Upsala J Med Sci 107: 61–70, 2002

Clinicopathological Study for Gastric Cancer with Liver Metastasis[1]

Shin Teshima, Kenji Kakizaki, Toshihiro Saito, Takashi Yokota and Hidemi Yamauchi

Department of Surgery, Sendai National Hospital, Sendai 983-8520, Japan

ABSTRACT

Clinicopathological characteristics, stromal volume (Vvf), nuclear DNA content and cell protein were retrospectively analyzed in order to scrutinize the risk factors of hepatic metastasis from carcinoma of the stomach. We conducted a clinicopathological study of 327 patients with gastric cancer, including 34 patients with liver metastasis (synchronous, 22; metachronous, 12) and 294 patients without liver metastasis. Univariate analysis revealed significant inter-group differences in tumor size ($p<0.001$), depth of invasion ($p<0.001$), lymph node metastasis ($p<0.001$), vascular involvement ($p<0.001$), lymphatic involvement ($p<0.001$), peritoneal dissemination ($p<0.05$), Vvf ($p<0.01$) and DNA content ($p<0.01$). Vvf and DNA content were estimated in the liver metastasis group (n=20) and in the group of patients with stage III–IVa carcinoma but without liver metastasis (n=11). In multivariate analysis, only Vvf and DNA content showed significant correlations with liver metastasis ($p<0.01$). A comparison of Vvf and expression of the amount of interstitial connective tissue showed that there as a significant correlation between them. Our results indicate that gastric cancer with low Vvf and high DNA content carries a high risk of hepatic metastasis. Therefore, mean DNA content and Vvf are useful indices for predicting liver metastasis from gastric carcinoma.

INTRODUCTION

Although the incidence of liver metastasis has been decreasing due to an increase in the number of diagnosed casas of early gastric cancer, Yonemura et al. reported in 1990 that it was found in 6–10% of all patients with gastric cancer (18).

The prognosis for gastric cancer with hepatic metastasis is quite poor, and the best method of treatment is not clear. Hepatic resection of metastatic tumors from colorectal cancer has been carried out as a standard treatment (3); however, patients with metastatic liver tumors from gastric cancer have rarely been considered as can-

1) Received 15 August 2001
Revised manuscript accepted 7 August 2002

didates for surgical treatment because in most cases there are multiple metastases or the malignancy is associated with peritoneal dissemination (17). If the risk factors of hepatic metastasis from gastric cancer could be determined, more meticulous monitoring and prophylactic intervention such as adjuvant chemotherapy could be considered in those patients with high risk factors.

In order to evaluate the risk factors of hepatic metastasis from gastric cancer, clinicopathological characteristics, stromal volume (Vvf), nuclear DNA content and cell protein were retrospectively analyzed.

PATIENTS AND METHODS

This study was comprised of two parts.

1) Clinicopathological study

A total of 327 patients with gastric cancer were treated between 1984 and 1988 at the Department of Surgery, Sendai National Hospital. Thirty-four (10.4%) of the 327 patients had hepatic metastasis, of which 22 cases (6.7%) were synchronous and 12 cases (3.7%) metachronous. In the synchronous group, gastrictomy was performed in 8 cases. According to the criteria set by the Japanese Classification of Gastric Carcinoma (6), clinicopathological characteristics were retrospectively analyzed to clarify risk factors of hepatic metastasis from carcinoma of the stomach.

2) Morphometric and molecular-biological study

Morphometric analysis of the volume fraction of the stromal elment, nuclear DNA and mean cell protein were studied retrospectively in selected patients in order to stratify the clinical staging between groups. In this part of the study patients were divided into a synchronous group (Group A, n=8), a metachronous liver metastasis group (Group B, n=12), and a group with no liver metastasis (Group C, n=11). Patients in group C were those who were classified as stage III–IVa and had survived for more than 3 years or had shown an absence of liver metastasis at the time of death. Clinicopathological characteristics of these selected case were analysed between patients with liver meta (group A and B) and these without liver metastasis (group C).

