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Lymph node staging in colon cancer

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Chapter 3

Effect of modified Davidson's fixative on examined number of lymph nodes and TNM-stage in colon carcinoma

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Abstract

Aims: We evaluated the effect of modified Davidson's Fixative (mDF) on the number of lymph nodes examined and staging in patients with colon carcinoma.

Methods: The results of two different fixation methods used in the pathological preparation of the resection specimens were analyzed. A traditional formalin preparation with manual dissection of all nodes was performed in 117 colon specimens between January 2003 and July 2004. After July 2004, the resected specimen of 125 patients were fixated in mDF. Differences in the retrieval and number of nodes and size of suspected nodal metastases were measured. All lymph nodes were stained with conventional H&E methods.

Results: The median number of examined nodes increased from 5 (0-17) to 13 (0-35) nodes after the introduction of mDF ($p < 0.001$). The type of resection and the T-stage influenced the number of retrieved nodes significantly. The percentage of node positive cases increased from 30% to 41% ($p = 0.077$) with mDF, the median size of the retrieved lymph nodes decreased from 9 mm before to 6 mm ($p < 0.001$) and more micrometastases were found (6% vs 16%, $p = 0.03$).

Conclusions: With mDF technique more lymph nodes were retrieved in the resected colon specimens. Smaller nodes and more micrometastases were found, leading to more node positive patients.

Introduction

The primary treatment for colon cancer is a radical surgical resection of the affected colon segment en-bloc with removal of related mesenteric lymph nodes. Adequate nodal staging is important for additional oncological treatment and to predict long-term survival based on the TNM classification.¹ In the assessment of nodal status the number of examined nodes is crucial. The impact of the surgeon and the surgical technique itself on quality and survival in patients with colorectal cancer have been described extensively.^{2,3} However, the number of nodes detected in a surgical specimen also depends on the diligence of the pathologist and the extent of the pathological examination.⁴⁻¹³ Numerous attempts have been made to estimate the minimal number of examined nodes for correct staging, varying from 6 to 18.^{4,8,13-15}

Several methods have been developed to increase lymph node yield, including xylene fat clearance, alcohol treatment and ether based clearance. Most of these methods require special equipment and the use of noxious volatile compounds and are time consuming with a delay in outcome (up to 3 weeks).^{5,6,11,16-20} Modified Davidson's fluid (mDF) is an acetic acid-alcohol-formalin based fixative that has been widely used for the preservation of different tissues for histological evaluation (www.histosearch.com, histonet archives, Davidson's fixative).^{21,22} It is a rapid, simple to use substance that provides no additional safety hazards or disposal problems compared to routine formalin solutions. (<http://members.aol.com/RSRICHMOND/histology.html>).

This report compares traditional neutral buffered formalin fixation and manual identification of lymph nodes with the use of mDF on number, size, and presence of metastases of detected lymph nodes in surgical resection specimens of colon cancer in a routine daily practice.

Patients and methods

Patients

All patients were treated in a Dutch teaching hospital between January 2003 and January 2006. Patients with evidence of distant metastatic disease were excluded from the study as the presence of distant metastases might have led to an unusual surgical and pathological approach that differed from standard recommendations. Patients with adenomas or polyps were excluded for the same reason. Since the number of detected lymph nodes is influenced by pre-operative radiotherapy which is routinely applied in rectal cancer in the Netherlands, patients with rectal cancer were excluded from the study. Rectal cancer was defined as a tumor situated within 15 cm from the anal verge located beneath the

peritoneal reflection. Patients with previous colorectal surgery were also excluded from the study.

All patients underwent a potential radical surgical resection according to the standard rules, based on the location of the primary tumor. The performed procedure was deduced retrospectively from the surgical and pathological reports.

Pathology

All five pathologists employed at the Martini Hospital routinely examined the resected specimens. From January 2003 to July 2004, all 117 specimens were examined using the traditional technique of manual dissection after overnight fixation in 10% neutral buffered formalin. From July 2004, after overnight fixation of the 125 specimens in 10% neutral buffered formalin, the pericolic fat and mesentery was removed and immersed in mDF containing 500 ml of 37% formalin, 750 ml of absolute ethanol, 25 ml of 1.2% glacial acetic acid and 750 ml tap water. After mDF fixation, lymph nodes turn white in the mesenteric fat. During the whole study period, lymph nodes were examined with conventional H&E staining at 5 mm intervals. The size of the lymph nodes and nodal metastases of node positive patients was determined by one of the pathologists (A.T.) retrospectively by measuring, in millimeters, the largest diameter of the lymph node tissue on H&E stained cross-sections of the lymph nodes.

