Pergamon

*European Journal of Cancer* Vol. 32A, No. 1, pp. 63–68, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

0959-8049(95)00504-8

## Original Paper

# The Prognostic Value of Serum and Immunohistochemical Tumour Markers in Advanced Gastric Cancer

A. Webb,[1] P. Scott-Mackie,[1] D. Cunningham,[1] A. Norman,[1] J. Andreyev,[1] M. O'Brien[1] and J. Bensted[2]

[1]Cancer Research Campaign Section of Medicine and the GI Unit; and [2]Department of Histopathology, The Royal Marsden Hospital and The Institute of Cancer Research, Sutton, Surrey SM2 5PT, U.K.

**Using a prospectively acquired database of 290 patients with advanced gastric adenocarcinoma, the prognostic significance of serum levels of carcinoembryonic antigen (CEA) (237 patients), alphafeto protein (AFP) (164 patients), β-human chorionic gonadotrophin (βHCG) (165 patients), CA19-9 (64 patients) and CA125 (104 patients) and tissue staining for C-erb B-2 (160 patients) and βHCG (160 patients) was investigated. Serum was taken prior to 5-fluorouracil (5FU)-based chemotherapy and immunohistochemistry was performed on diagnostic specimens. In the univariate analysis, tumour markers of poor prognosis were CEA ≥ 5 μg/l ($P = 0.01$; median survival (MS) 42 versus 35 weeks), serum βHCG ≥ 4 U/l ($P = 0.02$; MS 42 versus 25 weeks), CA125 ≥ 35 U/ml ($P = 0.03$; MS 43 versus 31 weeks) and CA125 ≥ 350 U/ml ($P = 0.001$; MS 42 versus 17 weeks). Other significant factors were poor performance status, the presence of metastases and poorly differentiated tumour histology. Tumours markers of poor prognosis in the multivariate analysis were serum βHCG ≥ 4 IU/l [hazard ratio (HR) 1.7; 95% confidence interval (CI) 2.8–1.1] and CA125 ≥ 350 U/ml (HR 2.2; CI 4.2–1.2). There was a degree of subgroup variability in this model but, in general, other factors correlating with a poor survival were poor performance status, metastases and poorly differentiated tumour histology. This is the largest prognostic study of each tumour marker in advanced disease and demonstrates that serum βHCG and CA125 in gastric cancer prior to chemotherapy do convey an independent poor prognosis which may reflect not just tumour burden but aggressive biology.**

**Key words: gastric cancer, tumour markers, prognostic significance, chemotherapy, carcinoembryonic antigen, alphafeto protein, β-human chorionic gonadotrophin, CA19-9, CA125, C-erbB-2**
*Eur J Cancer*, Vol. 32A, No. 1, pp. 63–68, 1996

## INTRODUCTION

THE MEASUREMENT of tumour markers is of proven benefit in a number of different cancers. However, in gastrointestinal malignancy, despite the weight of research, they are not generally useful in diagnosis or screening due to their low sensitivity and specificity. Their role in predicting prognosis and response to treatment is less well defined. Gastric cancer is the fifth most common cancer in both sexes and the fourth most common cancer cause of death [1, 2]. This tumour in the advanced setting is generally considered incurable but the role of palliative chemotherapy and radiotherapy is becoming more accepted. Tumour markers could be very valuable in identifying patients with a better prognosis and/or chance of treatment response and hence targeting palliative treatment more effectively.

Correspondence to D. Cunningham.
Revised 21 Jul. 1995; accepted 31 Aug. 1995.

Carcinoembryonic antigen (CEA) has been the most evaluated tumour marker in gastric cancer. However, its value in the prognosis of advanced gastric cancer is much less clear. A number of other serum markers has been shown to be elevated in this cancer and their roles established in other tumours; alphafetoprotein (AFP) and β-human chorionic gonadotrophin (βHCG) in germ cell tumours, AFP in hepatoma, βHCG in choriocarcinoma, CA125 in ovarian cancer and possibly CA19-9 in pancreatic tumours. However, their roles in gastric cancer have yet to be clearly evaluated. In addition, the immunohistochemical markers C-erbB-2 and βHCG have been reported as correlating with a poor prognosis [3–8] in gastric cancer. The aims of this study were to analyse seven different tumour markers to determine their role as prognostic markers and in predicting tumour responsiveness to chemotherapy in advanced gastric carcinoma.

