Lymph node revealing solutions in colorectal cancer: should they be used routinely?

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ABSTRACT

The Royal College of Pathologists (RCPath) and College of American Pathologists recommend that at least 12 lymph nodes should be harvested for adequate staging of colorectal carcinoma. Just one nodal tumour deposit upstages the malignancy from pN0 to pN1. This is critically important as node-positive patients (pN1) are considered for adjuvant chemotherapy whereas node-negative patients (pN0) may not be. It is not always easy to harvest the required number, especially in patients with rectal carcinoma who may have received neoadjuvant therapy—an increasingly common treatment. The use of neoadjuvant therapy is known to further decrease the number and size of identifiable lymph nodes within specimens, meaning that the lymph node harvest often fails to reach RCPath guidelines. Lymph node revealing solutions consisting of either single chemicals such as alcohol or acetone or compounds have been investigated to help improve the lymph node harvest in difficult specimens, for example, those received following neoadjuvant therapy. Published research evidence reviewed here suggests that lymph node revealing solutions significantly improve lymph node harvesting, and that glacial acetic acid, ethanol, water and formalin is advantageous in comparison with other revealing solutions in that it is safe, cheap, easy to use and relatively quick. However, the quantity of good evidence is limited and the clinical implications of improving lymph node harvesting require further research.

INTRODUCTION

Colorectal carcinoma (CRC) is the fourth most common cancer in the UK.[1] In 2010 alone, there were 40 695 new diagnoses and 16 013 deaths from the disease.[2] High quality histopathological assessment, including harvesting of an adequate number of lymph nodes, is required in order to accurately stage the patient and help deliver the most appropriate treatment postsurgery. The presence of metastases within lymph nodes is inextricably linked to the prognosis of the patient.[3]

Current recommendations are that at least 12 lymph nodes should be retrieved for adequate staging of CRC,[4 5] with all mesentery within the tumour vicinity searched. Just one nodal tumour deposit upstages the malignancy from pN0 to pN1.[4] This is important as node-positive patients (pN1) are considered for adjuvant chemotherapy whereas node-negative patients (pN0) may not be.[6]

The requirement for at least 12 lymph nodes is based on evidence demonstrating the prognostic significance of lymph node harvesting.[7 8] Some literature suggests that more lymph nodes should be harvested for adequate staging,[7] but 12 is the current consensus.[4 5] At our hospital, specimens are resampled when less than 12 lymph nodes are harvested at the first attempt.

Lymph node harvesting is traditionally performed by a manual technique of vision and palpation. In the majority of cases, harvesting a minimum of 12 lymph nodes should be achievable but this may become more difficult in the rectum, especially in patients who have received neoadjuvant chemotherapy as the size of lymph nodes may be reduced, making identification more challenging.[9] Use of neoadjuvant therapy is not the sole cause of an inadequate lymph node harvest. Other limiting factors are known to be fixation time,[3 10] experience of the surgeon and failure by the dissector to appropriately examine all nodes within a specimen, either due to lack of experience or poor technique.[11 12]

In response to this, a number of studies have been carried out to address the issue of lymph node harvesting, using a variety of methods. These have included extending the fixation time,[3 13 14] injecting dyes to accurately map lymph node chains,[15 16] transilluminating the mesentery to identify small nodes,[17–22] submitting residual mesenteric tissue in its entirety[23] and using a variety of different lymph node revealing solutions.[3 17–44] In many studies, these techniques have been combined.[17–24 27 28 31–34]

This review is based on a search of medical and scientific databases to identify all available literature written in English, and published within the last 30 years. The review focuses on the use of chemical lymph node revealing solutions in relation to CRC specimens only. Studies related to other carcinoma types are excluded from this review, as are those which use other adjunct techniques such as lymph node mapping. The studies within this review are mainly of cohort and case control design,[3 17–44] although there is also one randomised controlled trial.[38]