Equivalent to the description of nodal metastases in breast cancer, lymph node metastases <0,2mm were called isolated tumor cells, metastases between 0,2 and 2mm were called micrometastases, and metastases >2mm were called macrometastases.¹

Statistical methods

SPSS 12.01 for Windows (SPSS, Inc, Chicago, IL) was the statistical software used for all the analyses. The level of significance was set to 0.05 for all tests. The χ^2 test was applied to test differences in proportions between groups. The Mann-Whitney U test was used to calculate the significance of differences in continuous variables.

Factors that were considered to be possible determinants of the number of examined lymph nodes and lymph node status were tested with an ANOVA analysis or regression analysis depending on the type of variable. The influence of possible determinants was also tested in multiple stepwise regression analysis for continuous variables and binary logistic regression analysis for nominal variables.

Results

Patients

Characteristics of the included patients and techniques before and after the introduction of mDF are listed in Table 1. Both groups did not differ significantly with respect to patient gender, age, T-stage, type of resection, length of specimen and the pathologist who examined the specimen. Due to changes in the surgical staff, there was a difference in the operating surgeons before and after the introduction of the mDF fixation.

Number of examined lymph nodes

All results for the number of examined nodes are shown in tables 1 and 2. The median number of examined nodes for the whole group was 10 (0-35). With traditional formalin fixation the median number of nodes was 5 (0-17). After the introduction of mDF the median number of nodes increased significantly to 13 (0-35). The ANOVA test showed that T-stage, the type of resection and the operating surgeon also might have an effect on the number of nodes. No effect was found for the pathologist and the length of the specimen. Linear stepwise regression analysis showed that the fixation technique was the most important predictor for the number of examined nodes, followed by the type of resection, T-stage and the operating surgeon. In this multivariate analysis the effect of the operating surgeon and T-stage were not significant. There was no significant difference in the mean number of nodes per surgeon when corrected for the type of resection. More nodes were removed with a right or left hemicolectomy compared to the other types of resection. In patients with a T1 tumor less nodes were removed compared to the other T-stages (mean 5 vs mean 10).

N-stage

Table 2 shows the results of the statistical analysis for nodal status. Table 3 shows the N-stage before and after the introduction of mDF. The percentage of node positive cases increases from 30% to 41% after the use of mDF. The mean number of nodes was 9.9 in the node-negative group and 10.4 in the node-positive group. The χ^2 test showed a possible effect of T-stage and fixation technique on N-stage. The type of resection, the operating surgeon, the pathologist, the length of the resected specimen removed and the number of examined nodes showed no effect. Both T-stage and fixation technique were tested in a binary logistic regression analysis. T-stage reached significance, while the fixation technique did not.

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Using the cut off point of the required 12 examined nodes according to the Dutch Cancer Guidelines; the proportion of node positive patients was 34% if less than 12 nodes are examined versus 38% if 12 or more nodes were examined which is not significant. To determine the minimal number of nodes to be examined for an accurate prediction of the N-stage, we divided the patients in groups based on the number of nodes removed. However, no difference was noted in N-stage per group (table 4).

Table 1. Patient, surgical and pathological factors

	Total group	Traditional technique	mDF	P
Gender ratio (♂/ ♀)	137/105	64/53	73/52	NS
Mean Age	73 (35-95)	74 (42-91)	72 (35-95)	NS
T-stage				NS
Tis	4	2	2	
T1	13	7	6	
T2	34	16	18	
T3	167	84	83	
T4	24	8	16	
Median Nr of nodes	10(0-35)	5(0-17)	13(0-35)	P=0.000
Type of resection				NS
Right hemicolectomy	119	56	63	
Left hemicolectomy	25	8	17	
Transversectomy	8	4	4	
Sigmoidectomy	86	46	40	
Ileocecal resection	4	3	1	
Surgeon				p=0.01
1	30	18	12	
2	13	5	8	
3	20	9	11	
4	22	8	14	
5	73	44	29	
6	30	9	21	
7	30	18	12	
8	24	6	18	
Mean length of specimen	25 (6-73)	25cm (7-70)	24cm (6-73)	NS
Pathologist				NS
1	23	12	11	
2	80	35	45	
3	34	19	15	
4	29	14	15	
5	76	37	39	

