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Fixation Using Carnoy's Solution Enables Detection of More Lymph Nodes After Gastrectomy for Gastric Cancer

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Abstract. *Background: To compare the number of lymph nodes (LNs) detected when using Carnoy's solution (CS) versus 10% neutral buffered formalin (NBF) to fix specimens after radical gastrectomy for gastric cancer. Patients and Methods: LNs were routinely detected using NBF until 2020, since then, for the fixation procedure, residual fat was fixed in CS for 24 hours and dissected again for the detection of further LNs. Of 143 specimens, 117 were included in the NBF group and 26 in the CS group. Results: The mean numbers of LNs examined were 27.85±14.89 and 36.30±12.41 in the NBF and CS groups, respectively (p=0.008). The mean number of additional LNs detected using CS was 8.07±2.91, of which 0.38±1.02 were metastatic. Additional LNs were found in all patients of the CS group, and all were ≤3 mm. Of the 26 patients in the CS group, metastatic LNs were detected in four, disease in two of whom was up-staged. Conclusion: CS is an appropriate alternative to NBF for the fixation of gastric cancer specimens, and more LNs were detected in the resected specimens fixed when using CS compared with NBF.*

Gastric cancer is the fourth most common cause of malignancy and the second leading cause of cancer-related mortality worldwide (1). Among several factors related to the survival of patients with gastric cancer, nodal status is the most important prognostic factor for accurate staging and in the decision to perform adjuvant treatment after surgical procedures for gastric cancer (2-5). The pathological nodal (pN) stage is classified as pN0, pN1, pN2, pN3a, or pN3b according to the number of metastatic nodes. The presence of ≥16 metastatic lymph nodes (LN) is defined as pN3b, and the Union for International Cancer Control and American Joint Committee on Cancer recommend that ≥16 LNs should be harvested for assessment after gastrectomy for gastric cancer with curative intent (6). Nodal stage can be influenced by the absolute number of LNs harvested, and the examination of only a small number of LNs can lead to stage migration (2). Several studies demonstrated that other options for nodal staging, such as the LN ratio and log odds of positive LNs, are superior to the absolute number-based pN system (5, 7, 8).

Although extensive surgical LN dissection is the most important factor, the ability of the pathologist to identify metastatic LNs in the resected specimen is also crucial for accurate pathological analysis. Even small-sized LNs <1 mm may have cancer cell metastasis, and not detecting these LNs in the resected specimen may also lead to underestimation of nodal stage.

Carnoy's solution (CS) is a fixative composed of ethanol and glacial acetic acid which is used for dental treatment of keratocystic odontogenic tumors. It can also be used for enhancing LN detection during dissection (9).

The chemical cauterizing effect of CS on living tissue allows its use as an additional topical treatment after surgical enucleation of bone cysts, keratocystic tumors of the jaw, and certain other benign tumors such as ameloblastoma. In addition, CS can be used as an LN-revealing solution because the ethanol extracts phospholipids from the cells, which makes LNs much more visible in excised specimens (10-13).

This study was performed to determine whether more LNs would be detected in a sample when using CS compared with using 10% neutral buffered formalin (NBF) to fix specimens after radical gastrectomy for gastric cancer.

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Key Words: Carnoy's solution, lymph node, gastric cancer.