Photomicroscopic examination of 3-µm thick sections of cancer tissue stained with Elastica-Goldner stain was carried out. The volume fraction of the stromal element (Vvf, %) was morphometrically estimated by means of point counting (19).

Paraffin-embedded blocks obtained from the primary lesions were subjected to DNA and cell protein study. Fifty-µm-thick sections of carcinoma tissue were deparaffinized and minced. Then the cell suspension was smeared after staining with 4´,6-diamidino-2 phenylindole and hematoporphyrin for nuclear DNA and cell protein, respectively (12). Fluorescence intensities of 20 lymphocytes as a control and of 200 carcinoma cells of each specimen were measured by using an Olympus spectrophotometric microscope with an on-line computer system (OPS-1). Cancer

Table 1. Characteristics of gastric cancer with and without liver metastasis. (22 cases of synchronous and 12 cases of metachronous metastasis, 293 cases of no liver metastasis).

		With liver metastasis	Without liver metastasis	
Sex	male	26 (16:10)	195	
	female	8 (6:2)	98	N.S.
Age		63.5±11.5 63.5:64.5)	61.6	N.S.
Size		77.7±33 mm (81.2:71.8)	48.0	P<0.001
Depth	m, sm	3.8% (0:8.3)	43.1	
	mp, ss	15.4% (7.1:25)	29.5	
	se	65.4% (78.6:50)	24.4	
	si	15.4% (14.3:16.7)	3.0	P<0.001
P	(–)	70.6%	86.4	
	(+)	29.4%	13.6	P<0.05
n	(–)	2.9% (0:83)	51.6	
	(+)	97.1% (100:17)	48.4	P<0.001
ly	(–)	4% (0:9.8)	53.4	
	(+)	96% (100:90.2)	46.6	P<0.001
v	(–)	45% (22.7:90.9)	89.7	
	(+)	55% (77.3:9.1)	10.3	P<0.001
Stromal type	med.	44% (33.3:60.8)	42.2	
	int.	44% (50:38.5)	35.9	
	sci.	12% (16.7:10.7)	21.9	N.S.
INF	α	8% (0:15.4)	24.4	
	β	72% (75:69.2)	45.9	P<0.05
	γ	20% (25:15.4)	29.7	
Histological type	diff.	60% (54.5:63.2)	53.6	
	undiff.	40% (45.5:36.8)	46.4	N.S.

The values in brackets refer to the groups with synchronous and metachronous metastasis, respectively. Depth of tumor invasion: m=mucosa, sm=submucosa, mp=muscularis propria, ss=subserosa, s=serosa, si=invasion of adjacent strictures, P=peritoneal metastasis, n=extent of lymph node metastasis, ly=lymphatic invasion, v=venous invasion. Stromal thpe: med.=medullary, int.=intermediate type, sci.=scirrhous type, INF=pattern of tumor infiltration into the surrounding tissue. Histological type: diff.=differentiated type, undiff.=undifferentiated type.

cells were verified and selected for analysis to eliminate contamination of normal cells.

Statistical Analysis

Student's t-test and the χ^2-test were employed for inter-group comparison. Statistical significance of nuclear DNA and cell protein values was determined by one way ANOVA. Multiregression analysis was also used. A probability of less than 0.05 was considered statistically significant.

Table 2. Clinicopathologic features of gastric cancer in selected patients (second part of the study).

	With liver metastasis (n=20) synchronous: 8 metachronous: 12	Without liver metastasis (n=11)	
Age	63.0±12.1	56.3±14.7	N.S.
Size (mm)	69.0±33.0	62.5±21.0	N.S.
Histological type			
differentiated	15	8	
undifferentiated	5	3	N.S.
n (–)	1	0	
n (+)	19	11	N.S.
ly (–)	2	0	
ly (+)	18	11	N.S.
v (–)	17	10	
v (+)	3	1	N.S.
Stromal type			
medullary	9	2	
intermediate	9	3	
scirrhous	2	6	p<0.05

n=extent of lymph node metastasis, ly=lymphatic invasion, v=venous invasion.