Table 2. Determinants of number of nodes and nodal status

	Univariate (p)	Multivariate (p)
Number of nodes	ANOVA	Linear regression
Fixation technique	0.000	0.000
T-stage	0.022	NS
Type of resection	0.042	0.010
Surgeon	0.034	NS
Pathologist	NS	
Length of specimen	NS	
Nodal status	χ^2	Logistic regression (p)
Fixation technique	0.077	NS
T-stage	0.009	0.004
Type of resection	NS	
Surgeon	NS	
Pathologist	NS	
Number of nodes	NS	
Length of specimen	NS (ANOVA)	

Table 3. N-stage before and after introduction of mDF

Stage	Total	Traditional technique	mDF
N0	156	82	74
N+ ¹	86	35	51
N1	64	26	38
N2	21	9	13
Total	242	117	125

¹All node-positive cases

Table 4. Percentage of N+ patients per nr of nodes

Nr of nodes (nr pts)	Node-positive patients (%)
≤ 6 (88)	34.1
≤ 8 (111)	35.1
≤ 10 (131)	32.8
≤ 12 (163)	33.7
≤ 14 (193)	35.2
≤ 16 (207)	35.7
≤ 18 (221)	35.3
≤ 20 (226)	35.4

Number of positive nodes and size of metastases

Before the introduction of mDF the total number of positive nodes was 84 with 5 micrometastases (5.9%) and 79 macrometastases (94%). After mDF fixation there were 126 positive nodes with 2 isolated tumor cells (1.6%), 18 micrometastases (14.2%) and 106 macrometastases (84%). This difference in the percentage of micro- and macrometastases is significant ($p=0.03$). The median size of the positive nodes found before introduction of the fixation technique was 9 mm. After changing the technique the size decreased to 6 mm. This difference is significant ($p<0.001$). The size of the negative lymph nodes found in the specimens with positive lymph nodes also decreased significantly from a median of 6 mm before the change of technique to 4 mm after ($p<0.001$).

Discussion

Methods

The serial study set up is not ideal for comparing two fixation methods. However, both study groups were comparable with respect to patient gender, age, T-stage, type of resection, length of specimen and the pathologist who examined the specimen. Although there was a difference in operating surgeons before and after the introduction of the mDF fixation, it was not a significant factor in the multivariate analysis in relation to the number of examined nodes. Moreover, there was no significant difference in the mean number of nodes per surgeon when corrected for the type of resection. Therefore, the study set up is applicable in this particular situation.

Number of examined lymph nodes

The principle of radical surgical resection of colon cancer includes removal of the affected colon segment with adequate margins en bloc with all draining lymph nodes in the corresponding mesocolon. The 5-year survival rate is 70-80% for patients with node negative disease (stage I/II), in contrast to 45-50 % for patients with node positive tumors (stage III).²³ Adjuvant chemotherapy in patients with stage III colon cancer clearly improves survival.²⁴⁻²⁶ The number of examined lymph nodes in a colectomy specimen varies widely. This may be due to variations in the surgical technique or the pathologist's attempt in retrieving the nodes from the resected specimen. There is substantial evidence that the number of lymph nodes examined has an important impact on survival in patients with colon cancer.^{4,7,9,27} An oncological specialized surgeon probably performs a more extensive lymphadenectomy which yields more nodes in the specimen. In addition, a pathologist who performs a more precise examination of the specimen also provides more accurate staging. It has not been possible to identify a single mechanism for improved outcome with increased node count. In our study the type of resection and the fixation technique are significant factors in the number of recovered lymph nodes. It is known that generally less lymph nodes are found in a sigmoidectomy or transversectomy specimen than in a right or left hemicolectomy specimen. Regarding the fixation technique, comparisons of mDF with previously described methods are clearly in favor of mDF. It is neither time-consuming nor costly and does not involve the use of noxious substances like diethyl ether or xylene which are used in fat clearance techniques. (<http://members.aol.com/RSRICHMOND/histology.html>). In addition, mDF can be used with conventional ventilation devices. After 24-48 hours of fixation specimens can be processed or transferred to alcohol or formalin for storage. Due to this rapid effect, safety and low costs it is ideal for use in a busy primary or tertiary care hospital. With mDF lymph nodes turn white in the yellow mesenteric fat, making it easier for the pathologist to identify even small lymph nodes, thereby reducing the operator dependence in lymph node retrieval.²¹ Two studies showed that 72% of the metastatic lymph nodes are smaller than 5 mm in diameter.^{5,6} In our study indeed more and smaller lymph nodes are found with mDF, which may lead to an increase in lymph node metastases.

