Original article

doi:10.1111/codi.14335

Acetone clearance of mesocolic or mesorectal fat increases lymph node yield and may improve detection of high-risk Stage II colorectal cancer patients

C. A. W. Leung*, G. E. Fazzi*, J. Melenhorst†, D. Rennspiess* and H. I. Grabsch*‡

*Department of Pathology and GROW-School for Oncology and Developmental Biology, Maastricht University Medical Center+, Maastricht, The Netherlands, †Department of Surgery, Maastricht University Medical Center+, Maastricht, The Netherlands, and ‡Pathology and Tumour Biology, Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK

Received 15 February 2018; accepted 6 June 2018; Accepted Article online 10 July 2018

Abstract

Aim Lymph node (LN) status is key to determining the need for adjuvant therapy in colorectal cancer (CRC) and for disease which has progressed to Stage II (T3–T4, N0, M0). A yield of fewer than 12 LNs is considered a risk factor similar to high-grade histology and vascular, lymphatic and perineural invasion. The aim of this retrospective study was to investigate the effect of acetone fat clearance of the mesocolon or mesorectum on LN yield and the identification of patients with high-risk Stage II CRC.

Method After conventional LN retrieval, fatty tissue derived from the mesocolon or mesorectum of 80 CRC specimens was incubated in acetone for 24 h. A second dissection was then performed by a trained technician. The total number of LNs as well as tumour involvement (LNpositive and LNnegative) were assessed at each stage. In addition, LN morphology was assessed and clinicopathological data were extracted from existing pathology reports.

Results Eighty CRC specimens were available for study. 1548 (94%) LN were negative and 96 (6%) were positive. The median (range) LN yield per specimen was 12 (3–41) LN increasing to 18 (4–48) LN after fat clearance ($P < 0.001$). After fat clearance, 534 additional LNs were identified in 75 (94%) of the specimens, and all but 10 were negative. The pN stage did not change in six patients who were found to be LN positive after fat clearance. However, the number of high-risk Stage II CRC patients decreased from 11 to 7. Although important for these patients, this downstaging did not reach statistical significance ($P = 0.125$).

Conclusion Acetone clearance of mesocolic or mesorectal fat increases median LN yield and may in a larger study decrease the number of patients classified as having high-risk Stage II CRC.

Keywords Colorectal cancer, lymph node yield, fat clearance

What does this paper add to the literature?
This study is the first to suggest that acetone fat clearance of mesocolic or mesorectal fat could decrease the number of patients classified as having high-risk Stage II colorectal cancer and confirms that there is an increase in median LN yield.

Introduction

The lymph node (LN) status (cN or pN) is a key factor for predicting prognosis and guides potential adjuvant therapies for patients with colorectal cancer (CRC) [1–4]. The total number of dissected LNs is an independent risk factor for recurrence irrespective of the presence of LN metastasis [5,6]. The American Joint Committee on Cancer has suggested that a minimum of 12 LNs should be recovered for accurate LN staging [7]. A LN yield of less than 12 is considered a risk factor similar to high-grade histology and vascular, lymphatic and perineural invasion in Stage II CRC (T3–T4, N0, M0) [4].

Population-based studies suggest that conventional specimen dissection, which relies on using visual assessment and palpation, fails to yield the recommended 12 nodes in as many as 23–63% of CRC specimens [8–10]. Factors influencing the LN yield include patient age

Correspondence to: Heike I. Grabsch MD PhD FRCPath, PO Box 5800, 6202 AZ Maastricht, The Netherlands.
E-mail: h.grabsch@maastrichtuniversity.nl

and body mass index as well as tumour size and neoadjuvant treatment [11]. Irrespective of this, high-quality surgical resection and pathological analysis remain the most important and modifiable factors for optimal LN yield [12,13]. Previous studies of techniques which increased LN yield did upstage pN0 to pN1 but did not improve the identification of high-risk Stage II CRC patients [14–16].

In this study, we investigated whether or not 24-h incubation of mesocolic or mesorectal fat in acetone increased LN yield and therefore decreased the number of high-risk Stage II CRC patients.