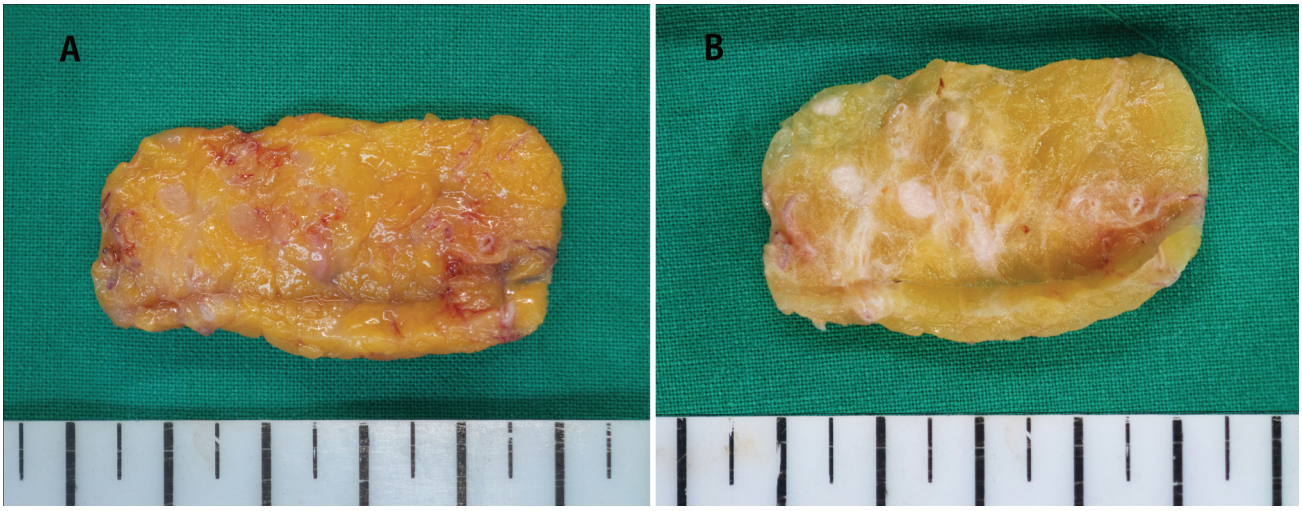

Figure 1. *Cut surface of a resected specimen before (A) and after (B) fixation with Carnoy's solution.*

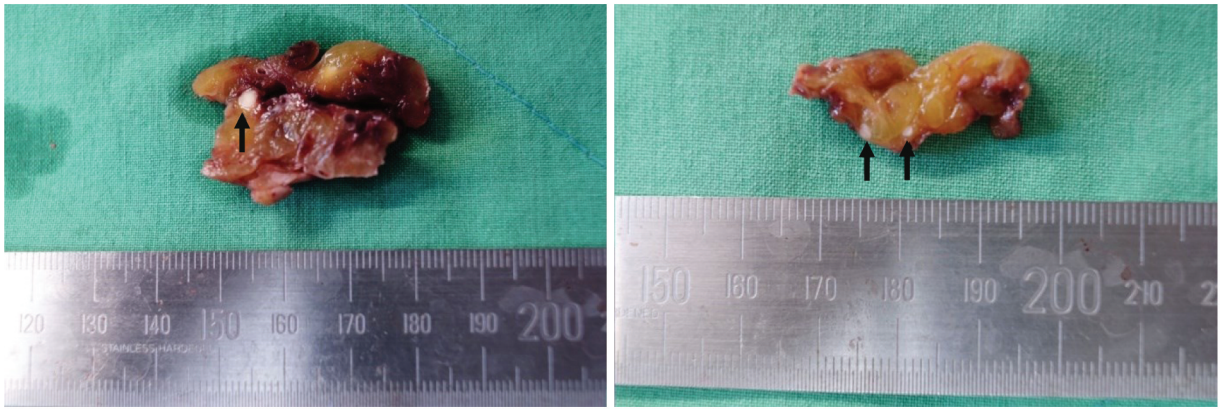

Figure 2. *Small-sized lymph nodes identified after fixation using Carnoy's solution.*

Patients and Methods

Patients. A total of 143 consecutive patients who had undergone radical gastrectomy with curative intent at our hospital between 2016 and 2020 were enrolled in the study. Six of these patients were diagnosed with stage IV disease because the results of washing cytology were positive for malignancy or metastatic LNs were found in omental tissue. Prior to 2020, resected specimens were fixed using NBF according to the standard procedure. Since 2020, following node detection using NBF, the residual fat tissue is fixed again in CS for 24 h and dissected again. Of the 143 specimens, 117 were included in the NBF group and 26 in the CS group.

Pathological examination. Initially, the perivisceral fat tissue was fixed in NBF solution for 24-48 h. Pathological examination was performed according to the College of American Pathologists protocol (14). All LNs retrieved were embedded in paraffin blocks and sent for microscopic examination. The remaining residual fat tissue was then submerged in CS for 24 h and dissected to detect additional small-sized LNs.

This study was approved by our Institutional Review Board (approval number: 2019-07-014-003) which waived the need for informed consent for the use of patient data due to the retrospective nature of the study. All methods were performed in accordance with the relevant guidelines and regulations.

Statistical analysis. Clinical outcomes and complication rates (Clavien–Dindo classification grade ≥II) (15) were compared between the groups using Student's *t*-test or the chi-square test, as appropriate. Statistical analyses were performed using SPSS (ver.