HISTORY OF LYMPH NODE REVEALING SOLUTIONS

Since the first fat clearance technique using dye injection and lymph node mapping with alcohol clearance was described by Gilchrist and David[24] in 1938, authors have studied a variety of lymph node revealing solutions.[17–43] A number of early studies investigated the use of alcohols, acetone and xylene,[17–19 21 22 26 28 30–33] but since 1997 when the first study was published,[41] there has been a greater focus on the use of glacial acetic acid, ethanol, water and formalin (GEWF) (table 1).[35–42]

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Table 1 Number of harvested lymph nodes

	Manual dissection		Fat clearance		
Lymph node revealing solution	No. cases	Mean no. lymph nodes	No. cases	Mean no. lymph nodes/(+) additional nodes	Statistically significant difference?
Acetone*[25]	34	15.4	34	+5.7	–
	80	6.9	80	+4.4	
Acetone/IPA/oil[26]	–	–	864	27.0	–
Acetone/xylene[22]	75	2.7	75	+7.5	–
Acetone/alcohol/xylene[18]	22	4.7	10	30.9	Yes (p<0.0001)
Acetone/alcohol/xylene[27]	15	20.9	15	+68.6	–
Acetone/alcohol/xylene[28]	–	–	311	74.3	–
Alcohol[23]	48	19.4	48	+23.6	No (p=0.177)
Alcohol†[29]	82	9.6	155	27.6	Yes (p<0.001)
		5.2		20.4	Yes (p<0.001)
Alcohol[33]	–	–	27	34	–
Alcohol‡[19]	37	18.1/21.2	140/182	76.4/73.7	–
Alcohol/xylene[31]	41	21	43	−9/−4	–
Alcohol/xylene[30]	10	18.7	10	−2.3	No (p=0.57)
Alcohol/xylene[32]	103	6.2	103	+12.4	–
Alcohol/xylene§[21]	221	10.5	51	23.1	Yes (p<0.001)
	50	13.1			
Alcohol/xylene[17]	41	7.3	41	+47.6	–
Alcohol/xylene[20]	–	–	48	50.2	–
GEWF[37]	40	18.30	45	19.96	No (p=0.53)
GEWF¶[42]	30	5.1	30	+1.73	Yes (p<0.01)
	12	6.25+1.6	12	+1.2	Yes (p<0.01)
GEWF[36]	32	6.8	35	10.2	Yes (p=0.002)
GEWF[41]	30	2.94	30	+8.6	–
GEWF[40]	35	6.26	35	+13.0	–
GEWF‡[38]	59	10	61	17	Yes (p<0.001)
		9		16	
GEWF[39]	117	5.0	125	13.0	Yes (p<0.001)
GEWF[35]	34	5.9	59	14.7	Yes (p=0.05)
GEWF[3]	423	11.4	423	+6.0	–
GEWF**[43]	76	–	62	–	–
GEWF[44]	8	7.6	8	+4.7	Yes (p<0.5)

*Two study groups.
†Non-neoadjuvant/neoadjuvant.
‡Colonic/rectal.
§Multiple sites with lymph node clearance performed at the main site only.
¶Two study groups, multiple dissections and multiple sites of tumour.
**Comparison of cases from different years; also includes assessment of improved surgical practice.
GEWF, glacial acetic acid, ethanol, water and formalin.

REVIEW OF THE LITERATURE

Number of lymph nodes retrieved

The most commonly described benefit of using lymph node revealing solutions is the pure increase in the numbers of lymph nodes harvested, many of which are of a smaller size than might be identified by manual dissection. Studies have shown a variable increase in harvested lymph nodes. In one study, a mean harvest of 76.4 and 73.7 lymph nodes was seen after application of alcohol in colonic and rectal resections, respectively.[19] In the same study, a secondary manual dissection identified a mean of 18.1 and 21.2 lymph nodes, respectively, but the authors did not clarify whether both sets of dissections were performed by the same individuals.[19] If manual dissections had been carried out by less experienced individuals then it is possible that this may have also affected the numbers of nodes harvested.

