Mechanised one-day fat clearance method to increase the lymph node yield in rectal cancer specimens

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Accepted: 20 May 2005

ABSTRACT

The Royal College of Pathologists' guidelines for reporting colorectal cancer state that all lymph nodes in a colorectal cancer specimen should be sampled, regardless of site or size. The only means by which one can be certain that all nodes have been sampled is to clear the fat and visualise the lymph nodes. Methods of fat clearance have been available for many years, but few are acceptable in routine practice. Here, a simple, effective and economical solution to the problem is described, which should be amenable to any laboratory with a spare or back-up tissue-processing machine.

KEY WORDS: Adipose tissue.
Colorectal neoplasms.
Lymph node excision.
Tissue preservation.

Introduction

Ever greater emphasis is being placed on the identification of adequate numbers of lymph nodes in colorectal cancer specimens. The current minimum dataset guidelines for colorectal cancer (1998) issued by The Royal College of Pathologists recommends that all lymph nodes of whatever site and size should be examined.[1] The TNM (6) classification states that a regional lymphadenectomy specimen will ordinarily contain 12 or more lymph nodes; however, if the lymph nodes are negative but the number ordinarily examined is not met, this is classified as pN0.[2] Often, this number can only be achieved after lengthy dissection. The question of how one can be certain that all lymph nodes have been sampled is seldom raised.

Fat clearance has long been known as a useful tool for the detection of small or inconspicuous lymph nodes, but it requires the use of sequential immersions of the tissue in baths of alcohol, followed by xylene. The fat is then dissected in a fume cabinet using transillumination from a light box to locate the lymph nodes.

Most pathologists are deterred from using the method because it is noxious and the clearance process takes several days (five to seven, depending on the specimen). In addition, laboratory staff disliked the traditional fat clearance methods for similar reasons, and for the wastefulness of using large quantities of alcohol and xylene just once. Thus, fat clearance has been a process reserved only for cases in which an unacceptably low number of lymph nodes are identified.

Here, a simple fat clearance method is described that identifies all lymph nodes in a specimen, involves minimal wastage and adds just one day to the reporting time. Other advantages of the method are also apparent.

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Materials and methods

The method used an old enclosed vacuum infiltration processor (VIP) tissue processor, retained in the laboratory in case of emergency, which was programmed to halt after 18 h, following the xylene immersion phase.

Twelve sequential anterior resection specimens, performed for rectal cancer, were compared with 15 archival anterior resections, matched for age, site and Dukes' stage, which had been dissected and sampled conventionally. By chance, the test group contained fewer patients who had undergone pre-operative irradiation with short-course DXT (4/12 *vs*. 12/15). This may be explained by the fact that selection of controls was made when short-course radiotherapy had been introduced at the hospital.

The rectal specimens were cut transversely in 8–10 mm thick slices. The fat from the test slices was placed in mega-cassettes, together with a pencil-labelled paper tag with details of the case number and the location of the slice. Depending on the size of the specimen, this necessitated the use of between 10 and 27 mega-cassettes. Control cases underwent conventional macroscopic lymph node dissection and the nodes and tumour samples were processed overnight.

The mega-cassettes from the test specimens were processed overnight and the machine was arrested at the xylene stage, prior to paraffin-wax impregnation. The

Table 1. Comparison of mechanised fat clearance and a control group dissected traditionally and without fat clearance.

	New method	No fat clearance
	n=12	(control) *n*=15
Mean (range) age	70.7 (46–89) years	66.4 (36–88) years
Gender	11 male, 1 female	11 male, 4 female
Pre-operative radiotherapy		
Yes	4	12
No	8	3
Dukes' stage		
A	1	2
B	1	2
C	10	11
Mean (range) no of involved nodes per specimen	4.9 (0–27)	3.1 (0–17)
Mean (range) total nodes per specimen	31.3 (19–46)	16.3 (5–30)*
Mean percentage of involved nodes	15.7	19
Mean (range) involved nodes per specimen in irradiated specimens	10.3 (3–27)	3.4 (0–17)†
Mean (range) total nodes per specimen in irradiated specimens	27.3 (19–42)	16.8 (8–30)‡
Mean percentage of nodes in irradiated specimens	37.7	20.2
*P=0.0001, †P=0.07, ‡P=0.04		

following day, the xylene-cleared tissue was removed from the mega-cassettes and examined in a fume hood by transillumination over a light box in order to visualise the lymph nodes (Fig. 1). However, the light box required protection (a sheet of glass) from the effects of xylene, and both the glass and the light box are bulky and required storage when not in use.

