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Acknowledgements—We thank Mr A. Cione, Department of Pathology, for technical assistance. This study was partly supported by a grant of the Ministero della Sanità and A.I.R.C./90.

Eur J Cancer, Vol. 27, No. 2, pp. 190–193, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Diagnostic Accuracy of Combination of Assays for Immunosuppressive Acidic Protein and Carcinoembryonic Antigen in Detection of Recurrence of Gastric Cancer

Norio Shimizu, Hiroshi Yamashiro, Atsunobu Murakami, Ryuichi Hamazoe and Michio Maeta

Two tumour markers, immunosuppressive acidic protein (IAP) and carcinoembryonic antigen (CEA), were assayed in gastric cancer patients. Levels of IAP and CEA were measured simultaneously in the preoperative and postoperative periods. The usefulness of the combined assay of these markers for detection of recurrence of cancer was investigated in terms of sensitivity, specificity and diagnostic accuracy. Sensitivity was not high (69.2%), but specificity and diagnostic accuracy were 96.7% and 86.9%, respectively. In cases with metastases in the liver, sensitivity (100.0%), specificity (100.0%) and diagnostic accuracy were high. In cases of peritoneal dissemination, these indices were low. The combination assay of IAP and CEA appears to be useful for detection of recurrence of gastric cancer, especially in patients with liver metastases.
Eur J Cancer, Vol. 27, No. 2, pp. 190–193, 1991.

INTRODUCTION

MANY TUMOUR markers have been used to detect malignancies, to predict staging or prognosis, to estimate the effects of treatment and to detect recurrence [1–5]. Carcinoembryonic antigen (CEA) has generally been used to predict the stage or prognosis of colorectal cancer and to detect recurrence. We have used CEA as a marker for gastric cancer [6]. Immunosuppressive acidic protein (IAP) was first found by Tamura *et al.* [7]. It is an α-1 acid glycoprotein and has been used as a marker for various malignancies, (e.g. gynaecological [8], testicular [9], colorectal [10], pancreatic and choledochal [10], and gastric cancers [11]). We have measured plasma levels of CEA and IAP in patients with gastric cancer and investigated the usefulness of these tumour markers for the detection of gastric cancer and of recurrence.

PATIENTS AND METHODS

Plasma levels of IAP and CEA were measured simultaneously in 349 patients with gastric cancer admitted to the Hospital of

Table 1. Sensitivity, specificity and diagnostic accuracy in gastric cancer and sensitivity in benign gastric disease of assays for IAP and CEA

	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
Resectable gastric cancer at:			
Stage I/II (n = 160)			
IAP	21.9 (a)	72.9	33.7 (c)
CEA	4.4 (b)	91.7 (d)	24.5 (f)
IAP + CEA	24.4 (c)	64.6 (d')	33.7 (g)
Stage III/IV (n = 161)			
IAP	52.2 (a')	72.9	56.9 (e')
CEA	22.4 (b')	91.7 (d)	38.3 (f')
IAP + CEA	62.1 (c')	64.6 (d')	62.7 (g')
Non-resectable gastric cancer (n = 28)			
IAP	85.7 (a'')	72.9	77.6 (e'')
CEA	39.3 (b'')	91.7 (d)	65.8 (f'')
IAP + CEA	85.7 (c'')	64.6 (d')	77.6 (g'')
Benign gastric disease (n = 48)			
IAP	27.1 (a''')	–	–
CEA	8.3 (b''')	–	–
IAP + CEA	35.4 (c''')	–	–

a–a', a–a'', a'–a'', a'–a''', b–b', b–b'', b'–b'', b'–b''', c–c', c–c'', d–d', g–g' and g–g'' : $P < 0.001$. c–c'', e–e', e'–e'', f–f' and f–f'' : $P < 0.01$. g'–g'' : $P < 0.02$.

Tottori University School of Medicine between 1984 and 1988. IAP and CEA were assayed preoperatively, and then 1 month after surgery and every 3 months thereafter. Levels were also measured in 48 patients with benign gastric disease (gastric or duodenal ulcer, gastric polyps or gastritis).

The assay methods for plasma CEA and IAP have been described [6, 11]; CEA was measured by the Z-gel method and IAP by the single radial immunodiffusion method. Values above 5.0 ng/ml CEA and 500 μg/ml IAP were recorded as positive. Sensitivity, specificity and diagnostic accuracy were calculated for each tumour marker and for the combined assay of CEA and IAP.

Stages of gastric cancer were classified according to the general rules for the gastric cancer study in surgery and pathology [12]. Statistical analysis was done with Student's t test.

RESULTS

Table 1 shows the sensitivity, specificity and diagnostic accuracy of assays for IAP and CEA in patients with gastric cancer and sensitivity in patients with benign gastric disease. Sensitivity in stage I and II gastric cancers was low, although it was somewhat higher in stage III and IV disease. In non-resectable gastric cancer, sensitivity was high for IAP (85.7%) and for IAP with CEA (85.7%). Specificity was very high in the assay for CEA for all patients (91.7%). Diagnostic accuracy was low in resectable gastric cancer and in cases of non-resectable cancer it was 77.6% at most with the assay for IAP or the combined assay. Sensitivity for all the cases of gastric cancer was 41.0% for IAP, 15.5% for CEA and 46.7% for the combined assay.