Metastatic incidence and upstaging

Metastatic incidence refers to the proportion of lymph nodes which contain tumour deposits. A decrease in metastatic incidence after the use of lymph node revealing solutions has been reported.[17 18 22 25 27 31 38 40–42] Saleki and Haeri[40] attributed significance to this finding, stating it to be due to the overall greater number of lymph nodes harvested after secondary dissection. In contrast, five studies showed an increase in metastatic incidence,[29 32 36 37 39] but not always with significance.[37 39]

Upstaging refers to an upwards change in pathological staging, which may then alter patient treatment if there is a shift from node-negative (pN0) to node-positive (pN1 or pN2). This is because node-positive patients receive chemotherapy, while node-negative patients may not.[45]

Nine studies reported upstaging after the use of lymph node revealing solutions,[17 23 25 27 31 32 40–42] ranging from 2.4% to 33% (table 2).[31 41] Six of these claimed the finding to be significant, in that upstaging from Dukes' B to Dukes' C was reported, prompting adjuvant therapy.[17 25 27 32 40 41] However, this may not have been a correct assumption because most of these studies had questionable underlying primary manual dissection

practice with fewer than the recommended minimum of 12 lymph nodes found on average (range 2.94–7.3).[17 25 32 40 41] These studies were therefore more likely to identify upstaging once a lymph node revealing solution had been applied. It is likely that upstaging would have been insignificant, or not present at all, had there been optimal primary manual dissection. In one study by Koren *et al*,[41] there was upstaging in 10 cases, and a further eight cases had the staging changed from Nx to N0, suggesting an underlying deficit in primary manual dissection technique. The case upstaged by Brown *et al*[27] was a soft tissue metastasis which the authors suggested may have been artefactual. The evidence in the literature is therefore questionable.

Does lymph node size matter?

Multiple studies have demonstrated smaller sized lymph nodes after lymph node revealing solutions are used (table 3).[3 18 19 22 25 36 39 40 42 44] Some of the more recent studies using GEWF have assessed and attributed statistical significance to this.[36 39 40 42] Brown *et al*[27] found that 83% of additional lymph nodes were ≤2 mm in size. Where GEWF is used this may be due to the white colour of lymph nodes which facilitates detection.[39] There is ongoing debate regarding the clinical significance of CRC metastases in small lymph nodes. Dhar *et al*[46] concluded that metastatic lymph node size is a strong prognostic variable in CRC, using two sample log rank testing to demonstrate that the prognostic impact decreased when lymph nodes were more than 10 mm in diameter. Dhar *et al*[46] did concede that their findings needed to be confirmed with a larger study before clinical application. In another recent study, Märkl *et al* concluded that 'minute lymph nodes [<1 mm] have virtually no role in correct histopathological lymph node staging'.[47] They did however agree that the detection of relatively small lymph nodes (1–5 mm) was an important factor for exact lymph node staging and was prognostically relevant, with an association between a high number of harvested lymph nodes and a favourable outcome in colon carcinoma.[47]

It is important to consider whether finding a greater number of smaller lymph nodes has the potential to change patient management. If the only significant finding is a greater number of smaller tumour-free lymph nodes, then the patient will remain node-negative and there will be no change in treatment. There will be no benefit to the patient but there will be a cost to the laboratory, both in terms of increased turnaround times and finances.

If metastases are prevalent in larger lymph nodes (ie, >5 mm), then they should be identified by manual dissection, providing the dissector is adequately experienced. If this is the case, then one might argue that the use of lymph node revealing solutions is not necessary. It may be that education is as important a tool as is the use of adjunct chemicals, but currently there remains a lack of evidence to prove or disprove this.