The dissected nodes were then placed in routine cassettes and returned to a processor, to resume processing through to paraffin wax. Use of a multiloculated cassette meant that lymph node position could be recorded and that the use of excessive numbers of cassettes was unnecessary (Fig. 2).

After blocking out the nodes, sections were cut and then stained with haematoxylin and eosin (H&E) for reporting the following day. Thus, an extra day's delay was incurred before the lymph nodes could be examined microscopically.

Unmarked mega-cassettes were cleaned and re-used, and processor reagents were used several times; thus, the technique involved minimal wastage of solvents.

Statistical analysis

All tests were performed using the SPSS Data Editor (version 10). The Mann Whitney U test was used to compare unpaired non-parametric groups. The level of significance was accepted as $P<0.05$ in all tests.

Results

Mean lymph node yield was significantly ($P=0.0001$) higher in the test group (31.3) than in the control group (16.3). Mean number of involved nodes detected was 4.9 using the mechanised fat clearance technique (15.6% nodes involved) compared to 3.1 (19% nodes involved) in the control group (Table 1, Fig. 3). Although higher, this was not statistically significant and the percentage of involved nodes was lower in the test group than the control group.

Subset analysis of post-irradiation patients revealed that total lymph node yield was higher in the study population (mean: 27.3; range: 19–42) compared to the irradiated controls (mean: 16.8; range: 8–30) ($P=0.04$). The positive lymph node yield was also higher in the irradiated study population (mean: 10.3; range: 3–27; 37.7% nodes involved) compared with the irradiated control group (mean: 3.4; range: 0–17; 20.4% nodes involved) ($P=0.07$) (Table 1, Fig. 4).

Discussion

The Royal College of Pathologists' minimum dataset guidelines for colorectal cancer (1998) state that all lymph nodes in a colorectal cancer resection specimen should be sampled.[1] However, if the fat is not cleared, how can a pathologist be certain that no node has remained unsampled? If fat clearance is performed, both the pathologist and surgeon can be certain that all lymph nodes submitted have been examined.

Guidelines notwithstanding, many laboratories are reluctant to undertake what is traditionally a time-consuming and environmentally noxious process. Against this background, the one-day method presented here is quicker than other fat clearance techniques, with far less risk

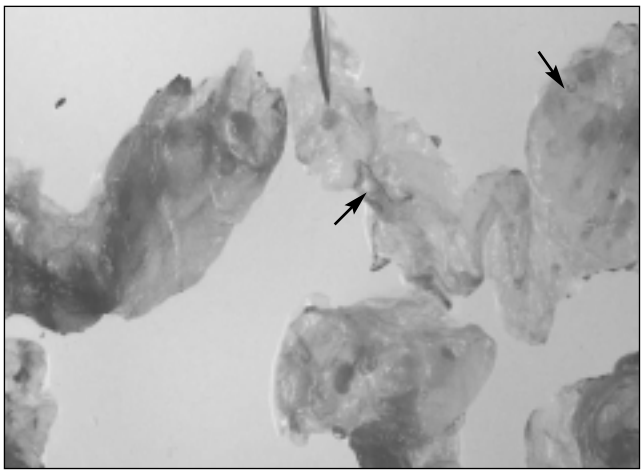

Fig. 1. Lymph nodes are clearly demonstrated in the pericolic fat (metal pointer) following the xylene phase of tissue processing. Blood vessels appear hollow in cross section and are obvious in longitudinal section (arrows).