Table 2. Main types of recurrence in patients with gastric cancer who had curative surgery

Recurrence	No. of patients
Liver metastases	11
Peritoneal dissemination	7
Lymph node metastases	4
Local recurrence	1
Other (lung metastases)	1
Total	24

Of 321 patients with gastric cancer who underwent surgery, 252 patients received curative surgery. In this group of 252, which included those patients with early gastric cancer, 24 had a recurrence (Table 2).

Sensitivity, specificity and diagnostic accuracy of the combination of the assays for IAP and CEA for the detection of recurrence were calculated (Table 3). The sensitivity of the combined assay was not high (69.2%), but specificity and diagnostic accuracy were very high (96.7% and 86.9%, respectively).

The three main types of recurrence of gastric cancer and the sensitivity, specificity and diagnostic accuracy of assays for IAP and CEA for the detection of recurrence of gastric cancer are shown in Table 4. Sensitivity was higher in cases of liver metastases than with peritoneal dissemination and lymph node metastases. Specificity was high in cases of metastases in the liver and lymph nodes, being 100.0% in for the liver for the combination assay. Diagnostic accuracy was also high in cases of metastases in the liver, but low with peritoneal dissemination.

DISCUSSION

Among the many tumour markers identified, CEA has achieved widespread acceptance as a marker in various malignancies for prediction of stage and prognosis, for assessment of the efficacy of cancer chemotherapy and for detection of recurrence. There are a few reports of the use of CEA in gastric cancer. Preoperative sensitivity of measurements of CEA in gastric cancer cases has been reported as 27.5% [13], 21.0% [14], 20.9% [15] and 18.7% [6], and at 15.5% in our study. Almost all the

Table 3. Sensitivity, specificity and diagnostic accuracy of assays of IAP and CEA in detection of recurrence of gastric cancer

Assay	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
IAP	50.0	91.3	90.5
CEA	44.1	95.9	88.9
IAP + CEA	69.2	96.7	86.9

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Received 1 Sep. 1990; accepted 5 Nov. 1990.

Table 4. Three main types of recurrence of cancer and sensitivity, specificity and diagnostic accuracy of assays for IAP and CEA in detection of recurrence of cancer

Assay	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
IAP			
Liver metastases (n = 11)	72.7 (a)	76.9	75.0
Peritoneal dissemination	14.3 (a')	53.8	33.3
(n = 7)	25.0	76.9	45.8
Lymph node metastases (n = 4)			
CEA			
Liver metastases (n = 11)	81.8 (b)	77.8	66.7
Peritoneal dissemination	28.6 (b')	44.4	25.0
(n = 7)	50.0	77.8	37.5
Lymph node metastases (n = 4)			
IAP + CEA			
Liver metastases (n = 11)	100.0 (c)	100.0 (d)	75.0 (e)
Peritoneal dissemination	28.6 (c')	28.6 (d')	16.7 (e')
(n = 7)	50.0 (c'')	71.4	29.2
Lymph node metastases (n = 4)			

a–a', c–c' and d–d' : $P < 0.001$. c–c'' and e–e' : $P < 0.01$. b–b' : $P < 0.02$.

reported results indicate that sensitivity becomes higher as the cancer progresses [13, 16, 17]. We found high sensitivity at later stages and the highest sensitivity in non-resectable gastric cancer. Thus, gastric cancer patients with a high CEA value should be considered as having advanced cancer.

IAP has an immunosuppressive effect *in vitro* and *in vivo* [18]. Pre-operative levels of IAP have been measured in patients with various malignancies, including gastric cancer, and sensitivity generally increases at later stages [8–11]. In our previous report, preoperative sensitivity of IAP assay increased with progression of stage, as it did in our present study. Various tumour markers have been measured in combination assays for gastric cancer to detect lesions, to predict cancer staging or prognosis, to assess the efficacy of therapy or to detect recurrence: for example CA 19-9, CEA and IAP [10], tissue polypeptide antigen (TPA), lipid-bound sialic acid (LBSA) and CEA [14]. Furukawa *et al.* have also used combined assay of IAP and CEA in gastric cancer [10].

Sensitivity, specificity and diagnostic accuracy of assays have been discussed as indices for the evaluation of tumour markers [19, 20], and we have used these criteria to assess the usefulness of IAP and CEA assay. IAP and CEA were assayed in an attempt to find patients with gastric cancer, but, as with other tumour markers, sensitivity and diagnostic accuracy were not high, even with the combined assay in resectable gastric cancer. For the detection of the recurrence of gastric cancer, the sensitivity of the assay for IAP was 50.0%, for CEA, 44.1%, and for the combined assay, 69.2%. Both specificity and diagnostic accuracy were high for all assays. Thus, periodic measurement of IAP and CEA was useful for confirming recurrence.

