Impact of Intraoperative Macroscopic Diagnosis of Serosal Invasion in Pathological Subserosal (pT3) Gastric Cancer

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Purpose: The macroscopic diagnosis of tumor invasion through the serosa during surgery is not always distinct in patients with gastric cancer. The prognostic impact of the difference between macroscopic findings and pathological diagnosis of serosal invasion is not fully elucidated and needs to be re-evaluated.

Materials and Methods: A total of 370 patients with locally advanced pT2 to pT4a gastric cancer who underwent curative surgery were enrolled in this study. Among them, 155 patients with pT3 were divided into three groups according to the intraoperative macroscopic diagnosis of serosal invasion, as follows: serosa exposure (SE(−)) (no invasion, 72 patients), SE(±) (ambiguous, 47 patients), and SE(+) (definite invasion, 36 patients), and the clinicopathological features, surgical outcomes, and disease-free survival (DFS) were analyzed.

Results: A comparison of the 5-year DFS between pT3_SE(−) and pT2 groups and between pT3_SE(+) and pT4a groups revealed that the differences were not statistically significant. In addition, in a subgroup analysis of pT3 patients, the 5-year DFS was 75.1% in SE(−), 68.5% in SE(±), and 39.4% in SE(+) patients (P<0.05). In a multivariate analysis to evaluate risk factors for tumor recurrence, macroscopic diagnosis (hazard ratio [HR], SE(−) : SE(±) : SE(+) = 1 : 1.01 : 2.45, P=0.019) and lymph node metastasis (HR, N0 : N1 : N2 : N3 = 1 : 1.45 : 2.20 : 9.82, P<0.001) were independent risk factors for recurrence.

Conclusions: Gross inspection of serosal invasion by the surgeon had a strong impact on tumor recurrence in gastric cancer patients. Consequently, the gross appearance of serosal invasion should be considered as a factor for predicting patients’ prognosis.

Key Words: Stomach neoplasms; Prognosis; Neoplasm staging

Introduction

Pathological results are occasionally different from intraoperative gross findings, particularly in the case of serosal invasion in patients with gastric cancer. Some studies have revealed the accuracy of preoperative or intraoperative staging. However, only a few studies have evaluated the true meaning of grossly overestimated or underestimated lesions as compared to the pathological findings in patients with subserosal gastric cancer. We evaluated the prognostic value of intraoperative findings for the presence or absence of serosal invasion in patients with subserosal gastric cancer.

Materials and Methods

This study protocol is approved by institutional review board of the Catholic Medical Center (XC13RIMI0093S).

A total of 954 patients who underwent gastric cancer surgery between January 2004 and April 2013 were identified from an institutional database. Among them, 494 patients with early gastric cancer, 12 patients with non-adenocarcinoma, 28 patients with tumors...
extending to an adjacent organ, 49 patients who underwent non-curative resections, and 1 pT3 patient in whom the intraoperative gross appearance of serosal invasion could not be evaluated, were excluded. Thus, 370 patients with locally advanced gastric cancer—88 pT2, 155 pT3, and 127 pT4a patients—were included. The 155 pT3 patients were divided into three groups for a subgroup analysis according to the intraoperative macroscopic serosal invasion findings, as follows: grossly negative [serosa exposure, SE(−)], ambiguous serosal invasion [SE(±)], and definite serosal invasion [SE(+)].

Clinicopathological characteristics, recurrence-free survival according to the pathologic T-stage, and the macroscopic diagnosis were analyzed in the pT3 cases. Recurrence was determined by examining either medical records or the National Cancer Institute (NCI) cancer patient registry. If, according to the NCI registry, the patient died due to gastric cancer, we regarded the case as a recurrence, although we could not define the exact recurrence site in these patients. Univariate and multivariate analyses were performed to elucidate the risk factors for recurrence in pT3 cases based on various factors, including the gross serosal invasion findings. Additionally, the recurrence patterns were analyzed. The gastric cancer stage was classified according to the seventh edition of the TNM Classification of Malignant Tumors given by Union for International Cancer Control (UICC).4

1. Determination of the gross appearance of serosal invasion

All subserosal lesions were divided into three subgroups according to their gross appearance, as follows:

1) SE(−): normal appearance of gastric serosa (Fig. 1A)
2) SE(±): discolored serosal surface without definite nodularity (Fig. 1B)
3) SE(+): serosal surface combined with discoloration and elevated nodularity (Fig. 1C)

2. Statistical analysis

All continuous variables are expressed as mean±standard deviation, and they were analyzed by using either the Student t-test or an analysis of variance in order to compare the different groups. The chi-square test or Fisher exact test was used for univariate analysis of nominal variables. Disease-free survival (DFS) was calculated by using the Kaplan–Meier method, and the log–rank test was used in the univariate analysis to identify significant factors influencing recurrence. The Cox–regression model was used to identify the independent contributors to DFS among the significant factors from the univariate analysis.

Statistical software was PASW SPSS ver. 18 (IBM Co., Armonk, LA, USA).

Results

The median follow-up period was 21 months (range: 1–105 months). Lymph node metastasis; tumor size; venous, lymphatic, and perineural invasion; Lauren classification; tumor growth pattern; extent of resection; and lymph node dissection were significantly different when stratified according to the depth of tumor invasion (Table 1). Significant differences were observed in the 5-year DFS according to the pathological T-stage between pT2 (83.2%), pT3 (64.5%), and T4a (28.0%) patients (Fig. 2A).

Among the pT3 patients, the tumor size, lymph node metastasis, and lymphatic invasion were significantly different among the three groups based on the macroscopic diagnosis (Table 2). The 5-year DFS in the pT3 patient subgroups were 75.1% for SE(−), 68.5% for SE(±), and 39.4% for SE(+) patients. The SE(+) subgroup had a significantly lower 5-year DFS compared with that of the SE(−) and SE(±) subgroups. In addition, no differences in the 5-year DFS were observed in the pT2 versus pT3 SE(−) groups, or the pT4a versus pT3 SE(+) groups (Fig. 2B).
Table 1. Clinicopathological features according to the pathological T-stage