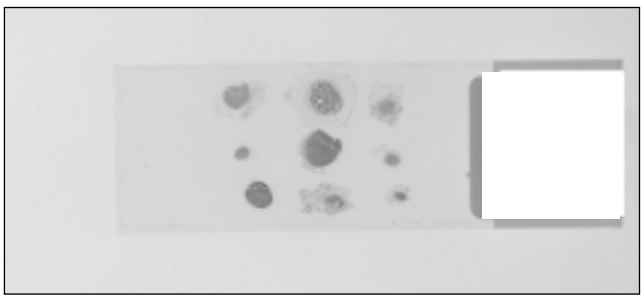

Fig. 2. If a multiloculated cassette is used, the position of particular lymph nodes can be recorded and up to nine nodes sampled at a time.

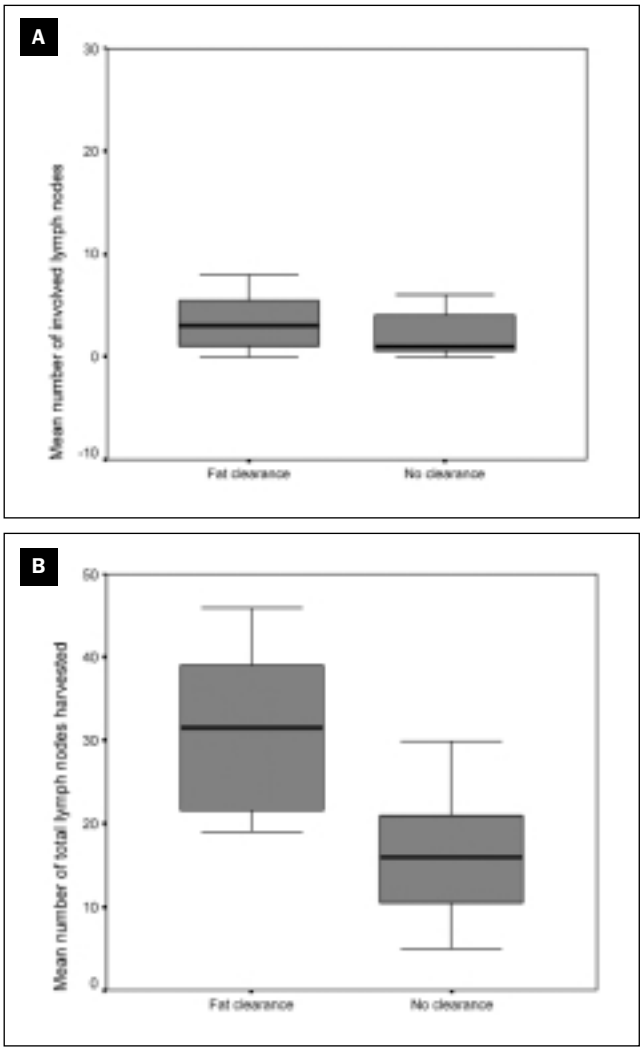

Fig. 3. Number of involved lymph nodes obtained (a) and total number of lymph nodes sampled (b) in the test and control groups.

of breaching health and safety guidelines. The amount of time taken to sample a fat-cleared specimen is equivalent to that usually devoted to a conventional lymph node dissection (20–40 min) in the authors' department.

Lymph node metastases commonly occur in small lymph nodes (<5 mm),[4,5] which are difficult to identify by conventional dissection but are relatively easy to find by fat clearance.[6] Here, a significantly higher lymph node yield was obtained using the one-day fat clearance technique, compared with that from the conventionally dissected control population. The results, although convincing, relate to a small number of specimens and an unequal distribution of irradiated cases, and therefore further study using a larger cohort is desirable.

Although the mean yield of lymph nodes containing deposits of metastatic carcinoma was found to be higher in the study population of 12 cases than in controls, it did not achieve statistical significance. The percentage of involved nodes was actually lower in the test group because more uninvolved nodes were sampled than in the control group. If the percentage of involved nodes were to be used for prognostic, surgical audit or management consideration, the results obtained by fat clearance would carry more weight than would results from a technique likely to miss a proportion of nodes.