N-stage

Not only found more lymph nodes were detected after the introduction of mDF, but we also found more and smaller positive nodes. This can be explained by the white color of regional nodes, which facilitates detection compared to conventional manual dissection

with non-white nodes. In addition, more micrometastases were noted with mDF. Both factors probably contributed to 11% more node positive patients after the introduction of this mDF. Although not significant with $p=0.077$, it does seem clinically relevant for nodal staging. It could be that our population is just too small to detect a significant difference. Therefore, larger studies are required to demonstrate the real impact of additional, smaller lymph nodes on prognosis and/or their therapeutic significance. It was not possible to find a cut off value in the number of lymph nodes to be examined to find more nodal metastases with this modified fixation method. Using the recommended cut off number of 12 nodes we did not find a significant difference in the percentage of node positive patients.^{1,14} Even when we used cut off points of 6, 14 or 18 lymph nodes as mentioned in most studies^{8,15,28}, no significant difference in node-positivity was found. Again, insufficient patient numbers might play a role. As Goldstein stressed the importance to examine even lymph nodes of 1 or 2 mm in diameter⁴ our study confirmed that the difference in N-stage seems to depend on the smaller metastases found after mDF fixation. Therefore, it is important to search also for smaller nodes and not only for the highest number of large nodes.⁵

In this single center study the number of nodes recovered, the surgeons involved in the operation and the pathologists were of no significant importance. The only important factors were T-stage and the use of mDF. The increase in node-positivity with higher T-stages is expected, as it represents a more advanced disease.

Effects of staging on adjuvant therapy

As the two patient groups are not related, we cannot state that there is any upstaging after mDF. We have only observed that with mDF 41% of the patients had lymph node metastases compared to 30% with formalin fixation. We have to wait for the survival data of both patient groups before we can draw any conclusions on the importance of this fixation technique for staging and prognosis. Hypothetically, it is interesting to calculate what could happen if 11% more patients would be offered adjuvant chemotherapy, keeping in mind that before July 2004 patients with less than twelve examined lymph node did not automatically receive chemotherapy in our region. In our hospital, we treat a part of the population covered by the Comprehensive Cancer Center North Netherlands (CCCNN). In this northern region, 625 colon resections are performed annually in colon cancer patients without proven metastases. An increase of 11% in lymph node metastases will lead to 69 more patients being referred for adjuvant chemotherapy. With the current chemotherapy regimens an increase in the 5-year survival rate of 15-20% can be expected compared to

no adjuvant therapy at all.²⁹ Considering this, about 10 to 14 people would benefit in overall survival, assuming that they all do receive adjuvant treatment.

Conclusion

After adequate surgical resection in patients with colon cancer, the pathologists may improve the staging procedure by using the mDF fixation technique which is simple, rapid and cheap. With this method more and smaller lymph nodes and smaller nodal metastases were detected. This may result in upstaging and a possible survival benefit as more patients will be offered adjuvant chemotherapy.

