滋賀医大誌, 10, 65−72, 1995

Evaluation of Peritoneal Lavage Cytology at the Time of Operation for Gastric Cancer

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Abstract: Cytologic examination of peritoneal lavage fluid obtained during surgery was performed in 120 patients with gastric cancer to assess the prognostic value of a positive cytology. Positive cytology was obtained in 19.2% of all cases, while 30.5% of cases with overt microscopic serosal invasion were positive. Degree of macroscopic or microscopic invasion to the gastric serosa, macroscopic peritoneal dissemination and stage of cancer were associated with a positive cytology. Patients with intraperitoneal free cancer cells demonstrated a significantly poorer prognosis than those without ($p<0.001$). In cases of positive lavage cytology, all patients except two died with peritoneal dissemination within 2 years after operation. Peritoneal metastasis was the most frequent site of recurrence. Therefore, this technique may provide a useful indicator of prognosis and for determining subpopulations of patients that might receive adjuvant chemotherapy for micrometastasis.

Key words: peritoneal lavage cytology, peritoneal dissemination, gastric cancer

INTRODUCTION

Considerable recent progress in treatment has improved long-term survival in gastric cancer patients. This success is largely attributed to the performance of extensive lymph node removal. Despite these efforts, postoperarive recurrence is still frequent. Disseminated peritoneal metastasis is the most frequent type of recurrence and a critical factor in the prognosis of patients with gastric cancer. This must develope from residual microscopic cancer cells despite surgery with curative intent. However, satisfactory attention has not been paid to cancer invasion into the gastric serosa, a phenomenon likely closely related to the development of peritoneal metastasis. We therefore conducted a cytologic examination for free cancer cells obtained by washing the peritoneal cavity. Since no effective method of treatment is now available for established peritoneal dissemination, cytology of lavaged saline from the Douglas pouch may be useful as a predictor of prognosis and an indicator for adjuvant therapies.

Accepted for publication December 1, 1994.

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MATERIALS AND METHODS

From January 1986 to December 1992 intraoperative, peritoneal lavage cytology was performed in 120 patients with gastric cancer. Gastrectomy was performed in 106 patients and laparotomy only in 14 patients.

The methods of peritoneal lavage and cytologic examination are performed as follows: Immediately after opening the peritoneal cavity, Douglas pouch was irrigated with 100 ml of saline, and this was aspirated by syringe with a Nelaton tube after mild stirring by hand. The aspirated fluid was centrifuged, and the sediment was smeared and stained by the Papanicolaou method and PAS reaction for microscopic examination. Evaluation was performed according to the Papanicolaou's classification in which class IIIb, IV and V were considered positive for malignant cells.

To explore the clinical significance of cytological examination of peritoneal lavage fluid, its correlation with other parameters of gastric cancer such as the histologic stage, grade of macroscopic serosal invasion by tumor, histologic extent of gastric wall invasion, macroscopic grade of peritoneal dissemination, histologic type, macroscopic Borrmann's classification, tumor size and survival rate, was analysed statistically.

Microscopic wall invasion (t), macroscopic peritoneal metastasis (p) and staging were defined in the General Rules for Gastric Cancer Study in Surgery and Pathology published by the Japanese Research Society for Gastric Cancer[1]. The degree of macroscopic serosal invasion (S) was also investigated according to the criteria of the previous edition[2] for purposes of comparison.

Survival was analyzed according to the actuarial and Kaplan-Meier method. The difference in survival rate was assessed statistically using the generalized Wilcoxon method. The significance of the incidence of positive cytology was determined with the Fisher exact test and the chi square analysis. A probability value smaller than 0.05 was considered significant.

RESULTS

Peritoneal lavage cytology was examined in relation to several other factors. These included staging, degree of macroscopic invasion, extent of gastric wall invasion, macroscopic peritoneal dissemination, histology, macroscopic type, tumor size and survival.

1) Staging

Free cancer cells were demonstrated in Douglas cavity in 23 of 120 (19.2%) patients. Table 1 shows the results of cytology at lap-

Table 1. Staging and Peritoneal Lavage Cytology

Stage	Papanicolaou's classification						Incidence of positive cytology §	
	I	II	IIIa	IIIb	IV	V		
I a, I b	13	11	3	0	1	0	3.6%	
II	12	12	1	0	0	0	0	*
IIIa, IIIb	11	12	1	1	1	9	31.4	
IVa, IVb	7	11	3	2	0	9	34.4	

* : $p<0.01$ by Fisher exact test

§ : According to Papanicolaou's classification, Class IIIb, IV and V were considered positive for malignant cells

arotomy in 120 patients in relation to their stage. Stage III (31.4%) and IV (34.4%) showed a significantly higher positive cytology rate as compared to other stages ($p<0.01$ by Fisher exact test).