Table I. *Baseline characteristics of specimens stained with neutral buffered formalin (NBF) and with Carnoy's solution (CS).*

Characteristic		Whole cohort	NBF group (n=117)	CS group (n=26)	*p*-Value
Age, years	Mean±SD	66.45±10.68	67.30±10.68	62.61±9399	**0.042**
Gender, n (%)	Male	104 (72.7)	84 (71.8)	20 (76.9)	0.638
	Female	39 (27.3)	33 (28.2)	6 (23.1)	
BMI, kg/m^2	Mean±SD	24.34±3.95	24.16±3.98	25.18±3.80	0.236
ECOG PS, n (%)	0	95 (66.4)	72 (61.5)	23 (88.5)	**0.029**
	1	43 (30.1)	40 (34.2)	3 (11.5)	
	2	5 (3.5)	5 (4.3)	0 (0)	
Comorbidity, n (%)	No	52 (36.4)	39 (33.3)	13 (50)	0.120
	Yes	91 (63.6)	78 (66.7)	13 (50)	
CEA, ng/ml	Mean±SD	4.30±6.42	4.03±5.80	5.40±8.61	0.341
Approach, n (%)	Open	60 (42.0)	52 (44.4)	8 (390.8)	0.265
	LAG	2 (1.4)	2 (1.7)	0 (0)	
	TLG	77 (53.8)	59 (50.4)	18 (69.2)	
	Robotic	4 (2.8)	4 (3.4)	0 (0)	
Resection, n (%)	TG	33 (23.1)	2 (23.9)	5 (19.2)	0.793
	DSG	107 (74.8)	86 (80.8)	21 (80.8)	
	PG	2 (1.4)	2 (1.7)	0 (0)	
	Whipple	1 (0.7)	1 (0.9)	0 (0)	
Residual tumor, n (%)	R0	135 (94.4)	110 (94.0)	25 (96.2)	>0.999
	R1	8 (5.6)	7 (6.0)	1 (3.8)	
Node dissection, n (%)	D1	13 (9.1)	13 (11.0)	0 (0)	0.078
	D1+	84 (58.7)	70 (59.8)	14 (53.8)	
	D2	46 (32.2)	34 (29.1)	12 (46.2)	
Operative time, min	Mean±SD	169.79±53.94	169.28±51.18	169.61±66.10	0.986
EBL, ml	Mean±SD	120.48±133.74	119.40±133.24	125.38±138.57	0.837
Tumor size, cm	Mean±SD	3.84±3.11	3.69±3.05	4.51±3.32	0.230
PRM, cm	Mean±SD	4.34±2.90	4.36±2.97	4.26±2.62	0.865
DRM, cm	Mean±SD	6.23±4.26	6.02±4.13	7.11±4.80	0.243
T Stage, n (%)	T1	85 (59.9)	70 (60.3)	15 (57.7)	0.292
	T2	10 (7.0)	9 (7.8)	1 (3.8)	
	T3	25 (17.6)	22 (19.0)	3 (11.5)	
	T4a	22 (15.5)	15 (12.9)	7 (26.9)	
N Stage, n (%)	N0	106 (74.6)	86 (74.1)	20 (76.9)	0.579
	N1	7 (4.9)	7 (6.0)	0 (0)	
	N2	13 (9.2)	11 (9.5)	2 (7.7)	
	N3a	7 (4.9)	6 (5.2)	1 (3.8)	
	N3b	9 (6.3)	6 (5.2)	3 (11.5)	
M Stage, n (%)	M0	136 (95.8)	111 (95.7)	25 (96.2)	>0.999
	M1	6 (4.2)	5 (4.3)	1 (3.8)	
Pathologic stage, n (%)	I	89 (62.7)	73 (62.9)	16 (61.5)	0.958
	II	24 (16.9)	20 (17.2)	4 (15.4)	
	III	23 (16.2)	18 (15.5)	5 (19.2)	
	Iv	6 (4.2)	5 (4.3)	1 (3.8)	
LN, mean±SD (median)	Harvested	29.40±14.80 (27)	27.85±14.89 (24)	36.30±12.41 (33)	**0.008**
	Metastatic	2.73±8.75 (0)	2.58±8.98 (0)	3.38±7.75 (0)	0.676
	Additional		0	8.07±2.91 (7)	**<0.001**
	Additional metastatic		0	0.38±1.02 (0)	0.067
Differentiation, n (%)	Differentiated	63 (45.7)	52 (46.0)	11 (44.0)	>0.999
	Undifferentiated	75 (54.3)	61 (54.0)	14 (54.3)	
Complication, n (%)	No	115 (81.0)	95 (81.9)	20 (76.9)	0.584
	Yes	27 (19.0)	21 (18.1)	6 (23.1)	
Postoperative stay, days	Mean±SD	11.57±6.81	12.01±7.31	9.61±3.34	0.105

BMI: Body mass index; CEA: carcinoembryogenic antigen; DRM: distal resection margin; EBL: estimated blood loss; ECOG: Eastern Cooperative Oncology Group; LAG: laparoscopy-assisted gastrectomy; LN: lymph node; PRM: proximal resection margin; TLG: totally laparoscopic gastrectomy. Statistically significant *p*-values are shown in bold.