Reference List

1. Greene, F. L. Page D. L. Fleming I. D. et al. American Joint Committee on Cancer - Cancer staging handbook, TNM classification of malignant tumors. 129. 2002. New York: Springer.
2. Gunnarsson U. Quality assurance in surgical oncology. Colorectal cancer as an example. *Eur J Surg Oncol* 2003; 29: 89-94.
3. Kapiteijn E, Putter H, van de Velde CJ. Impact of the introduction and training of total mesorectal excision on recurrence and survival in rectal cancer in The Netherlands. *Br J Surg* 2002; 89: 1142-9.
4. Goldstein NS. Lymph node recoveries from 2427 pT3 colorectal resection specimens spanning 45 years: recommendations for a minimum number of recovered lymph nodes based on predictive probabilities. *Am J Surg Pathol* 2002; 26: 179-89.
5. Haboubi NY et al. The novel combination of fat clearance and immunohistochemistry improves prediction of the outcome of patients with colorectal carcinomas: a preliminary study. *Int J Colorectal Dis* 1998; 13: 99-102.
6. Hida J et al. Metastases from carcinoma of the colon and rectum detected in small lymph nodes by the clearing method. *J Am Coll Surg* 1994; 178: 223-8.
7. Jestin P, Pahlman L, Glimelius B, Gunnarsson U. Cancer staging and survival in colon cancer is dependent on the quality of the pathologists' specimen examination. *Eur J Cancer* 2005; 41: 2071-8.
8. Joseph NE et al. Accuracy of determining nodal negativity in colorectal cancer on the basis of the number of nodes retrieved on resection. *Ann Surg Oncol* 2003; 10: 213-8.
9. Le Voyer TE et al. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003; 21: 2912-9.
10. Liefers GJ et al. Micrometastases and survival in stage II colorectal cancer. *N Engl J Med* 1998; 339: 223-8.
11. Scott KW, Grace RH, Gibbons P. Five-year follow-up study of the fat clearance technique in colorectal carcinoma. *Dis Colon Rectum* 1994; 37: 126-8.
12. Tepper JE et al. Impact of number of nodes retrieved on outcome in patients with rectal cancer. *J Clin Oncol* 2001; 19: 157-63.
13. Wong JH, Severino R, Honnebiel MB, Tom P, Namiki TS. Number of nodes examined and staging accuracy in colorectal carcinoma. *J Clin Oncol* 1999; 17: 2896-900.
14. Fielding LP et al. Clinicopathological staging for colorectal cancer: an International Documentation System (IDS) and an International Comprehensive Anatomical Terminology (ICAT). *J Gastroenterol Hepatol* 1991; 6: 325-44.

15. Hernanz F et al. Colorectal adenocarcinoma: quality of the assessment of lymph node metastases. *Dis Colon Rectum* 1994; 37: 373-6.
16. Cawthorn SJ, Gibbs NM, Marks CG. Clearance technique for the detection of lymph nodes in colorectal cancer. *Br J Surg* 1986; 73: 58-60.
17. Pickren JW. Current concepts in cancer. Nodal clearance and detection. *JAMA* 1975; 231: 969-71.
18. Brown HG, Luckasevic TM, Medich DS, Celebrezze JP, Jones SM. Efficacy of manual dissection of lymph nodes in colon cancer resections. *Mod Pathol* 2004; 17: 402-6.
19. Koren R et al. Lymph node-revealing solution: simple new method for detecting minute lymph nodes in colon carcinoma. *Dis Colon Rectum* 1997; 40: 407-10.
20. Svec A, Horak L, Novotny J, Lysy P. Re-fixation in a lymph node revealing solution is a powerful method for identifying lymph nodes in colorectal resection specimens. *Eur J Surg Oncol* 2006; 32: 426-9.
21. Newell KJ, Sawka BW, Rudrick BF, Driman DK. GEWF solution. *Arch Pathol Lab Med* 2001; 125: 642-5.
22. Latendresse JR, Warbritton AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol Pathol* 2002; 30: 524-33.
23. Hermanek P. pTNM and residual tumor classifications: problems of assessment and prognostic significance. *World J Surg* 1995; 19: 184-90.
24. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. *Lancet* 1995; 345: 939-44.
25. Gill S et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004; 22: 1797-806.
26. Taal BG, Van Tinteren H, Zoetmulder FA. Adjuvant 5FU plus levamisole in colonic or rectal cancer: improved survival in stage II and III. *Br J Cancer* 2001; 85: 1437-43.
27. Berger AC et al. Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. *J Clin Oncol* 2005; 23: 8706-12.
28. Wong JH, Bowles BJ, Bueno R, Shimizu D. Impact of the number of negative nodes on disease-free survival in colorectal cancer patients. *Dis Colon Rectum* 2002; 45: 1341-8.
29. Andre T et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; 350: 2343-51.