Method

This was a retrospective single-centre study performed at Maastricht University Medical Center+ (MUMC+) in the Netherlands. Specimens from 80 consecutive colorectal resections performed between May 2015 and February 2016 were examined in the Department of Pathology at MUMC+. The mesocolon or mesorectum was first dissected conventionally by one of the nine pathologists specializing in gastrointestinal pathology or trainee pathologists and LNs were identified by direct vision or palpation as per national CRC guidelines [17]. Fat clearance was achieved by incubation in acetone (Klinipath 4066-9005, Breda, the Netherlands) for 24 h (Fig. 1). A second dissection of the acetone-treated tissue was performed by a trained and certified technician (DR) blinded to the results of the first preparation. The results of both, the first and second dissection were reported in the final pathology report.

Archived haematoxylin and eosin stained LN slides from all 80 cases were scanned at 600 dpi using a HP Scanjet N6310. After manually selecting the area, the LN characteristics (area, length, width and shape) were measured using Leica image analysis (Qwin 3.5.1). Length was recorded at the maximum axis and width where the axis was perpendicular to the length (Fig. 2). Pathological LN tumour involvement was recorded.

Clinicopathological data including age at time of resection, dissecting pathologist (senior versus trainee), surgical procedure, TNM stage (according to TNM 5th edition [7]), total number of LNs, total number of positive LNs, tumour differentiation and neoadjuvant treatment status were retrieved. There were no missing data.

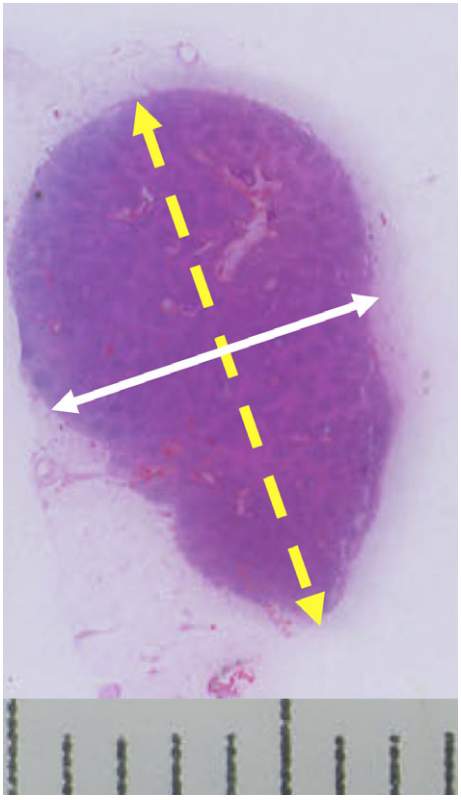

Figure 2 Scanned haematoxylin and eosin stained slide with a lymph node. The dotted line represents length (largest diameter) and the uninterrupted line represents width.

Statistical analyses were performed using IBM SPSS Statistics for Macintosh, Version 23.0 (IBM Corp., Armonk, New York, USA). A P-value < 0.05 was accepted as significant. The difference in number of LNs found per case before and after fat clearance was compared using the Wilcoxon signed rank test. The Kruskal–Wallis, Mann–Whitney U-test, chi-square test and Fisher exact tests were used to compare LNs before and after fat clearance and to explore the relationship between clinicopathological variables, pathologist experience and the number of positive LNs.

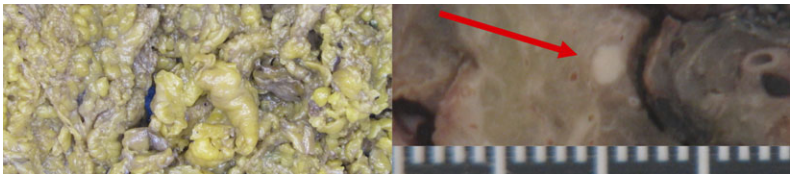

Figure 1 Pericolorectal fat before (left panel) and after (right panel) 24 h acetone incubation. The arrow points towards a lymph node.

Results

Patient characteristics

The patient and tumour characteristics of the study population are shown in Table 1. The median (range) age was 67 (43–84) years, with men comprising 57.5% (n = 45) and women 42.5% (n = 34) of the population.