## MATERIALS AND METHODS

*Patient selection*

Patients were identified for this study using a prospectively acquired database and the study period was from February 1990 to March 1994. All the patients had either inoperable locally advanced or metastatic biopsy-proven adenocarcinoma of the stomach or gastro-oesophageal junction. Locally advanced disease was defined as inoperable primary tumour or partially resected primary without evidence of distant metastases. There were 290 patients with gastric cancer identified as suitable.

*Chemotherapy*

In most patients, the chemotherapy given was first-line chemotherapy. 5-Fluorouracil (5FU)-based chemotherapy was given to all patients and the most common regimens used were ECF: epirubicin (50 mg/m$^2$ 21-day cycle), cisplatin (60 mg/m$^2$ 21-day cycle), continuous infusional 5FU (200 mg/m$^2$) and FAMTX: 5FU (1500 mg/m$^2$ day 1), methotrexate (1500 mg/m$^2$ day 1), doxorubicin (30 mg/m$^2$ day 15 28-day cycle).

*Response evaluation*

Tumour site and size was evaluated before treatment using computed tomography (CT) scans and endoscopy. Response to chemotherapy was assessed similarly. A complete response (CR) was defined as disappearance of all bidimensional measurable disease, a partial response (PR) was defined as at least a 50% reduction in measurable disease, progressive disease (PD) was defined as an increase of 25% of measurable disease and stable disease (SD) was a state between partial response and progressive disease. An overall response is represented by the addition of CR and PR. Response to chemotherapy was not evaluable in 21 patients.

*Serum and immunohistochemical markers*

Serum was taken for tumour markers prior to treatment with differing numbers of patients in each tumour marker group. The patient pretreatment characteristics for each group were consequently analysed separately. CEA, AFP, βHCG, CA125 and CA19-9 serum levels were measured using a commercially available solid-phase enzyme immunoassay (Cobas kit from Roche, London, U.K.). The cut-off values were 5 μg/l for CEA, 10 IU/ml for AFP, 4 IU/ml for βHCG, 35 U/ml for CA125 and 37 U/ml for CA19-9. Immunohistochemical studies were performed on 164 gastric biopsies taken prior to treatment. An indirect alkaline phosphatase technique was used and this involved 3-μm cut sections of formalin-fixed paraffin wax-embedded tissue which was then dewaxed in histoclear and rehydrated in alcohol. This was treated in 20% acetic acid for 5 min to destroy endogenous alkaline phosphate and then washed in phosphate-buffered solution (PBS) at pH 7.4. The sections were then incubated with either 1:500 dilution of the antibody against C-erbB-2 protein (in-house product) or a 1:2000 dilution of the antibody against βHCG (Dako, U.K.) for 1.5 h at room temperature. After washing in PBS, the sections were then incubated with the secondary antibody, α-rat immunoglobulin (Serotec) diluted to 1:100 for the C-erbB-2 stain or α-rabbit immunoglobulin (Dako) at 1:50 dilution for the βHCG stain for 1.5 h at room temperature. Following further washing, the sections were incubated with the substrate solution for 1 h at room temperature and then the nuclei counterstained and mounted routinely. Sites of immunocytochemistry activity showed red against the blue nuclei counterstain. Positive control sections from a breast cancer for C-erbB-2 and normal placental tissue for βHCG were included with each batch. A positive stain for βHCG was defined as cells with staining in either cytoplasm or membrane or both but for a C-erbB-2 positive result, the stain had to include the membrane as this is reported to be correlated to *C-ERB B-2* gene amplification [9].

*Statistical methods*

Statistical analysis was performed in March 1994. The correlation between tumour marker and pretreatment characteristics/response to chemotherapy was analysed using the $\chi^2$ test. Survival curves were drawn using the Kaplan–Meier method and analysed by the log rank test. Multivariate analysis was performed on pretreatment prognostic factors using the proportional hazards model. Results were considered significant if the $P$ value was less than 0.05.

## RESULTS

*Carcinoembryonic antigen*

Pretreatment serum CEA levels were measured in 82% of the patients ($n$ = 237). Serum CEA levels were elevated in 119 patients (50%) with a range of 2–47783 μg/l. Elevated CEA levels correlated with liver metastasis and metastasis in general (as opposed to locally advanced disease), but there was no correlation with sex, performance status or histology differentiation (Table 1). Sixty-nine per cent of patients with liver metastases had an elevated CEA. The univariate analysis demonstrated a worse survival in the group with elevated CEA at a cut-off of 5 μg/ml ($P$ = 0.01; median survival 42 versus 35 weeks) but at the higher cut-off of 50 μg/l, there was no significant difference in survival (Table 2). Multivariate analysis indicated that CEA was not an independent prognostic factor but the presence of liver metastasis (hazard ratio, HR 2.0), performance status 3 or 4 (HR 1.8) and poorly differentiated tumour histology (HR 1.6) were all poor prognostic factors in the model.