| Variable                                      | pT2 (n=88) | pT3 (n=155) | pT4a (n=127) | P-value |
|-----------------------------------------------|------------|-------------|--------------|---------|
| Sex                                           |            |             |              | 0.549   |
| Male                                          | 63 (71.6)  | 103 (66.5)  | 82 (64.6)    |         |
| Female                                        | 25 (28.4)  | 52 (33.5)   | 45 (35.4)    |         |
| Age (yr)                                      | 60.6±11.0  | 62.4±12.1   | 62.6±2.8     | 0.450   |
| Tumor size (cm)                               | 3.4±1.5    | 6.0±2.6     | 7.9±3.6      | <0.001  |
| No. of retrieved nodes                        | 36.8±16.9  | 38.9±16.6   | 43.1±19.8    | 0.250   |
| Lymph node metastasis (UICC 7th ed)           |            |             |              | <0.001  |
| N0                                            | 55 (62.5)  | 53 (34.2)   | 23 (18.1)    |         |
| N1                                            | 16 (18.2)  | 28 (18.1)   | 13 (10.2)    |         |
| N2                                            | 8 (9.1)    | 32 (20.6)   | 25 (19.7)    |         |
| N3                                            | 9 (10.2)   | 42 (27.1)   | 66 (52.0)    |         |
| Tumor stage (UICC 7th ed)                     |            |             |              | <0.001  |
| IB                                            | 55 (62.5)  | 0 (0.0)     | 0 (0.0)      |         |
| IIA                                           | 16 (18.2)  | 53 (34.2)   | 0 (0.0)      |         |
| IIB                                           | 8 (9.1)    | 28 (18.1)   | 23 (18.1)    |         |
| IIIA                                          | 9 (10.2)   | 32 (20.6)   | 13 (10.2)    |         |
| IIIIB                                         | 0 (0.0)    | 42 (27.1)   | 25 (19.7)    |         |
| IIIIC                                         | 0 (0.0)    | 0 (0.0)     | 66 (52.0)    |         |
| Resection margin (cm)                         |            |             |              |         |
| PRM                                           | 4.2±2.5    | 3.9±2.5     | 3.7±2.7      | 0.371   |
| DRM                                           | 6.7±3.9    | 5.0±3.7     | 5.0±4.3      | 0.003   |
| Differentiation                               |            |             |              | 0.001   |
| Differentiated                                | 40 (45.5)  | 68 (43.9)   | 31 (24.4)    |         |
| Undifferentiated                              | 48 (54.5)  | 87 (56.1)   | 96 (75.6)    |         |
| Venous invasion                               |            |             |              | <0.001  |
| Present                                       | 1 (1.1)    | 5 (3.2)     | 26 (20.5)    |         |
| Absent                                        | 87 (98.9)  | 150 (96.8)  | 101 (79.5)   |         |
| Lymphatic invasion                            |            |             |              | <0.001  |
| Present                                       | 38 (43.2)  | 124 (80.0)  | 114 (89.8)   |         |
| Absent                                        | 50 (56.8)  | 31 (20.0)   | 13 (10.2)    |         |
| Perineurial invasion                          |            |             |              | <0.001  |
| Present                                       | 20 (22.7)  | 98 (63.2)   | 101 (79.5)   |         |
| Absent                                        | 68 (77.3)  | 57 (36.8)   | 26 (20.5)    |         |
| Lauren classification                         |            |             |              | <0.001  |
| Intestinal                                    | 50 (56.8)  | 74 (47.7)   | 42 (33.1)    |         |
| Mixed                                         | 15 (17.0)  | 35 (22.6)   | 20 (15.7)    |         |
| Diffuse                                       | 23 (26.1)  | 46 (29.7)   | 65 (51.2)    |         |
| Growth pattern                                |            |             |              | <0.001  |
| Expansile                                     | 45 (51.1)  | 46 (29.7)   | 28 (22.0)    |         |
| Intermediate                                  | 1 (1.1)    | 6 (3.9)     | 6 (4.7)      |         |
| Infiltrative                                  | 42 (47.7)  | 103 (66.5)  | 93 (73.2)    |         |
| Extent of resection                           |            |             |              | <0.001  |
| TG                                            | 16 (18.2)  | 46 (29.7)   | 60 (47.2)    |         |
| DSG                                           | 72 (81.8)  | 109 (70.3)  | 67 (52.8)    |         |
| Extent of dissection                          |            |             |              | <0.001  |
| D1+                                           | 11 (12.5)  | 3 (1.9)     | 3 (2.4)      |         |
| D2                                            | 77 (87.5)  | 152 (98.1)  | 124 (97.6)   |         |

Values are presented as number (%) or mean±standard deviation. The sum of the percentages does not equal 100% because of rounding. UICC = Union for International Cancer Control; PRM = proximal resection margin; DRM = distal resection margin; TG = total gastrectomy; DSG = distal subtotal gastrectomy.
The macroscopic diagnosis for serosal invasion and lymph node status were significant risk factors in a univariate analysis of the pT3 group (Table 3). The multivariate analysis revealed that the macroscopic diagnosis of serosal invasion (hazard ratios [HRs]: SE(−) : SE(±) : SE(+) = 1 : 1.01 : 2.45, P=0.019) and lymph node status (HRs: N0 : N1 : N2 : N3 = 1 : 1.45 : 2.20 : 9.82, P<0.001) were independent risk factors for tumor recurrence (Table 4).

On examining the pattern of recurrence, peritoneal metastasis was the most common pattern in all the patients and particularly in pT4a patients. There were no differences in the pattern of recurrence between the three pT3 patient subgroups (Table 5).

**Discussion**

According to the seventh edition of the UICC classification and the third edition of the Japanese classification of gastric cancer, tumor stage is defined mainly by the tumor depth and lymph node status. The presence or absence of serosal invasion is a very important factor for patient prognosis after surgery. In particular, serosa-positive gastric cancer has a great risk of peritoneal recurrence due to exfoliating and proliferating cancer cells in the peritoneal cavity. The T- and N-stages are determined by microscopic rather than macroscopic findings. However, some discrepancies exist between macroscopic and microscopic results for both T-stage and N-stage. Some investigators believe that even when the peritoneal surface has been penetrated focally, the peritoneum can resurface over exposed cancer cells. Thus, the significance of gross serosal invasion without histological invasion needs to be evaluated to reveal its prognostic impact.