Efficacy of fat clearance

A total of 1644 LNs were dissected (96 positive; 1548 negative). Ten per cent (n = 10) of the positive LNs were found after fat clearance. LNs found macroscopically after fat clearance resulted in a median (range) of 3 (1–10) additional paraffin blocks per case with a median (range) total number of 10 (2–26) cassettes per patient.

Before fat clearance 39 (49%) patients had a LN yield of less than 12, after fat clearance this dropped by 30% to 15 (19%). This led to the detection of an additional median (range) of 5 (0–20) LNs. These were found in 75 (94%) specimens. In six patients the LNs were positive. However, this did not result in a change in the pN stage (Table 2).

LN characteristics before and after fat clearance

LN width

The median (range) LN width after fat clearance was significantly reduced from 2.6 (0.21–17) mm to 1.8 (0.42–6.3) mm (P < 0.001). Although there was some overlap (Fig. 3), the median width of positive LNs was greater than that of negative LNs [positive LN: 4.0 (2.1–6.3) mm; negative LN: 1.7 (0.4–0.6) mm; P < 0.001] (Table 3).

Table 1 Patient and tumour characteristics.

	n	%
Tumour location		
Colon	47	58.8
Rectum	33	41.3
Neoadjuvant treatment		
Yes	21	26.3
No	59	73.8
Depth of invasion (pT)		
T0	2	2.5
T1	24	30
T2	24	30
T3	26	32.5
T4	4	5.0
Lymph node status (pN)		
N0	52	65
N1	21	26.3
N2	7	8.8

Table 2 Number of lymph nodes (LNs) per specimen before and after clearance of pericolorectal fat.

	No. of LNs before fat clearance, median (range)	No. of LNs after fat clearance, median (range)	P-value
All LNs	12 (3–41)	18 (4–48)	< 0.001
Positive LNs*	2 (1–20)	2 (1–21)	0.024
Negative LNs	11 (3–38)	17 (4–45)	< 0.001

*pN0 cases excluded.

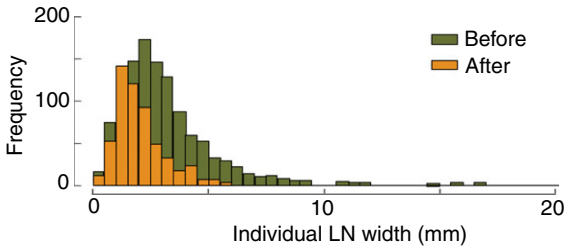

Figure 3 Histogram of lymph node (LN) widths before and after clearance of mesocolic and mesorectal fat.

Table 3 Lymph node (LN) width (in mm) before and after clearance of pericolorectal fat.

	Width before fat clearance, median (range)	Width after fat clearance, median (range)	P-value
Positive LNs (n = 96)	5.0 (1.1–17.0)	4.0 (2.1–6.3)	0.064
Negative LNs (n = 1548)	2.5 (0.2–9.5)	1.7 (0.4–6.0)	< 0.001

Number of LNs and identification of high-risk Stage II patients before and after fat clearance

Before fat clearance, 11 patients were classified as Stage II high-risk disease. A LN yield of fewer than 12 was the only risk factor in 6 (55%) of these patients. Increasing the LN yield by the use of fat clearance led to 7 patients being downstaged.

LN status and neoadjuvant treatment

There was no evidence that fat clearance led to proportionately more LNs being harvested in the 21 patients

Table 4 Positive lymph node (LN) yield before and after fat clearance (FC) by junior and senior pathologists.

	Junior		Senior	
	Before FC	After FC	Before FC	After FC
Total LNs	586	271	524	263
Positive LNs	47	8	39	2
Negative LNs	539	263	485	261

Table 5 Clinicopathological variables of patients with additional positive lymph nodes found after fat clearance.

Case	Gender	Age (years) at time of resection	Neoadjuvant treatment	Tumour location	pT
1	Male	71	No	Colon	4
2	Male	64	Yes	Rectum	2
3	Male	68	No	Colon	1
4	Male	64	Yes	Rectum	1
5	Male	65	No	Colon	3
6	Female	68	No	Rectum	1

who had neoadjuvant treatment. As might be predicted, the median LN width decreased from 2.4 (0.25–17.0) mm in patients without neoadjuvant treatment to 1.7 (0.21–10.7) mm ($P < 0.001$) in patients with neoadjuvant treatment.

