectal cancer who have undergone Japanese D2/D3 lymph node dissection: final results of JCOG0205. Eur J Cancer. 2014;50(13):2231–40.

Publisher's Note