2) Degree of macroscopic serosal invasion

The relationship between peritoneal lavage cytology and the grade of macroscopic serosal invasion by the primary lesion is shown in Table 2. The incidence of free caccer cells in the peritoneal cavity generally correlated with increasing degree of serosal invasion — S0 (3.7%), S1 (0%), S2 (20%), S3 (41.7%) (S0, S1 vs S2, S3: $p<0.01$ by Fisher exact test).

3) Extent of gastric wall invasion

In term of the microscopic depth of wall invasion, it was null in tl (m and sm), only one patient without any serosal involvement (t2: mp and ss) and 30.5% (22/72) in patients with overt serosal involvement (20.4% t3 (se), 52.2% t4 (si)) (Table 3). The difference in incidence of positive cytology between cases with and without serosal involvement was statistically significant ($p<0.01$).

4) Macroscopic peritoneal dissemination

The incidence of positive cytology in patients with macroscopic peritoneal dissemination was 4.5% (4/88) in P0 patients, but averaged 59.4% (19/32) in groups P1, P2 and P3 combined (Table 4). The greater the increase in the P-

Table 2. Degree of Macroscopic Serosal Invasion and Peritoneal Lavage Cytology

Grade of macroscopic serosal invasion (S)	Papanicolaou's classification						Incidence of positive cytology	
	I	II	IIIa	IIIb	IV	V		
0*	13	11	2	0	1	0	3.7%	
1	7	12	0	0	0	0	0 %	**
2	18	17	5	1	1	8	20 %	
3	5	6	1	2	0	10	41.7%	

**$p<0.01$ by Fisher's exact test

*0 : intact mucosa

1 : serosal invasion suspected in small area

2 : definite invasion of the serosa

3 : invasion of organs adjacent to primary serosal lesion

Table 3. Extent of Gastric Wall Invasion and Peritoneal Lavage Cytology

Extent of gastric wall invasion	Papanicolau's classification						Incidence of positive cytology	
	I	II	IIIa	IIIb	IV	V		
t1§(m,sm)	11	6	2	0	0	0	0 %	
t2 (mp,ss)	15	11	2	0	1	0	3.4%	*
t3 (se)	13	23	3	1	1	8	20.4%	
t4 (si)	4	6	1	2	0	10	52.2%	

* : $p<0.01$ by Fisher exact test

§ : t1 Lamina propria(m), submucosa(sm)

t2 Muscularis propria (mp), subserosa(ss)

t3 Penetrates serosa (se)

t4 Adjacent structure (si)

Table 4. Macroscopic Peritoneal Dissemination and Peritoneal Lavage Cytology

Macroscopic peritoneal dissemination	Papanicolau's classification						Incidence of positive cytology
	I	II	IIIa	IIIb	IV	V	
p0	36	41	7	0	1	3	4.5%
p1	3	3	0	1	0	5	50.5%
p2	3	2	0	0	1	3	44.4%
p3	1	0	1	2	0	7	63.6%

** $p<0.01$ by Fisher exact test

p0 : no macroscopic dissemination

p1 : small number of disseminated lesions in perigastric, upper abdomen (above transverse colon)

p2 : moderate number of lesions in entire abdomen

p3 : numerous lesions in entire abdomen

Table 5. Histology and Peritoneal Lavage Cytology

Histology	Papanicolau's classification						Incidence of positive cytology
	I	II	IIIa	IIIb	IV	V	
pap§	1	2	0	0	0	0	0 %
tub1	6	5	0	0	1	2	21.4%
tub2	14	15	2	1	0	5	13.5%
por	21	20	3	1	1	10	21.4%
muc	1	2	2	1	0	0	16.7%
sig	1	2	1	0	0	1	20.0%

§ pap : papillary adenocarcinoma,
tub1 : tubular adenocarcinoma, well differentiated type
tub2 : tubular adenocarcinoma, moderately differentiated type
por : poorly differentiated adenocarcinoma
muc : mucinous adenocarcinoma
sig : signet-ring cell carcinoma

number, the larger was the number of cases of positive cytology. There was a significant difference between cases with and without macroscopic dissemination ($p<0.01$). It is noteworthy that these four who did not have macroscopic peritoneal dissemination were gastric cancer patients with positive cytologies.