LN yield and seniority of pathologist

A junior pathologist was defined as a trainee in his or her first 3 years of training, whilst senior pathologist status was given to final-year trainees or consultants. Nine pathologists were involved in this study. Junior pathologists missed 8 (15%) and senior pathologists missed 2 (5%) of the positive LNs during the first dissection ($P > 0.05$) (Table 4).

Relationship between clinicopathological variables and positive LN yield after fat clearance

The clinicopathological variables of the six patients with positive LN yield after fat clearance are summarized in Table 5. There was no difference in the median age, gender, neoadjuvant treatment, surgical resection or pT stage when compared with the rest of the study group.

Discussion

In our study we used acetone to improve LN yield after surgical resection for CRC which led to a reduction in the total number of high-risk Stage II (pT3–T4, pN0) patients from 12 to 7.

An increase in the total number of LNs per specimen has been reported with the use of a number of fat clearing techniques, including acetone [14,15,18–22]. All these studies reported changes in the lymph node status (pN). In our study, we hypothesized that the increase in LN yield after fat clearance would decrease the number of patients with high-risk CRC. This is important, because the total number of LNs harvested can influence whether or not a patient with CRC is considered 'high-risk' Stage II (pT3–T4, pN0) or not. Indeed, in many centres clinicians consider adjuvant therapy for CRC patients with a LN yield of fewer than 12 LNs in the absence of other risk factors [4]. Even though fat clearance did not result in a change in the pN status (overall presence or absence of positive LNs) in our study population, fat clearance did have a clinical impact by decreasing the number of patients with high-risk Stage II tumours from 11 to 7.

Relationship between pathologist experience, patient gender and LN yield

In our study, the positive LNs were substantially larger than the negative LNs, which has been reported previously [23,24]. To our surprise, there was an overlap in size irrespective of fat clearance, indicating that 'larger' LNs can be missed during conventional dissection. This finding has also been observed in two previous studies using GEWF (glacial acetic acid, ethanol, water and formalin) to try to improve LN yield [15,16].

Although 10% of the positive LNs were found after fat clearance, none of them led to an increase in pN stage as these cases were already staged as pN1 or pN2 before fat clearance. This is in contrast to previous reports [14].

Previous studies suggested that the experience and education of the dissecting pathologists had a positive effect on conventional LN yield [13,25]. Our results do not support this hypothesis: although junior pathologists missed more positive LNs than senior pathologists, this did not reach statistical significance.

Further work in a larger study population is needed to better understand the influence of patient gender and pathologist experience.

Toxicity and cost-effectiveness

Acetone, a relatively safe agent, can be used for fat clearing without the need for any special equipment, in contrast to the compression technique described by Scheel and colleagues [22,26]. It can be used with standard ventilation and has no adverse effect on routine special stains.

The pathologists' workload for macroscopic dissection did not increase during our study as a trained technician performed the second dissection. Studies have shown that trained technicians produce LN yields equivalent or even superior to those of junior pathologists [27,28].

In our institution, the total cost for the extra acetone clearing of the mesocolic or mesorectal fat was 12.50 EUR per specimen. This included both material and labour costs. The increase in pathologist workload for the review of the additional blocks was minimal (three extra blocks per case). Methods that process the complete mesocolon or mesorectum in paraffin result in 25 additional blocks/cases – a significant burden for the reviewing pathologist [19,22].

Limitations of the study

We acknowledge the limitations of single-centre retrospective studies. The relatively small sample size of 80 patients might explain why there was no statistical difference in the clinicopathological variables.

Conclusion

This study has shown that acetone clearance of mesocolic or mesorectal fat increases the median LN yield and may decrease the number of patients being classified as having high-risk Stage II CRC and receiving unnecessary adjuvant treatment. Since acetone clearance is cost-effective and easy to implement, we have introduced acetone for processing mesocolic or mesorectal tissue and we would recommend other histopathology laboratories to follow suit.

Acknowledgement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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