Estimating the cancer stage intraoperatively as well as preoperatively is very important in order to decide the extent of resection or postoperative adjuvant treatment. Many clinicians have studied the accuracy of intraoperative staging, and compared it to pathological staging. Korenaga et al. evaluated the prognostic value of intraoperative serosal invasion assessment. In that study, they included patients with all gastric cancer stages and found that, among patients with proper muscle and subserosal invasion, patients who had gross serosal invasion had a poor 5-year survival rate. This result is similar to the findings from our study. Yasuda et al. also investigated the prognostic significance of macroscopic serosal invasion in patients with advanced gastric cancer and showed a poor prognosis for those with macroscopic serosal invasion. However, because those studies followed the UICC sixth staging system, no specific data were provided for each layer. Ichiyoshi et al. compared the macroscopic findings of serosal invasion with histological results. In that study, the authors focused on the significance of underestimating serosal invading gastric cancer as gross non-invasion. Our study focused on the significance of overestimating serosal invasion in patients with pathologic subserosal gastric cancer.

Although some authors insist that tumor size is an independent prognostic factor in survival analysis, it is not generally accepted as a prognostic factor in patients with gastric cancer. However, tumor size is very important in overestimating serosal invasion. Jeong et al. found that a tumor mass of ≥4 cm and a preoperative overestimation of serosal-positive cancer on multi-detector computed tomography were independent risk factors for overestimating serosal invasion during surgery. In our study, among the three groups in pT3 patients, tumor size was significantly larger in the SE(+) group. However, tumor size was not associated with the 5-year DFS.
Table 2. Clinicopathological differences according to the macroscopic findings in patients with pT3 gastric cancer

| Variable                              | SE(−) (n=72) | SE(±) (n=47) | SE(+) (n=36) | P-value |
|---------------------------------------|--------------|--------------|--------------|---------|
| Sex                                   |              |              |              |         |
| Male                                  | 48 (66.7)    | 32 (68.1)    | 23 (63.9)    | 0.921   |
| Female                                | 24 (33.3)    | 15 (31.9)    | 13 (36.1)    |         |
| Age (yr)                              |              |              |              | 0.513   |
| <60                                   | 35 (48.6)    | 24 (51.1)    | 14 (38.9)    |         |
| ≥60                                   | 37 (51.4)    | 23 (48.9)    | 22 (61.1)    |         |
| Tumor size (cm)                       | 4.9±2.2      | 6.7±2.9      | 7.2±2.3      | <0.001  |
| <3                                    | 15 (20.8)    | 1 (2.1)      | 0 (0.0)      | <0.001  |
| 3~6                                   | 34 (47.2)    | 17 (36.2)    | 13 (36.1)    |         |
| ≥6                                    | 23 (31.9)    | 29 (61.7)    | 23 (63.9)    |         |
| Tumor location                        |              |              |              | 0.799   |
| Min                                   | 39 (54.2)    | 28 (59.6)    | 19 (53.8)    |         |
| Maj                                   | 8 (11.1)     | 7 (14.9)     | 6 (16.7)     |         |
| AW                                    | 12 (16.7)    | 8 (17.0)     | 7 (19.4)     |         |
| PW                                    | 13 (18.1)    | 4 (8.5)      | 4 (11.1)     |         |
| No. of retrieved nodes                | 36.9±15.4    | 40.3±18.0    | 40.9±17.1    | 0.398   |
| Lymph node/tumor stage                |              |              |              | 0.010   |
| N0/Iia                                | 32 (44.4)    | 13 (27.7)    | 8 (22.2)     |         |
| N1/Iib                                | 13 (18.1)    | 8 (17.0)     | 7 (19.4)     |         |
| N2/Iiia                               | 17 (23.6)    | 11 (23.4)    | 4 (11.1)     |         |
| N3/IIib                               | 10 (13.9)    | 15 (31.9)    | 17 (47.2)    |         |
| Resection margin (cm)                 |              |              |              |         |
| PRM                                   | 4.3±2.9      | 3.3±1.9      | 3.9±2.3      | 0.081   |
| DRM                                   | 5.3±3.8      | 5.2±3.6      | 4.1±3.7      | 0.220   |
| Differentiation                       |              |              |              | 0.061   |
| Differentiated                        | 35 (48.6)    | 14 (29.8)    | 19 (52.8)    |         |
| Undifferentiated                      | 37 (51.4)    | 33 (70.2)    | 17 (47.2)    |         |
| Venous invasion                       |              |              |              | 0.458   |
| Present                               | 1 (1.4)      | 2 (4.3)      | 2 (5.6)      |         |
| Absent                                | 71 (98.6)    | 45 (95.7)    | 34 (94.4)    |         |
| Lymphatic invasion                    |              |              |              | 0.049   |
| Present                               | 52 (72.2)    | 39 (83.0)    | 33 (91.7)    |         |
| Absent                                | 20 (27.8)    | 8 (17.0)     | 3 (8.3)      |         |
| Perineural invasion                   |              |              |              | 0.101   |
| Present                               | 41 (56.9)    | 31 (66.0)    | 26 (72.2)    |         |
| Absent                                | 31 (43.1)    | 16 (34.0)    | 10 (27.8)    |         |
| Lauren classification                 |              |              |              | 0.429   |
| Intestinal                            | 35 (48.6)    | 18 (38.3)    | 21 (58.3)    |         |
| Mixed                                 | 15 (20.8)    | 14 (29.8)    | 6 (16.7)     |         |
| Diffuse                               | 22 (30.6)    | 15 (31.9)    | 9 (25.0)     |         |
| Growth pattern                        |              |              |              | 0.902   |
| Expansile                             | 23 (31.9)    | 13 (27.7)    | 10 (27.8)    |         |
| Intermediate                          | 3 (4.2)      | 1 (2.1)      | 2 (5.6)      |         |
| Infiltrative                          | 46 (63.9)    | 33 (70.2)    | 24 (66.7)    |         |
| Resection                             |              |              |              | 0.140   |
| TG                                    | 17 (23.6)    | 19 (40.4)    | 10 (27.8)    |         |
| DSG                                   | 55 (76.4)    | 28 (59.6)    | 26 (72.2)    |         |
| Dissection                            |              |              |              | 0.340   |
| D1+                                   | 1 (1.4)      | 2 (4.3)      | 0 (0.0)      |         |
| D2                                    | 71 (98.6)    | 45 (95.7)    | 36 (100.0)   |         |