5) Histologic type

There was no relation between the histologic differentiation and the incidence of positive peritoneal cytology (Table 5).

6) Macroscopic type

According to the Borrmann's classification, the incidence of positive lavage cytology was 33.3 % in type IV (Table 6). There was no correlation between this classification and lavage cytology.

7) Tumor Size

The relationship between the incidence of intraperitoneal free cancer cells and tumor size in 106 patients is shown in Table 7. The rate of detection increased sharply with tumor size greater than 8 cm in diameter ($p<0.01$).

8) Survival

Those patients with normal cytologic findings had a significantly better prognosis than

Table 6. Macroscopic Type and Peritoneal Lavage Cytology

Macroscopic type	Papanicolau's classification						Incidence of positive cytology
	I	II	IIIa	IIIb	IV	V	
superfical type	8	6	1	0	1	0	6.3%
I §	2	0	0	0	0	1	33.3%
II	7	10	1	0	0	2	10.0%
III	9	15	3	2	0	5	20.6%
IV	7	5	2	1	1	9	44.0%
V	10	10	1	0	0	1	4.5%

§ : I……Polypoid carcinoma often called fungating variety
II……Ulcerating lesion surrounded by an elevated wall which is demarcated sharply all around against the surrounding mucosa
III……Ulcerative variety, described often as the ulcerating-infiltraring type
IV……Diffusely infiltrating vareiety
V……Unclassified type

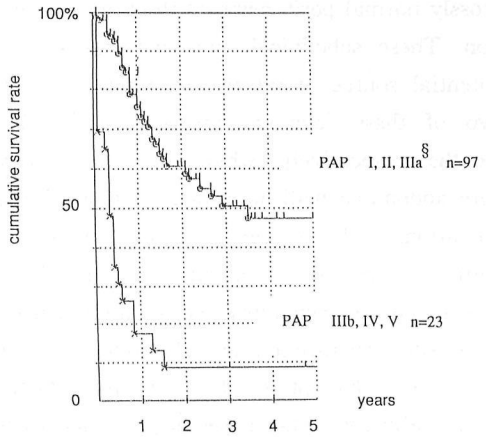

Fig 1. Survival as a fashion of peritoneal lavage cytology
★ : significant difference between two groups by generalized Wilcoxon analysis ($z=6.14519$ $p<0.001$)
§ : Papanicolaou's classification class I, II, IIIa

the patients with positive cytologies ($p<0.001$) (Figure 1). The cumulative 5-year survival rate in patients with positive peritoneal lavage cytologies was 8.7%, while in those without it was 46.7 %.

All but two patients died as a result of peritoneal dissemination within 2 years of operation (mean, 5.0±4.76 months), in cases of positive lavage cytology, even if the primary tumor was completely resected,

DISCUSSION

Free cancer cells shed from the serosal surface of the primary lesion ultimately give rise to disseminated peritoneal disease. Therefore, the majority of intaperitoneal free cancer cells can be assumed to be derived from the area of gastric serosal invasion. According to this hypothesis, the incidence of intraperitoneal free cancer cells will be dependent on the degree of invasion of cancer to the gastric serosa. In fact, we have shown that invasion to the serosa markedly influences the incidence of positive cytology as well as the prognosis in patients with gastric cancer.

Another factor involved in the determination of the presence or absence of intraperitoneal free cancer cells is the size of the tumor mass. The rate of detection of free malignant cells increased to 42.9% in patients with a tumor greater than 8 cm in diameter.

In the present study, 23 out of 120 (19%) patients with gastric cancer had a positive cyto-

Table 7. Tumor Size and Peritoneal Lavage Cytology

Tumor size (cm)	Papanicolaou's classification						Incidence of positive cytology
	I	II	IIIa	IIIb	IV	V	
< 2	3	7	1	0	0	0	0 %
2− 4	11	8	1	0	0	1	4.8%
4− 6	10	10	2	1	0	1	8.3%
6− 8	9	9	1	0	1	2	13.6%
8−10	3	3	1	0	0	4	36.4%
>10	5	5	2	0	1	4	29.4%

**p<0.01 by chi square test

logy. This value is slightly smaller than findings in previous reports by Nakajima et al (30%)[4], but is slightly greater values suggested by Hirano et al (4.0%)[5] and Koga et al (13.5%)[6]. The difference in the percentage of positive cytologies among these several reports may be due to somewhat different patients populations, the method of peritoneal lavage and the procedure for aspirating lavage fluid. A high incidence of positive cytology tends to depend on lavage of both upper abdomen and Douglas pouch[3], rather than Douglas pouch[5][6].