Table II. *Cases with additional metastatic lymph nodes (LNs) detected using Carnoy's solution (CS).*

Case no.	NBF	N Stage	CS	NBF plus CS	N Stage
4	15/22	N3a	3/15	18/37	N3b
8	25/34	N3b	4/17	29/51	N3b
13	19/41	N3b	2/9	21/50	N3b
18	2/15	N1	1/7	3/22	N2

Data are expressed as the number of metastatic LNs/harvested LNs; NBF: neutral buffered formalin.

12.0; SPSS Inc., Chicago, IL, USA). In all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

It was much easier by naked eye to distinguish the LNs from the fat tissue after 24 hours of fixation using CS because the loss of phospholipids in the adipose tissue increased the contrast between the fat and LNs (Figure 1). It was possible to discriminate even small-sized LNs (<2 mm) in cross-sectional views of perivisceral fat tissue (Figure 2).

The NBF and CS groups were similar in terms of baseline characteristics except for age, Eastern Cooperative Oncology Group performance status, and the number of harvested LNs. The mean ages of the patients were 67.30±10.68 and 62.61±93.99 years for the NBF and CS groups, respectively ($p=0.042$). The proportion of patients with an Eastern Cooperative Oncology Group performance status of 0 was higher in the CS group than the NBF group ($p=0.029$) (Table I). As this was not a prospective randomized controlled study, more cases would have been necessary to match the clinical factors between groups.

The mean number of LNs examined was 27.85±14.89 (median=24) and 36.30±12.41 (median=33) in the NBF and CS groups, respectively ($p=0.008$). The mean number of additional LNs detected using CS was 8.07±2.91 (median=7), of which 0.38±1.02 were metastatic. Additional LNs were found in all patients of the CS group, and all were ≤3 mm. Of the 26 patients in the CS group, metastatic LNs were detected in four, in two of whom disease was up-staged (Table II).

Discussion

As radical gastrectomy including D2 dissection during gastric cancer surgery is becoming more commonly performed, the number of harvested LNs is steadily increasing. The American Joint Committee on Cancer recommends that at least 16 LNs should be harvested for accurate nodal staging. In addition, the consensus on stage migration, also known as the 'Will Rogers' phenomenon, is that the number of metastatic LNs can be influenced by the number harvested (16). If the number of resected LNs is small, stage underestimation may occur. However, even if radical surgery is performed in the same manner, the number of LNs examined can differ among institutions. Although the extent of surgical LN dissection is important, the ability of the pathologist to identify metastatic LNs in the resected specimen is also crucial for accurate pathological analysis. LN dissection in the specimen is crucial for precise staging but is a labor-intensive and time-consuming process. LNs <1-2 mm are often difficult to identify with the naked eye. The results of this study showed that fixation of resected specimens with CS allowed detection of additional small-sized LNs.

Many studies demonstrated that fixation using CS significantly increases the total number of LNs identified in many malignant diseases. Pereira *et al*. (13) and Flynn *et al*. (11) reported that CS is adequate for routine utilization in surgical and molecular pathology. In addition, Dias *et al*. demonstrated that CS increased LN detection and allowed more accurate pathological staging following gastrectomy for gastric cancer (12). Pereira *et al*. reported that CS is an adequate fixative for preserving cell morphology and molecular integrity (13). Therefore, CS may provide an alternative to NBF that allows easier handling of resected specimens for identification of LNs. CS also provides acceptable preservation of tissue morphology and antigenic and molecular integrities using standard surgical pathology procedures (9-12).

However, it is not clear whether the detection of additional LNs, which may result in upstaging, plays a decisive role in determining patient prognosis. Ghezzi *et al*. (10) suggested that examination of a greater number of LNs using CS fixation did not result in significant changes in patient staging or treatment. However, in the present study, additional metastatic LNs were found in four of 26 patients, resulting in disease in two being up-staged. The aim should be to identify all metastatic LNs, regardless of whether survival is affected.

This study had some limitations including the small sample size and lack of long-term survival data. Further long-term observations are needed to determine whether stage migration determined by CS fixation of resected specimens has an effect on the actual survival rate.

In conclusion, CS is an adequate alternative to NBF for the fixation of gastric cancer specimens, and its use allows the detection of more LNs in resected specimens.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

JWL proposed the topic and conceived and designed the study. SHH performed the pathological examinations, including node dissection. SH and JHH analyzed the data and helped with the interpretation. HSK collaborated with JWL in the writing of the article. All Authors read and approved the final article.

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