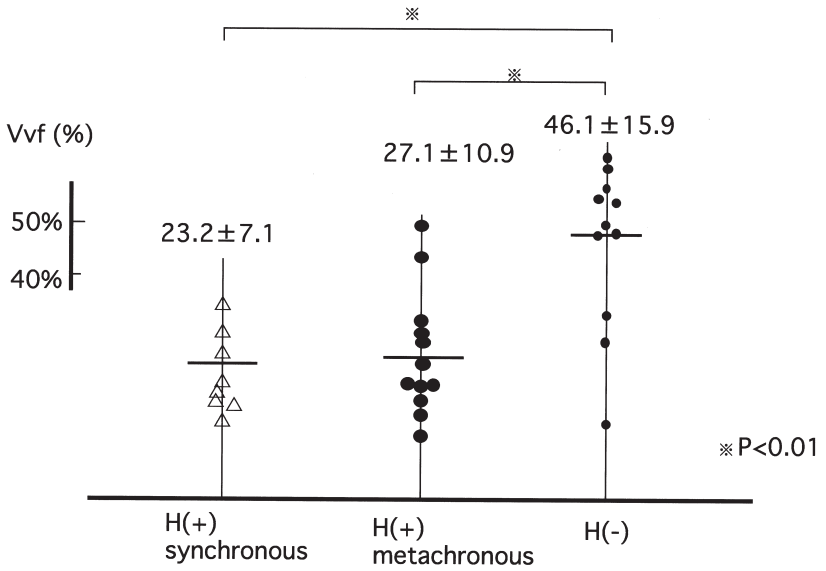

Fig. 1. Volume fraction of stromal element (Vvf). There were significant differences between groups A, B and C (p<0.01). All of the patients in group A had Vvf values of less than 40%, and all of the patients in group B had Vvf values of less than 50%. On the other hand, Vvf values were over 40% in eight out of the 11 patients (73.3%) in group C.

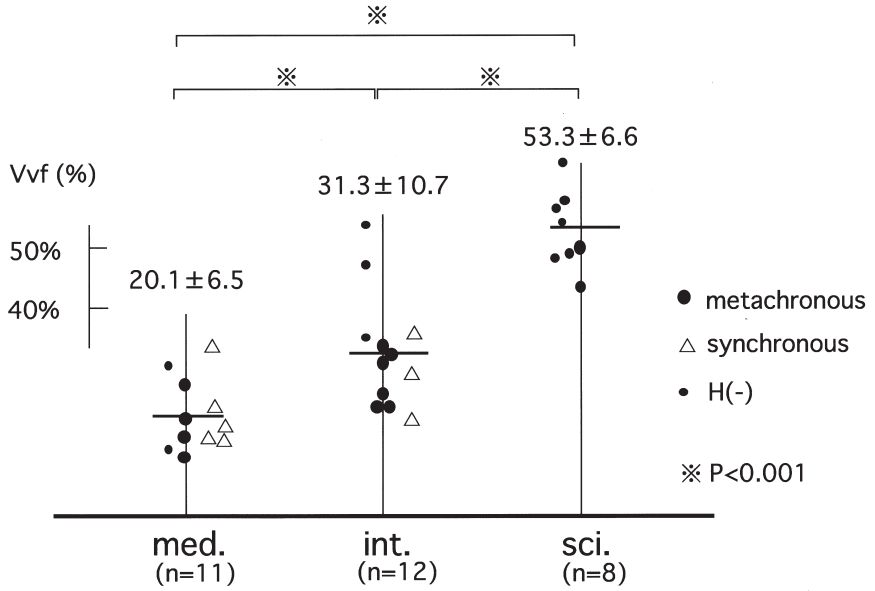

Fig. 2. Comparison of Vvf and expression of the amount of the interstitial connective tissue in the cancer tissue (medullary, intermediate, and scirrhous types). There were multiple overlaps of Vvf values between the groups divided by the usual classification of stromal type. med.=medullary, int.=intermediate type, sci.=scirrhous type.

