GEWF Solution

An Inexpensive, Simple, and Effective Aid for the Retrieval of Lymph Nodes From Colorectal Cancer Resections

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• ***Background.*—Lymph node status is an important prognostic factor in the staging of colorectal carcinoma. Several adjunctive solutions have been used to increase the yield of pericolic lymph nodes from colorectal cancer resection specimens.**

***Methods.*—During 1998 at the Grey Bruce Regional Health Centre (Owen Sound, Ontario), 67 colonic resections were performed for colorectal cancer. Lymph nodes were identified using GEWF solution (glacial acetic acid, ethanol, distilled water, and formaldehyde) in 35 cases, and by the conventional method of sectioning, inspection, and palpation in 32 cases.**

***Results.*—There were no significant differences between GEWF and non-GEWF cases with respect to patient age, length of resection, size of tumor, tumor histologic type, tumor differentiation, or depth of tumor penetration into the bowel wall. Use of GEWF led to a significant increase in the number of lymph nodes found (10.2 ± 4.9 per case) compared with non-GEWF cases (6.8 ± 3.9 per case) (P = .002). In GEWF cases 358 lymph nodes were identified, 82 with metastases, whereas in the non-GEWF cases 218 lymph nodes were found, 41 with metastases. The size of positive lymph nodes in the GEWF group (0.5 ± 0.2 cm) was significantly smaller than in the non-GEWF group (0.7 ± 0.4 cm) (P = .046). A greater percentage of positive lymph nodes in the GEWF cases (49/82, 60%) were 0.5 cm or smaller compared with the non-GEWF cases (17/41, 41%).**

***Conclusions.*—GEWF increases the yield of lymph nodes recovered from colorectal cancer specimens and may lead to improved staging of this cancer; it is inexpensive and simple to use.**

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Pathologic staging is the most important factor in assessing prognosis in patients with colorectal cancer (CRC), and lymph node status has a pivotal role in the staging process. The issue as to what constitutes an adequate or minimum number of lymph nodes to be found during the pathologic examination of CRC resection specimens has been confusing. Some authors have prescribed that a minimum number of lymph nodes (ranging from 6 to 17 per case) is required for adequate staging.[1–6] Others have recommended simply that as many lymph nodes as possible should be examined.[7] It has also been suggested that a complete search of the pericolic fat should include lymph nodes measuring 2 mm or less in greatest dimension.[8] Studies with and without pericolic fat clearing have shown that 44% to 78% of lymph nodes containing metastatic tumor measure 5 mm or less.[9–13] It is therefore important that as many lymph nodes as possible be found to stage CRCs adequately, and it is also important to find small lymph nodes, as these may harbor metastatic disease.

The ability to identify lymph nodes in the pericolic fat is dependent on several factors, not the least of which is the pathologist's skill and enthusiasm. The standard method (serial sectioning, inspection, and palpation) may be regarded as arduous, and lymph nodes may be missed, especially those 5 mm or less in size. A number of adjunctive methods have been developed to increase lymph node yield. These include fat stretching,[14] alcohol treatment,[10] xylene clearance,[6,9,15,16] wintergreen oil/cedar oil clearance,[12,13,17] and most recently ''lymph node revealing solution,'' an ether-based method.[18] Most methods require special equipment, the use of noxious volatile compounds, and/or prolonged treatment of pericolic fat (up to 3 weeks). In some studies, the additional positive lymph nodes have increased the stage of some cancers (Dukes B, TNM stage II to Dukes C, TNM stage III).[6,9,16,18] These results suggest that the use of adjunctive techniques to identify lymph nodes may lead to improved staging of CRCs. In addition, the most recent protocol for the examination of CRC specimens from the Cancer Committee of the College of American Pathologists recommends that if fewer than 12 nodes are found with traditional methods, then the use of ''visual enhancement techniques'' should be considered.[19]

In this article, we report the results of a retrospective study on the usefulness of a novel solution (GEWF solution) for highlighting lymph nodes in the pericolic fat from CRC resection specimens. This method is inexpensive and