Table 2 Incidence of upstaging

Lymph node revealing solution	Findings
Acetone[25]	2/34 (5.9%) upstaged from pN1 to pN2* 2/80 (2.5%) upstaged†: 1 upstaged from pN0 to pN1 1 upstaged from pN1 to pN2
Alcohol and xylene[17]	3/41 (7.3%) cases upstaged from Dukes' B to Dukes' C
GEWF[41]	10/30 (33%) upstaged‡: 4 upstaged from Nx to N1 4 upstaged from N0 to N1 2 upstaged from N1 to N2
GEWF[42]	4/30 (13.0%) upstaged—no colorectal cancer cases upstaged§
Alcohol and xylene[32]	5/58 (8.6%) upstaged (Dukes' B to Dukes' C)
GEWF[40]	3/35 (8.6%) upstaged from Dukes' B to Dukes' C
Acetone, alcohol and xylene[27]	4/15 (26.7%) upstaged: 1 upstaged from pN0 to pN1 3 upstaged from pN1 to pN2
Alcohol and xylene[31]	Stage changed in 2/84 (2.4%) of cases
Alcohol[23]	2/10 (20.0%) upstaged from pN1 to pN2

*Control group.
†Study group.
‡Stage also changed from Nx to N0 in eight cases.
§Upstaged one breast carcinoma and three bladder carcinoma cases.
GEWF, glacial acetic acid, ethanol, water and formalin.

CHALLENGES IN STUDY DESIGN

Quality of evidence

The greatest challenge in assessing the true value of lymph node revealing solutions in CRC surrounds the quality of the existing evidence. The majority of existing studies are open to at least one type of bias which may invalidate the conclusions. Different types of bias which may have affected the existing studies are summarised in box 1.

Underlying primary dissection practice

Many of the studies did not achieve the recommended targets during primary manual dissection,[17 22 25 29 32 35 36 38 39–42] with the mean number of lymph nodes harvested ranging from 2.7[22] to 21.2.[19] Kelder *et al*[39] only found a mean of 5.0 lymph nodes by primary manual dissection in 117 colonic specimens, even though their study was relatively recent. The highest number of lymph nodes found in any specimen in their study was only 17,[39] which was lower than the average number found by primary manual dissection in a number of other studies.[19 23 27 30 37] In the study by Schmitz-Moormann *et al*,[22] routine primary dissection yielded a mean nodal count of 2.7, and failed to identify any nodes in six out of the 75 cases. This issue is supported by a number of studies where the importance of enthusiasm and skill of both pathologist and surgeon is noted because it directly affects the quality of the specimen and subsequent nodal harvest.[18 35 42] Gregurek and Wu[37] found that educating pathologists in appropriate primary manual dissection practice gave more powerful results than the use of lymph node revealing solutions; however, there was potential bias in their study (box 1). Additionally, failing to consider the experience of dissectors may also introduce sampling bias, perhaps via the involvement of inexperienced dissectors who might miss smaller lymph nodes in comparison with dissectors who are highly experienced in manual dissection. It was often unclear in the case control studies who performed the secondary dissection.[17–24 26–29 31–43] The exceptions to this were the studies by Jass *et al*[30] and Vogel *et al*,[25] where secondary dissection was performed by the first author or one of three pathologists not aware of the outcome of the primary dissection, respectively. Only one of the studies included true randomisation of specimens into study groups.[38] Gregurek and Wu[37] claimed that cases were alternately enrolled into study and control groups; however, pathologists were given the opportunity to change this, which weakened their study design.