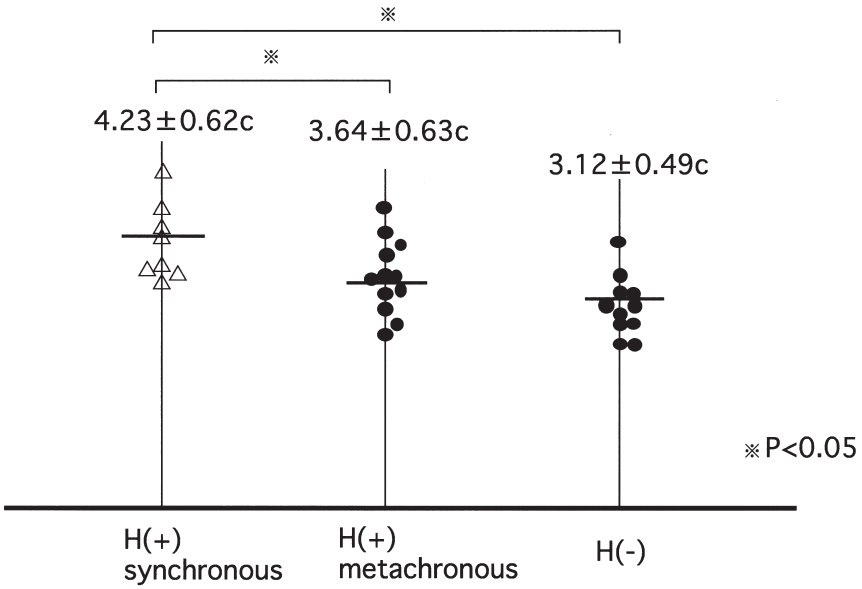

Fig. 3. Mean DNA content in each group. There were significant differences between the groups with and without liver metastasis ($p<0.01$).

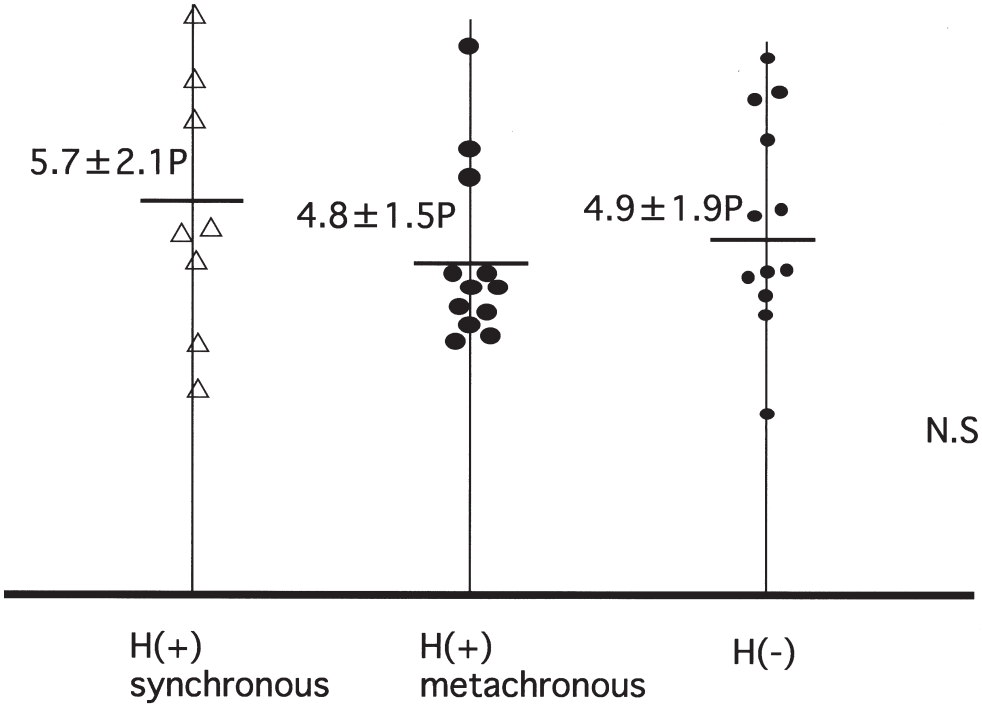

Fig. 4. Mean cell protein in each group. No significant difference was found between the three groups.