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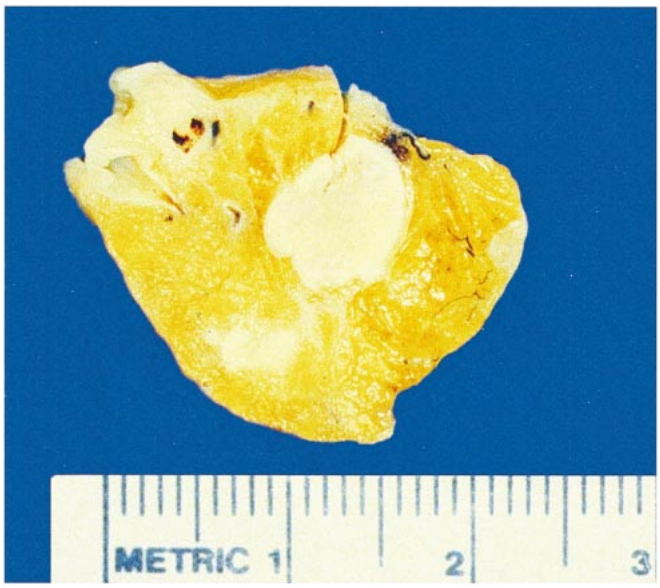
Table 1. Materials Used in the Preparation of GEWF*	
Reagent	**Volume, L**
Absolute ethanol	10.0
Distilled water	3.4
Formaldehyde (40%)	1.6
Glacial acetic acid	1.0

* The reagents are mixed in the order listed.



Two lymph nodes in a fragment of pericolic fat following GEWF treatment.

simple to perform, and the solution does not involve the use of noxious chemicals or additional equipment.

MATERIALS AND METHODS

The pathology database of the Grey Bruce Regional Health Centre, a community hospital in Southwestern Ontario, was searched for colonic resections for adenocarcinoma for the dates January 1, 1998 through December 31, 1998. This search yielded 67 cases, all of which were included in this study. Final reports for all of the cases were issued at least 8 months prior to the conception and initiation of the study, which was undertaken as a quality assurance project. None of the pathologists who signed out the cases were aware that a future retrospective study to assess the usefulness of GEWF would be performed.

GEWF is a modified Lillie acetic acid–alcohol–formalin fixative (Table 1)[20] and is prepared by mixing the reagents in the following order: alcohol, distilled water, formaldehyde, and glacial acetic acid. On average, 1 L of solution is used per resection, at a cost of Can $2.70 (US $1.76). GEWF does not require the use of additional equipment and can be used with standard grossing bench ventilation units without detectable odor.

All CRC specimens were received in the pathology department, where they were opened, pinned, and allowed to fix overnight (16–24 hours) in 10% neutral buffered formalin. Standard sections (excluding lymph nodes) were then taken. In 32 (non-GEWF) of the 67 cases, pericolic fat was then removed, serially sectioned at 5- to 8-mm intervals, and lymph nodes were identified by inspection and palpation (standard technique). For the remaining 35 (GEWF) cases, the pericolic fat was removed and immersed in approximately 1 L of GEWF (corresponding to 2–3 volumes of pericolic fat) for 12 to 18 hours. Following GEWF treatment, the pericolic fat was serially sectioned at 5- to 8-mm intervals, and lymph nodes were identified as firm chalky white nodules (Figure).

Sections were processed using standard techniques and embedded in paraffin. Sections cut at 5 μm were stained with hematoxylin-eosin. The size of positive lymph nodes was determined retrospectively by measuring the largest dimension of the lymph node tissue on the hematoxylin-eosin–stained sections. To determine if the use of GEWF had any adverse effects on special stains (periodic acid–Schiff and periodic acid–Schiff plus diastase) or immunohistochemistry (cytokeratin and leukocyte common antigen), the staining characteristics for 3 randomly selected positive lymph nodes from both the GEWF and non-GEWF cases were compared by 2 independent observers (K.J.N., B.F.R.). Statistical analyses were performed using 2-tailed Student *t* tests. A 2-sided *P* value less than .05 was considered significant. Results are expressed as mean ± SD where appropriate.

RESULTS

GEWF was used in the processing of 35 cases; 32 cases were used without any enhancement technique. The non-GEWF and GEWF groups had similar demographics with respect to sex and age (Table 2). There was no difference with respect to tumor location or length of colon resected (Table 2). With respect to the characteristics of the resected tumors (size, histologic type, grade, and depth of penetration into the bowel wall), there were no differences between the 2 groups (Table 2).

The use of GEWF lead to a significant increase in the number of lymph nodes found, 358 (10.2 ± 4.9 per case), compared with the number identified with the conventional method, 218 (6.8 ± 3.9 per case) ($P = .002$) (Table 3). There were 82 positive lymph nodes in the GEWF cases compared with 41 positive lymph nodes in the non-GEWF cases. The average size of the lymph nodes containing metastatic tumor in the GEWF cases (0.5 ± 0.2 cm) was significantly smaller than in the non-GEWF cases (0.7 ± 0.4 cm) ($P = .046$). Also, there was a greater percentage of positive lymph nodes measuring 5 mm or less in greatest dimension in the GEWF group (60%) compared with the non-GEWF group (41%). For the GEWF cases, 46% (16/35) were classified as Dukes C (TNM stage III) compared with 31% (10/32) in the non-GEWF cases.