The relation between types of recurrence of cancer and the sensitivity of the assays for IAP and CEA was examined. In cases of metastases in the liver, sensitivity of assays for IAP or CEA was high (72.7% and 81.8%, respectively) and it was 100.0% for the combined assay. In such cases, at the time of detection of recurrence, either the level of IAP or that of CEA was always positive. In cases of peritoneal dissemination, the sensitivity of assays for IAP or CEA was low (14.3% and 28.6%, respectively), and for the combined assay, sensitivity was still only 28.6%. For such cases, no useful tumour markers have yet been found, even though many investigators are searching for good markers of peritoneal recurrence. Intermediate results were obtained in cases with lymph-node metatases. According to the sensitivity, specificity and diagnostic accuracy of assays for IAP and CEA, liver metastases should be easily detectable by the combined assay. However, the detection of peritoneal dissemination was difficult.

In a previous report, elevated levels of CEA were identified about 4.8 months before detection of recurrence [6]. In this study, positive levels of CEA were observed about 3.9 months before detection of recurrence in 15 patients and positive levels of IAP were observed about 3.0 months before detection of recurrence in 11 patients. With the combined assay, positive values were observed about 2.8 months before detection of recurrence in 17 patients.

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Acknowledgement—We thank Miss A. Shimada for technical assistance.

Eur J Cancer, Vol. 27, No. 2, pp. 193–197, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Human Papillomavirus DNA in Cervical Intraepithelial Neoplasia Detected by *in situ* Hybridisation

Maria Rosaria Cardillo, Raffaele Marino and Vincenzo Pozzi

Human papillomavirus (HPV) infection was investigated by *in situ* hybridisation in histological sections from 38 women with abnormal Papanicolaou smears. 13 patients had condylomatous lesions without atypia, 15 cervical intraepithelial neoplasia (CIN) I, 4 CIN II, 3 CIN III and 2 carcinoma *in situ* (CIS). HPV DNA was detected in 29 cases (78%) (1 specimen was technically inadequate). HPV 16 and 18, and 31, 33 and 35 were both present (67%) in CIN III. HPV 6 and 11 were more frequent in CIN I (56%) and in condylomatous lesions (38%). 31% of the condylomatous lesions without atypia contained HPV 31, 33, and 35 and 31% of those with CIN I were infected with HPV 16 and 18. These data confirm the frequent association of HPV infection with cervical cancer and CIN, and indicate that *in situ* hybridisation can identify patients with specific types of HPV infection at risk for cervical cancer.
Eur J Cancer, Vol. 27, No. 2, pp. 193–197, 1991.

INTRODUCTION

SINCE 1976, when Meisel [1] first described cytohistological changes caused by human papillomavirus (HPV) in cervical epithelium, this infection has been diagnosed in 1–2% of women undergoing general screening [2] and in 5–10% of high-risk women being screened for cancer of the cervix [3]. In younger women, condylomatous changes generally occur without atypia; in women over 30, koilocytes are usually associated with various degrees of cervical intraepithelial neoplasia (CIN) and carcinoma *in situ* (CIS) [4].

Many low-grade lesions regress to normal or remain stable; others progress to dysplasia or invasive carcinoma. Although the mechanism underlying malignant conversion is unknown, current evidence implicates the type of HPV. Whether integration of HPV DNA by DNA in the host cell, other infective or viral agents, genetic predisposition, or the host's immunological defences influence the course of the infection is not known [5]. Because CIN develops faster in women infected with HPV [6], this high-risk group requires regular follow-up. With the use of DNA hybridisation, more than 60 HPV DNA types so far isolated have been detected in the uterine cervix. HPV 6 and 11 frequently coexist with cervical condylomata [7] and with low-grade intraepithelial neoplasia (CIN I) [8], but rarely with carcinoma. HPV 16, 18 and 31 have been found in all grades of CIN and also in invasive carcinomas of the cervix [9]. In worldwide reports, the virus most strongly associated with cervical intraepithelial neoplasia is HPV 16 [10], which is also associated with an increased risk of conversion from low-grade to high-grade CIN [11]. HPV was detected in 20% of CIN cases in the USA [12].

We have analysed HPV DNA in specimens from 38 intraepithelial cervical lesions, by *in situ* DNA hybridisation. The study was designed to detect HPV, identify the types present (HPV 6 and 11, 16 and 18, and 31, 33 and 35) and correlate the results with the histological appearance of the lesions.

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Revised 22 Oct. 1990; accepted 5 Nov. 1990.

MATERIALS AND METHODS

Biopsy specimens were taken from 38 women aged 20–46 years, who were undergoing colposcopy because two consecutive Papanicolaou tests had disclosed atypia (CIN I–III) or CIS. The smears were taken by cervical scraping. Biopsy specimens taken