Table 3 The effect of lymph node revealing solutions on the size of lymph nodes

Lymph node revealing solution	Control group	Study group	Statistically significant difference?
Acetone[25]	Average diameter 2.7 mm	Average diameter 2.0 mm	–
Acetone, IPA and oil[26]	–	–	–
Acetone and xylene[22]	9.7% nodes ≤2 mm, 10% metastatic nodes ≤2 mm	83.6% nodes ≤2 mm 0.6% metastatic nodes ≤2 mm	–
Acetone, alcohol and xylene[18]	4.8% nodes <5 mm 100% metastatic nodes >5 mm	89% nodes <5 mm 40% metastatic nodes <5 mm	–
Acetone, alcohol and xylene[27]	–	50% nodes <1 mm 82% nodes <2 mm 83% metastatic nodes <3 mm In both groups, 75% metastatic nodes <2 mm	–
Acetone, alcohol and xylene[28]	–	–	–
Alcohol[23]	–	88.6% nodes ≤2 mm 78.6% metastatic nodes ≤2 mm	–
Alcohol[29]	–	–	–
Alcohol[33]	–	75.5% metastatic nodes <5 mm 24.5% metastatic nodes >5 mm	–
Alcohol[19]	49.5% nodes <4 mm 14.8% metastatic nodes <4 mm	77.9% nodes <4 mm 32.6% metastatic nodes <4 mm	–
Alcohol and xylene[31]	–	–	–
Alcohol and xylene[32]	–	–	–
Alcohol and xylene[21]	–	–	–
Alcohol and xylene[17]	–	77% of metastatic nodes ≤5 mm In 7 cases metastases only found in nodes ≤5 mm	–
Alcohol and xylene[20]	–	94% nodes ≤5 mm 6% nodes >6 mm 71.8% metastatic nodes ≤5 mm	–
GEWF[42]	Mean diameter 6.8 mm (±4.13)	Mean diameter 4.2 mm (±3.46)	Yes (p<0.01)
GEWF[36]	Average diameter of metastatic nodes 7 mm (±4 mm) 41% nodes ≤5 mm	Average diameter of metastatic nodes 5 mm (±2 mm) 60% nodes ≤5 mm	Yes (0.046)
GEWF[41]	–	Diameter 0.5–7.0 mm	–
GEWF[40]	Mean diameter 0.429 mm (minimum 0.1 mm) All nodes >0.9 mm identified by standard technique Mean diameter metastatic nodes 0.568 mm 26% nodes <5 mm identified by standard practice 55.3% metastatic nodes ≤5 mm	Mean diameter 0.268 mm (0.2–0.9 mm) Mean diameter metastatic nodes 0.35 mm	Yes (p<0.000001)
GEWF[39]	Median diameter non-metastatic nodes 6 mm Median diameter metastatic nodes 9 mm	Median diameter non-metastatic nodes 4 mm Median diameter metastatic nodes 6 mm	Yes (p<0.001) Yes (p<0.001)
GEWF*[35]	–	86% nodes (246/286) <3 mm 11.5% nodes (33/286) 3–6 mm 1.4% nodes (4/286) >6 mm 6 metastatic nodes <3 mm (5 from neoadjuvant therapy cases)	–
GEWF[3]	Mean diameter 4.3 mm	Mean diameter 2.5 mm	–
GEWF[44]	Mean diameter 2.6 mm (1–15 mm)	Mean diameter 2.1 mm (1– 4 mm)	No (p>0.11)

*Only assessed 30.5% of cases in the study group.
GEWF, glacial acetic acid, ethanol, water and formalin.

Blinding

Studies involving GEWF will always have an immediate detection bias, caused by an inability to use blinding. Iversen *et al*[38] described GEWF as having 'its own characteristic macroscopic appearance, which is impossible to hide'. This could then either consciously or unconsciously give dissectors the ability to alter their practice which could skew any potentially significant findings. Newell *et al*[36] admit to this limitation, commenting that 'those pathologists using the standard technique would likely examine pericolic fat more thoroughly'.

Time and cost

The most rapid treatments took 6 h to complete and all used GEWF.[40–42] In contrast, the longest treatment using a combination of alcohol and xylene took a minimum of 3 weeks.[32] Unsurprisingly, many of the more lengthy treatments have been associated with multistep studies, where more than one chemical has been used in the lymph node revealing solution.[27 28 32] Many studies taking a day or less of additional time to harvest lymph nodes used GEWF.[35–38 40–42] With the need to modernise National Health Service (NHS) histopathology departments,[48–51] it is unsurprising that focus appears to be