Values are presented as number (%) or mean±standard deviation. The sum of the percentages does not equal 100% because of rounding. SE = serosa exposure; Min = lesser curvature; Maj = greater curvature; AW = anterior wall; PW = posterior wall; PRM = proximal resection margin; DRM = distal resection margin; TG = total gastrectomy; DSG = distal subtotal gastrectomy.
A significantly different survival pattern was observed among the pT3 patient subgroups classified by gross serosal invasiveness. The 5-year DFS in the SE(−) group was similar to that of pT2 (proper muscle invasion). The SE(±) group had significantly better 5-year DFS than the SE(+) group. In addition, 5-year DFS in the SE(+) group was not different from that of pT4a patients. Although there were discrepancies in the nodal status and staging among the three pT3 subgroups, the multivariate analysis revealed that the gross appearance of serosal invasion independently influenced the 5-year DFS.

Efforts have been made to identify risk factors that distinguish the prognosis for a specific depth of invasion. Song et al. sub-divided subserosal gastric cancer lesions into three categories according to the histological growth patterns, and revealed that the infiltrative type had a poorer prognosis and a higher rate of carcinomatosis development. In our study, the tumor growth pattern was not a risk factor for recurrence in pT3 patients. Therefore, further studies are needed to reveal the significant factors influencing prognosis among pT3 patients.