There were no observable differences in the staining of tissues with hematoxylin-eosin, special stains, or immunohistochemistry between the 2 groups.

COMMENT

The use of GEWF in this series of 67 CRCs led to a 1.5-fold increase in the number of lymph nodes found per case compared with the conventional method (10.2 vs 6.8), and a greater percentage of positive lymph nodes found using GEWF (60% vs 41%) were 5 mm or less in greatest dimension. Other adjunctive techniques have increased lymph node yields to a greater degree (2.2- to 6.5-fold),[6,14,15,16,18] but interestingly have reported similar percentages (44%–78%) of positive lymph nodes 5 mm or less in greatest dimension.[9–13] The increased yield with these solutions comes at the expense of greater inconvenience and cost compared with GEWF. Furthermore, finding more than 10 lymph nodes may not confer significant benefits; it has been shown that if 10 pericolic lymph nodes are examined, there is a 99% probability of correctly discriminating between Dukes B (TNM stage II) and Dukes C (TNM stage III) cancers.[4] The protocol for the examination of CRC specimens from the Cancer Committee of the College of American Pathologists states that 12 negative lymph nodes predicts for regional node negativity.[19] The data in the present study indicate that with the rou-

Table 2. Summary of Characteristics of GEWF and non-GEWF Colorectal Cancer Cases		
	GEWF (n = 35)	Non-GEWF (n = 32)
Sex		
Male	18	14
Female	17	18
Age, y*	71 ± 11	73 ± 9
Tumor location		
Right	19	16
Left	13	14
Rectal	3	2
Length of resection, cm*	22 ± 8	29 ± 27
Tumor dimension, cm*	4.4 ± 1.3	3.9 ± 1.6
Tumor type		
Adenocarcinoma, nos (well differentiated)	5	0
Adenocarcinoma, nos (moderately differentiated)	18	21
Adenocarcinoma, nos (poorly differentiated)	6	7
Mucinous adenocarcinoma	6	4
Depth of penetration		
Submucosa	1	1
Muscularis propria	2	5
Pericolic fat	26	20
Serosal surface	6	6

* Values presented as mean ± SD.

Table 3. Lymph Node Status of Cases		
	GEWF (n = 35)	Non-GEWF (n = 32)
Total no. of lymph nodes found	358	218
No. of lymph nodes per case,* mean ± SD	10.2 ± 4.9	6.8 ± 3.9
Total no. of positive lymph nodes	82	41
Mean size of positive lymph nodes, cm,† mean ± SD	0.5 ± 0.2	0.7 ± 0.4
No. (%) of positive lymph nodes ≥0.5 cm	49 (60)	17 (41)
TNM lymph node status	19 N0	22 N0
	8 N1	5 N1
	8 N2	5 N2

* $P = .002$.
† $P = .046$.

tine use of GEWF, it should be possible to obtain an adequate number of pericolic lymph nodes to identify Dukes C cancers appropriately.

GEWF is a modification of a fixative described in 1949 by Lillie.[20] Although other adjunctive methods were not evaluated in this study, comparisons based on previous reports show advantages in the use of GEWF over other methods. GEWF acts rapidly and does not involve the use of volatile noxious substances as used in other methods, for example, ether,[18] xylene,[6,9,15,16] wintergreen oil, or cedar oil.[12,13,17] GEWF does not require the use of additional equipment, it can be used with conventional ventilation, and the additional cost of Can $2.70 (US $1.76) per case is minimal.

Studies that compare the ability of novel versus standard methods to identify additional lymph nodes in CRC specimens are subject to bias. Although this study was retrospective, we believe that this approach provides the best method to study the usefulness of GEWF under standard working conditions. In a prospective study, pathologists would certainly be aware of whether they were using the novel method, and those pathologists using the standard technique would likely examine pericolic fat more thoroughly. This bias would apply even if pathologists grossly examined half of the specimens with the novel method and half without.

This study, which should not be the final word on the use of visual enhancement techniques vis-à-vis the identification of lymph nodes in fat, has shown that GEWF is simple and nontoxic to use, has no effect on routine special stains or immunohistochemistry, and is therefore ideal for use in a busy primary or tertiary care hospital. By way of visual enhancement of lymph nodes within fat, GEWF serves to reduce, albeit not entirely, the operator dependence on lymph node retrieval. In the case of CRC resection specimens, the routine use of GEWF may lead to improved staging of CRC. Anecdotal evidence reveals that GEWF also works efficiently as an aid to lymph node retrieval in other specimens, for example, axillary dissections for breast carcinoma. Finally, we suggest that a longitudinal study is required to demonstrate that the identification of additional lymph nodes using GEWF or any of the previously described adjunctive techniques has prognostic and/or therapeutic significance in cases of CRC.

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