It is widely acknowledged that lymph node yield is low in patients who have received pre-operative radiotherapy.[7] In specimens that had been irradiated, the total number of nodes harvested and the number of involved lymph nodes found using the one-day mechanised technique described here were both higher in the test group than in the control group. This mirrors the results of studies using more time-consuming fat-clearance methods.[3]

The Health and Safety aspects of fat clearance need to be borne in mind. All tissue in the authors' laboratory is processed under Control of Substances Hazardous to Health (COSSH) guidelines. Previously, when traditional fat clearance methods were used, laboratory staff endured significant exposure to alcohol and xylene fumes, as lymph nodes were transferred from one solvent bath to another, and the process was repeated daily for five to seven days. Using the 24-h 'closed' method described here, the only exposure to solvents is during dissection, which takes place in a fume hood.

As the inked resection margins are visible when the fat is processed in megaslices rather than shredded, the tissue orientation is maintained. Lymph nodes revealed in this way can be identified with regard to their location in the specimen, which is important because involved nodes close

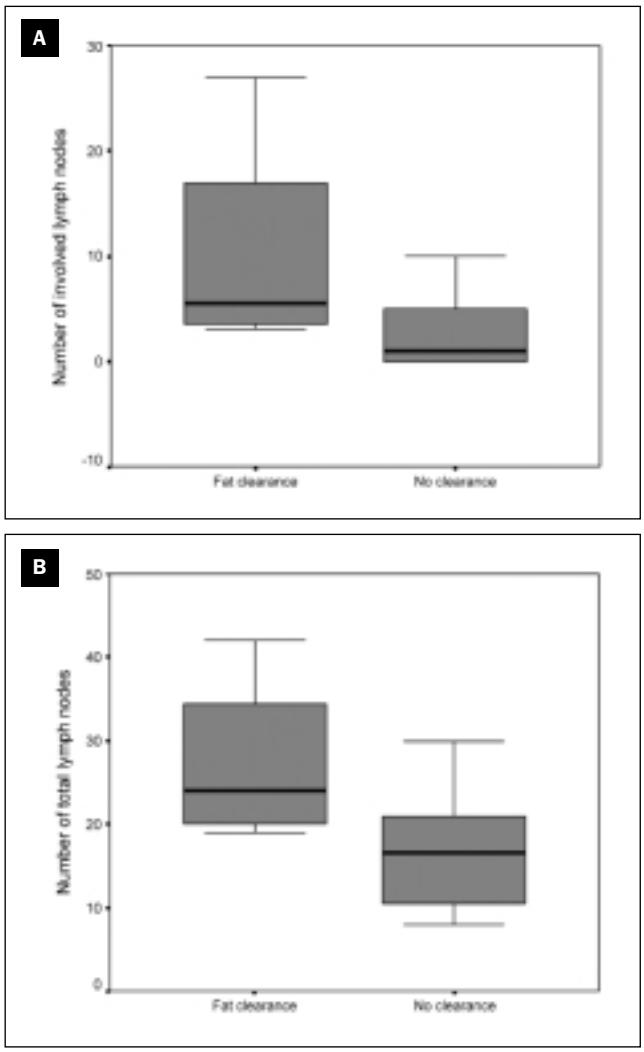

Fig. 4. Patients with preoperative radiotherapy: involved lymph node yield (a) and total lymph node yield (b) in the test and control groups.

to the mesorectal excision may have prognostic and treatment implications.

There are some minor disadvantages. For example, the laboratory must run an extra processing machine, the light box used for transillumination is bulky and must be stored when not in use (together with a glass shield to protect it from the effects of xylene), and an extra day's delay in reporting may be incurred. However, transillumination is required for other fat-clearance methods and the storage issues are the same, and the 24-h delay is unlikely to inconvenience either patient or surgeon in the immediate post-operative period.

In summary, the advantages of this technique are that it is cheap, easy to use and acceptable to biomedical scientists, pathologists and surgeons. In addition to the fact that the technique identifies all nodes in a specimen, it is quicker and less environmentally hazardous than are methods published previously, and has the extra advantage of being able to localise the site of involved lymph nodes relative to the excision margin. Disadvantages are that a separate processor is required, as the schedule involved differs slightly from routine processing, and the process adds a day to the reporting schedule. □

The authors are grateful for the financial assistance given to this project by the Pathology Private Fees Fund. An abstract based on this work was presented at the Annual Scientific Meeting of the Association of Coloproctology of Great Britain & Ireland, in Manchester in July 2002. Abstract: Colorectal Disease *2002; 4 (S 1): 56–7.*

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