Moreover, in spite of serosal invasion by the tumor, not so high incidence of positive cytologies (only 30.5% in our paper) can be obtained. Such a negative diagnosis does not always result from technical failure as already described above, because the possibility that positive cytology is derived from characteristics of cancer cell itself, cannot be ruled out. Some investigators have reported parallel correlation between positive cytologic findings and histologic type[4][7] or macroscopic classification of serosal surface[8]. According to these authors, tumor cells of pooly differentiated carcinomas, which invaded the serosal layer of the stomach, seems to detach and to be liberated into the peritoneal cavity more easily than those of well differentiated carcinomas. But these factors cannot sufficiently account for low incidence of positive cytology in serosal invasion cases. This question is at present difficult to assess.

We demonstrated four cases in which intraperitoneal free cancer cells were detected by peritoneal cytologic exploration despite a grossly normal peritoneum at the time of operation. These subclinical micrometastases are a potential source of future recurrence. Actually, two of these four patients died in 4 and 10 months, respectively, while one remains alive with abdominal wall metastasis 46 months after operation, and the remaining patient is alive without recurrence. This alive patient without recurrence received intravenous mitomycin C 3 days after operation and 5-fluorouracil per os for 6 years. Except for these two patients that remain alive, all other patients (21 patients with positive cytology) died within 2 years. Thus, the majority of patients with free peritoneal cancer cells detected at the time of surgery will not escape postoperative peritoneal recurrence. However, patients who have only a few intraperitoneal free cancer cells may escape peritoneal recurrence if prophylactic manipulations are used during surgery. Peritoneal lavage cytology will facilitate the effective selection of patients for postoperative adjuvant therapy.

A positive cytologic specimen was observed in one case in which the serosal layer of the stomach was not involved microscopically. This patient was described above because he did not

have macroscopic peritoneal dissemination. There have been several recent reports in which positive cytology was not accompanied by serosal invasion. Imada et al.[7] have reported one case out of 225 in which cancer invasion was contained within the mp, and Miwa et al[9]. have reported four cases with mp invasion. These authors have suggested that these positive cytologies may in some cases be false positive. For example, macrophages and mesothelial cells may sometimes be mistaken for malignant cells[10]. This is also the possibility that the malignant cells may be derived from metastasis in lymph nodes or primary tumors of other organs[11]. We should also consider that cases with exceptionally long survival, despite positive cytology, has been reported. Miwa et al. have stated that one can not conclude that a positive cytology is always associated with a poor prognosis[9].

Previous reports[3,6,9] have shown that the prognosis in surgically treated patients with gastric carcinoma is significantly affected by the presence or absence of intraperitoneal free cancer cells at the time of surgery. We have shown that the postoperative 5-year survival rate was only 8.7% in patients with microscopic evidence of intraperitoneal free cancer cells, compared with 46.7% in patients without detectable tumor cells in the peritoneal washings. Thus, the data suggest that cytology of lavaged saline from Douglas pouch can, although requiring a somewhat complicated procedure, be used as a prospective indicator for the risk of postoperative peritoneal metastasis. Based on these data, we are now conducting a study of prophylactic intraperitoneal administration of cytocidal anticancer drugs to prevent the recurrence of disseminated malignant cells.

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〈和文抄録〉

胃癌手術時の腹腔洗浄細胞診の評価

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教室の胃癌手術症例120例について，術中腹腔洗滌細胞診を行い，予後との関連について検索を行った．全症例での腹腔洗滌細胞診の陽性率は19.2%であったが，組織学的漿膜浸潤症例の陽性率は30.5%であった．胃壁漿膜への癌浸潤程度，肉眼的腹膜播種，癌進行程度などは腹腔洗滌細胞診の陽性率と密接な関連が見られた．細胞診陽性症例は陰性症例に比較し有意に生存率の低下が見られた（$p<0.001$）．細胞診陽性症例22例は2例を除いたすべての症例で2年以内の死亡が見られた．胃癌では腹膜播種は最も頻度の高い再発形成であることから，この診断手技は予後を予想する指標として，また，肉眼的には確認できない組織学的腹膜播種転移に対して補助的癌化学療法を施行する症例を選択する指標として有用である．