Our study has some limitations regarding the study design. First, our study was a retrospective analysis. However, our data were collected prospectively, and serosal positivity was evaluated in three categories by a single surgeon and reported in each operation record. The second limitation was that no definite categorical guidelines are available for gross serosal invasiveness to be fully objective, although we presented our own indications and examples. However, many other studies that considered the gross appearance of serosal invasion did not present their indications to detect gross serosal invasion. Finally, because the follow-up data for some pa-

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**Table 3. Univariate analysis for recurrence in patients with pT3 gastric cancer according to the patient characteristics**

| Variable                              | 5-Year DFS (%) | P-value |
|---------------------------------------|----------------|---------|
| Sex                                   |                |         |
| Male                                  | 61.2           |         |
| Female                                | 71.8           |         |
| Age (yr)                              |                |         |
| <60                                   | 63.0           | 0.622   |
| ≥60                                   | 65.8           |         |
| Gross appearance                      |                | <0.001  |
| SE(−)                                 | 75.1           |         |
| SE(±)                                 | 68.5           |         |
| SE(+)                                 | 39.4           |         |
| Lymph node status (UICC 7th ed)       |                | <0.001  |
| N0                                    | 85.9           |         |
| N1                                    | 82.4           |         |
| N2                                    | 73.1           |         |
| N3                                    | 16.5           |         |
| Tumor size (cm)                       |                | 0.708   |
| <3                                    | 61.5           |         |
| 3~6                                   | 64.9           |         |
| ≥6                                    | 63.3           |         |
| Tumor location                        |                | 0.996   |
| Min                                   | 64.5           |         |
| Maj                                   | 49.7           |         |
| AW                                    | 67.0           |         |
| PW                                    | 62.9           |         |
| Differentiation                       |                | 0.534   |
| Differentiated                        | 60.9           |         |
| Undifferentiated                      | 67.4           |         |
| Lauren                                |                | 0.584   |
| Intestinal                            | 64.8           |         |
| Mixed                                 | 59.2           |         |
| Diffuse                               | 69.6           |         |
| Growth pattern                        |                | 0.668   |
| Expansile                             | 77.7           |         |
| Intermediate                          | 53.3           |         |
| Infiltrative                          | 62.0           |         |
| Resection                             |                | 0.630   |
| TG                                    | 69.3           |         |
| DSG                                   | 62.4           |         |

DFS = disease-free survival; SE = serosa exposure; UICC = Union for International Cancer Control; Min = lesser curvature; Maj = greater curvature; AW = anterior wall; PW = posterior wall; TG = total gastrectomy; DSG = distal subtotal gastrectomy.

**Table 4. Multivariate analysis of recurrence according to surgical T-stage and pathological N-stage among pT3 patients**

| Variable                              | Hazard ratio      | P-value |
|---------------------------------------|-------------------|---------|
| Gross appearance                      |                   | 0.019   |
| SE(−)                                 | 1                 |         |
| SE(±)                                 | 1.01 (0.45~2.27)  | 0.981   |
| SE(+)                                 | 2.45 (1.17~5.10)  | 0.017   |
| Lymph node status (UICC 7th ed)       |                   | <0.001  |
| N0                                    | 1                 |         |
| N1                                    | 1.45 (0.43~4.87)  | 0.545   |
| N2                                    | 2.20 (0.73~6.58)  | 0.160   |
| N3                                    | 9.82 (3.79~25.44) | <0.001  |

SE = serosa exposure; UICC = Union for International Cancer Control.

A significantly different survival pattern was observed among the pT3 patient subgroups classified by gross serosal invasiveness. The 5-year DFS in the SE(−) group was similar to that of pT2 (proper muscle invasion). The SE(±) group had significantly better 5-year DFS than the SE(+) group. In addition, 5-year DFS in the SE(+) group was not different from that of pT4a patients. Although there were discrepancies in the nodal status and staging among the three pT3 subgroups, the multivariate analysis revealed that the gross appearance of serosal invasion independently influenced the 5-year DFS.

Efforts have been made to identify risk factors that distinguish the prognosis for a specific depth of invasion. Song et al. sub-divided subserosal gastric cancer lesions into three categories according to the histological growth patterns, and revealed that the infiltrative type had a poorer prognosis and a higher rate of carcinomatosis development. In our study, the tumor growth pattern was not a risk factor for recurrence in pT3 patients. Therefore, further studies are needed to reveal the significant factors influencing prognosis among pT3 patients.
In conclusion, although the depth of gastric cancer invasion definitely depends on a pathological investigation, serosal involvement, as determined upon gross inspection by the surgeon, had a strong impact on lymph node metastasis and patients’ DFS. Thus, the gross appearance of serosal invasion should be considered as a factor for predicting patients’ prognosis.

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