RESULTS

Clinicopathological features of the whole cases. Table 1 shows the clinicopathological features of cases in the liver metastasis group compared with those without liver metastasis. There were statistically significant inter-group differences in tumor size, depth of invasion, nodal involvement, lymphatic invasion, venous invasion, peritoneal dissemination and type of infiltrative growth (INF).

Clinicopathologial features of the selected cases. Table 2 shows the clinicopathological features of selected patients (second part of the study). In these patients, no statistically significant differences were found between cases with (group A and B) and those without liver metastasis (group C) in tumor size, depth of invasion, nodal involvement, lymphatic invasion, venous invasion, peritoneal dissemination and type of INF. A significant difference was found only in tromal type.

Ten of the 12 cases (83.3%) in the metachronous metastatic group (group B) belonged to clinical stage III or IV at the time of primary surgery and only 2 cases (16.7%) were classified as stage II.

Volume fraction of stromal element (Vvf). Fig. 1 shows Vvf in each group. The mean ± s.d. values of Vvf were 23.2±7.1% in group A, 27.1±10.9% in group B and 46.1±15.9% in group C. There were significant differences between the mean val-

Table 3. Multiregression analysis of risk factors for liver metastasis of gastric cancer.

	Correlation Coefficients	P
Size	–.012	.9219
ly	.332	.0681
v	–.130	.3347
n	–.084	.5024
Stromal type	.282	.3776
INF	–.150	.4518
P	.260	.1218
Vvf	–.758	.0024
DNA	.387	.0092
Cell protein	–.029	.8294

ly=lymphatic invasion, v=venous invasion, n=extent of lymph node metastasis, INF=pattern of tumor infiltration, P=peritoneal metastasis, Vvf=volume fraction of the stromal volume.

ues of Vvf in the groups with liver metastasis and the group without liver metastasis ($p<0.01$). The individual Vvf values of all patients in group A and group B were less than 40% and 50%, respectively. On the other hand, Vvf was over 40% in eight of the 11 patients (73.3%) in group C. As expected, a comparison of Vvf and the usual expression of the amount of interstitial connective tissue in cancer tissue (medullary, intermediate and scirrhous types) showed that there was a significant relationship ($p<0.001$) between them. However, there were multiple overlaps of Vvf values between the groups according to the usual classification of stromal type (Fig. 2).

Mean DNA content and cell protein. Fig. 3 shows the mean DNA content in each group. The mean ± s.d. value of DNA content in groups A, B and C were 4.23±0.62 C, 3.64±0.63 C and 3.12±0.49 C, respectively. There were significant differences between these three groups ($p<0.01$). The nuclear DNA pattern was mainly (in 70% of cases) aneuploid in the liver metastatic groups (groups A and B), and an aneuploid pattern was found in 5 cases (45.5%: N.S.) in group C. The mean ± s.d. values of mean cell protein in groups A, B and C were 5.66±2.15 P, 4.86±1.53 P and 4.92±1.90 P, respectively (Fig. 4). No significant inter-group differences were found.

The results of multiregression tests showed that only Vvf and mean DNA content were significant risk factors for development of liver metastasis (Table 3).

DISCUSSION

In the present study, clinicopathological characteristics of 327 patients with gastric cancer were analyzed according to the criteria set by the Japanese Research Society for Gastric Cancer (6), and a comparative study of patients with and those without liver metastasis was carried out. Significant differences were formed in tumor size,

depth of invasion, nodal involvement, lymphatic invasion, venous invasion, peritoneal dissemination and type of INF by univariate analysis. Some investigators have reported that gastric cancer of Borrmann type 1 or 2 and medullary type tend to metastasize to the liver (7, 10). The results of the present study agree well with those. In Japan, gastric cancer of the undifferentiated or medullary type is frequently accompanied by hepatic metastasis, although the number of cases of these types of gastric cancer is small.