Box 1 Types of bias within the literature

- Anatomical variation in numbers of lymph nodes within the colorectum[17 19 20 23 25 26 28 32 34 36–38 40–42]
- Suboptimal underlying manual dissection practice[3 17 22 25 29 32 35 36 38 39–42]
- Inappropriate or unclear sample size[17 20 27 29 30 33 37 44]
- Unclear or unbalanced study groups[17–19 22 23 25 29 40 42]
- No sample size calculation[19 22 25 27 28 30 31 33 37 39 40 44]
- Exclusion criteria unclear or absent[17 36 38]
- No randomisation used/strategy unclear[22 23 27 31 32 35]
- Inability to use blinding[22 35 36 38]
- No statistics used or described[3 17 22 25 31 33]
- Statistics used but methods not defined or discussed[40]
- Intervention and comparison compared during different study periods[29 37]
- Unclear or varying fixation time[29 35–37]
- Unclear length of time in lymph node revealing solution[39]
- Lengthy/unclear timescale of study[22 27 29]
- Staff allowed to choose which technique to use[37]

shifting towards GEWF, which acts as a fixative while also clearing fat in a shorter period of time than other lymph node revealing solutions. In the 21st century, focusing on lengthy techniques cannot be justified.[48–51] Even if lengthy multistep techniques are deemed to provide significant findings, it would be inappropriate to substantially delay reporting. In order to maintain and improve turnaround times, a quicker and more effective method of fat clearance is required if it is to be used routinely. As well as adding a diagnostic delay, older lymph node revealing solutions are also said to be expensive.[42]

Toxicity

Many older studies used noxious substances, most notably the aromatic hydrocarbon xylene.[17–22 28 30–34] Xylene was once ubiquitous in histopathology laboratories as a clearing agent used routinely in processing and staining. Laboratories are now seeking to eliminate the use of xylene in processing,[52–54] due to its known carcinogenic potential.[52] This has been facilitated by the introduction of xylene-free processing technology.[55 56] As a result of this, only one study in the last 10 years has included xylene.[27] In contrast, there have been a number of recent studies assessing the use of GEWF, which is seen as a better lymph node revealing solution than its predecessors because it is safe, cheap, easy to prepare and handle,[3 35 36 38–41 43 44] odourless, can be used with standard ventilation, and has no adverse effect on routine special stains or immunohistochemistry.[36]

CONCLUSIONS

As yet, there is no clear evidence to indicate whether one lymph node revealing solution is better than another from the current literature; however, the use of carcinogenic chemicals is inappropriate in terms of health and safety.[17–22 25–28 30–34] Lengthy lymph node revealing techniques which add significant reporting delays[19 27 28 32] are inappropriate in a modern NHS.[48–51] A number of studies have claimed that GEWF is a safe and efficient lymph node revealing solution,[19 36–42] which is quick, cheap, easy to prepare and handle.[18 39 41] In their prospective case control study, Ustün *et al*[42] stated that historical fat clearance techniques were difficult to handle and expensive while GEWF was easier to use with better results. GEWF could be further investigated with appropriately designed studies, adopting randomisation of cases and minimisation of any potential bias which has been an issue in the existing literature. It is difficult to determine whether the use of GEWF or any other lymph node revealing solution leads to upstaging from node-negative to node-positive; bias in existing studies limits their conclusions. Until evidence can show that the use of lymph node revealing solutions significantly affects patient management, their routine use cannot be recommended as no benefit to the patient has yet been proven. The next steps should be to design appropriate studies in order to look for statistically significant differences in lymph node harvest associated with the use of these solutions. This would help to test the hypothesis that the use of lymph node revealing solutions contributes to patient management and would ensure that the most appropriate evidence-based treatment options are available to patients.

Take home messages

- The use of lymph node revealing solutions leads to a significant increase in the number of harvested lymph nodes in colorectal carcinoma resection specimens.
- The use of lymph node revealing solutions leads to detection of significantly smaller lymph nodes and may lead to upstaging, which can change patient management by prompting adjuvant therapy. It has yet to be shown whether these findings have any clinical significance and therefore whether they can enhance patient management.
- Glacial acetic acid, ethanol, water and formalin is a safe and efficient lymph node revealing solution and its potential utility should be investigated further. Other older lymph node revealing solutions such as xylene have cost implications—in terms of finance, turnaround times and health effects; therefore, studies of their use are no longer relevant to modern practice.

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