*Alphafetoprotein*

Serum AFP levels were measured in 57% of the patients ($n$ = 164). AFP was raised in 25 patients (15%) with a range of 2–20667 IU/ml. A raised AFP correlated with the presence of liver metastases and metastases in general. Tumours with a poorly differentiated histology were less likely to secrete AFP (Table 1). An elevated AFP was not significant in both univariate and multivariate analysis for survival and the most important prognostic factor in the model were performance status 3 or 4 (HR 3.1) and the presence of metastases (HR 1.9) (Table 2).

*β-Human chorionic gonadotrophin*

Serum βHCG levels were measured in 57% of the patients ($n$ = 165). βHCG was raised in 34 patients (21%) with a range of 2–571 IU/l. An elevated serum βHCG correlated with liver metastases and poor performance status (Table 1). In the univariate analysis, an elevated βHCG at the lower cut-off value of 4IU/l correlated with poorer survival ($P$ = 0.02, median survival 42 versus 25 weeks) but at the higher cut-off value of 40 IU/l, there was no correlation with survival. Multivariate analysis confirmed that the independent prognostic importance of an elevated βHCG at this lower cut-off rate [HR 1.7; 95% confidence interval (CI) 2.1–1.8] but not at the higher cut-off value. Other factors which were important in the model were the presence of metastases (HR 1.8), poor performance status 3–4 (HR 2.9) and poorly differentiated tumour histology (HR 1.6) (Table 2). 85 patients had both serum βHCG and βHCG histochemistry performed but there was no correlation between these results.

*Table 1. Pretreatment and response characteristics*

| Pretreatment characteristics | CEA ≥ 5 μg/l ($n$ = 237) | AFP ≥ 10 IU/l ($n$ = 164) | βHCG ≥ 4 IU/l ($n$ = 165) | CA 125 ≥ 35 U/ml ($n$ = 104) | CA 19-9 ≥ 37 U/ml ($n$ = 64) | BHCG + ve stain ($n$ = 160) | C-erbB-2 + ve stain ($n$ = 160) |
|---|---|---|---|---|---|---|---|
| Total number | 119(50%) | 25(15%) | 34(21%) | 57(55%) | 35(55%) | 22(14%) | 12(8%) |
| Males | 94 | 18 | 21 | 34 | 26 | 18 | 10 |
| Performance status > 2 | 12 | 3 | 4* | 5* | 1 | 3 | 3 |
| Poorly differentiated | 59 | 7* | 13 | 32 | 15 | 14 | 5 |
| Tumour histology | | | | | | | |
| Liver metastases | 60‡ | 16† | 19† | 25† | 19* | 12 | 6 |
| Krukenberg metastases | — | — | — | 5 | — | — | — |
| All metastases | 85* | 22* | 26 | 46‡ | 27‡ | 14 | 9 |
| Chemotherapy response | 59 | 12 | 14 | 20* | 12 | 15 | 9 |

*$P < 0.05$; †$P < 0.005$; ‡$P < 0.0005$. Statistical analysis using the $\chi^2$ test correlating pretreatment characteristics with the elevated tumour marker group and the normal tumour marker group. Numbers in parentheses are the percentage of the total.

*Table 2. Univariate and multivariate analysis of pretreatment prognostic factors in advanced gastric cancer*

| Pretreatment characteristics | CEA | AFP | βHCG | CA125 | CA19-9 | βHCG stain | C-erbB-2 |
|---|---|---|---|---|---|---|---|
| Marker elevation | 0.01 | ns | 0.02* | 0.03 | ns | ns | ns |
| Marker elevation × 10 | ns | ns | ns | 0.001* | ns | ns | ns |
| Sex distribution | ns | ns | ns | ns | ns | ns | ns |
| Performance status > 2 | 0.002* | 0.02† | 0.02† | ns† | ns | ns | ns |
| Poorly differentiated tumour | 0.005* | ns | ns* | ns | ns | 0.01* | 0.01* |
| Histology | | | | | | | |
| Liver metastases | 0.0001* | 0.01 | 0.01 | 0.003 | ns | 0.008* | 0.008* |
| All metastases | 0.009 | 0.02* | 0.02* | 0.005* | ns | ns | ns |

*Hazard ratio (HR) > 1.5 < 2.5, †HR > 2.5 < 3.5. Multivariate analysis of pretreatment characteristics with the results expressed as ns, non-significant.