The development of distant metastasis depends on complex interactions between tumor cells, surrounding connective stroma, vascular endothelium, and parenchymal cells of the target organ (1, 14). The first step of metastasis is characterized by the ability of tumor cells to degrade the extracellular matrix (ECM) (8). As a consequence, metastatic tumor cells may easily pass through the ECM, thus reaching blood vessels. The main components of the ECM are laminin, fibronectin, and collagen. Fibronectin is involved in fibrous tissue formation, and laminin in promotion of venous spreading of cancer cells (9).

The activity of type IV collagenase might be associated with vascular invasion of cancer through disruption of basement membranes (2). In the present study, the volume fraction of the stromal element (Vvf, %) was morphometrically estimated by means of point vounting. It is well known that well-differentiated adenocarcinoma tends to metastasize more frequently to the liver than the scirrhous type of poorly differentiated adenocarcinoma (10). In our previous study of Vvf in carcinoma of the pancreas, the rate of liver metastasis in cases of scirrhous carcinoma of the pancreas was low (16). In the present study, Vvf in gastric cancer with hepatic metastasis (25.6%) was significantly lower than that in gastric cancer without hepatic metastasis ($p<0.01$). These results confirm those of our previous study on carcinoma of the pancreas. Moreover, it is remarkable that the values of Vvf were less than 50% in all patients with hepatic metastasis and over 40% in most patients without hepatic metastasis.

In most malignant neoplastic diseases in humans, there is a relationship between the cytometrically assessed nuclear DNA distribution pattern of tumor cells and the length of patient survival (4, 5). We also found a correlation between the prognosis and nuclear DNA content in pancreas cancer patients (15). Therefore, the analysis of nuclear DNA in gastric cancer was performed by cytofluorometry in which the fluorescence intensity of 200 carcinoma cells was measured after the cells had been identified as being cancerous in order to eliminate contamination by non-cancerous cells. In this study, the mean value of nuclear DNA content was higher in the liver metastatic group.

Multiregression analysis showed that patients with low levels of Vvf and mean DNA content were in the high-risk group for liver metastasis. These results indicated that DNA and Vvf may be useful biochemical tumor markers representing the growth and metastatic pattern. Further studies are needed to evaluate the significance of the extracellular matrix of gastric cancer in hepatic metastasis.

Recent improvements in surgical techniques and diagnostic procedures, especial-

ly intraoperative ultrasonography, have enabled hepatic resection to be performed safely and with exclusion of residual metastasis in the liver. This justifies hepatectomy as a strategy for metastatic liver tumors, especially in cases of colorectal cancer. The reason why gastric cancer with liver metastasis is believed to have a poorer prognosis is due to a combination of non-curative factors such as peritoneal dissemination, high-grade lymph node metastasis, or distant metastasis (17). In the present study, 81.2% of the patients with liver metastasis had these non-curative factors.

Alternative treatments for hepatic metastasis are systemic or intra-arterial chemotherapy. Many studies have shown the efficacy of chemotherapy via the hepatic artery, but long-term survivors are relatively rare. On the other hand, systemic chemotherapy or intra-arterial chemotherapy for liver metastasis was shown to be effective in patients treated by reduction surgery but not in patients who did not undergo gastrectomy (11, 13).

The problem is the regimen of postoperative adjuvant chemotherapy. The therapeutic strategy should be selected according to the predicted type of recurrence.

In conclusion, Vvf and nuclear DNA may be useful indices for predicting hepatic metastasis in cases of gastric carcinoma. Further investigation is needed to clarify the relation between the clinical outcome of patients and Vvf and nuclear DNA, and to evaluate whether this relation may be of importance for the therapeutic strategy for patients with gastric carcinoma.

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Adress for reprints: Shin Teshima, M.D.
Department of Surgery, Sendai National Hospital
Miyaginoku, Sendai 983-8520, Japan
Fax: 022-291-8114
E-mail address: shin-525@kg7.so-net.ne.jp