*CA125*

Serum CA125 levels were measured in 36% of patients ($n$ = 104). Serum CA125 was raised in 57 patients (55%) with a range of 20–3266 U/ml. A raised CA125 was correlated with a poorer performance status, liver metastases and metastases in general but not the presence of Krukenberg metastases (Table 1). In the univariate analysis, an elevated CA125 at a cut-off of 35 U/ml ($P$ = 0.03, median survival 43 versus 31 weeks) and at a higher cut-off 350 U/ml ($P$ = 0.001, median survival 42 versus 17 weeks) were both indicators of poor prognosis. However, on multivariate analysis only a CA125 above the higher cut-off value was significant for prognosis (HR 2.2; CI 1.2–4.2). Other more important prognostic factors in this model were metastases (HR 2.5) and a poor performance status (HR 3.7) (Table 2).

*CA19-9*

Serum CA19-9 levels were measured in 22% of the patients ($n$ = 64). Serum CA19-9 levels were raised in 35 patients (55%) with a range of 0–51425 U/ml. An elevated CA19-9 was correlated with the presence of metastases both in the liver and generally (Table 1). In the univariate and multivariate analysis, CA19-9 was not a significant prognostic factor (Table 2).

*βHCG immunohistochemistry*

Staining for βHCG was performed on samples from 55% of patients ($n$ = 160). Twenty-two specimens (14%) stained positively for βHCG and this did not correlate with any of the pretreatment characteristics (Table 1). On univariate and multivariate analysis there was no correlation with survival. Important prognostic factors in the multivariate model were liver metastases (HR 1.7) and poorly differentiated tumour histology (HR 1.5) (Table 2).

*C-erbB-2 immunohistochemistry*

Membrane staining for C-erbB-2 was performed in 55% of the patients ($n$ = 160). Positive staining was found in 12 patients (8%) and it did not correlate with any of the pretreatment characteristics (Table 1). C-erbB-2 staining was not a significant prognostic factor on the univariate or multivariate analysis. Important prognostic factors in the multivariate analysis were liver metastases (HR 1.7) and poorly differentiated tumour histology (HR 1.5) (Table 2).

*Prediction of chemotherapeutic response*

228 patients received ECF chemotherapy with a response rate of 60% and the remaining patients received a range of chemotherapy regimens with a response rate of 25%. In gastric cancer, CEA, AFP, βHCG and CA19-9 and both immunohistochemical stains did not predict response to chemotherapy, but an elevated CA125 correlated with a reduced response rate ($P$ = 0.04) (Table 1).

## DISCUSSION

CEA is the most studied tumour marker in gastrointestinal malignancies. It is a highly glycosylated cell surface glycoprotein

and may be expressed at increased levels in a variety of cancers and benign conditions. The half-life of CEA has not been determined. The liver is probably the major site of metabolism [10]. Following complete resection in colorectal cancer, the National Institutes of Health consensus conference stated that levels should return to normal in 6 weeks [11] but it has been reported to occasionally take 3 months [12]. This explains why raised levels are seen in benign liver disease and patients with liver metastases very commonly have an elevated CEA. This was confirmed by our findings that 69% of patients with liver metastases had an elevated CEA and that there was a high correlation between the two.

Evidence that CEA is elevated in gastric cancer has been available for a number years yet relatively little information has been published. An elevated CEA has been reported in 31–67% of patients with gastric cancer [13–18] and we confirm this with our findings of 50%. This value is less than in colorectal cancer probably because of the higher incidence of poorly differentiated tumours. Previous studies have concentrated on the value of CEA as a diagnostic or screening marker but have demonstrated low sensitivity and specificity. However, one study reported the value of postoperative CEA monitoring to facilitate the detection of recurrences in patients after gastrectomy [19]. Our results demonstrated that although statistically significant in the univariate analysis, CEA was not important in the multivariate model. Hence CEA does not have a prognostic role in advanced gastric cancer. AFP, like CEA, is an oncofetal antigen and is produced in the fetal liver, yolk sac and gastrointestinal tract. It is the major serum protein of the fetus and has a half life of 3.5–6 days. In adults there is a physiological elevation during pregnancy and moderate elevations (up to 500 IU/l) are found in up to 25% patients with benign liver diseases [20] and it has been hypothesised that AFP is synthesised during hepatic regeneration [21]. The role of AFP as a tumour marker for diagnosis and monitoring in malignant hepatoma and germ cell tumours is well established.

Fifteen per cent of our gastric cancer patients had an elevated AFP and 4 patients had values over 1000 IU/l, which compares with a previously reported range of 3.9–18% [22–25]. A raised AFP was highly correlated with liver metastasis and this has been reported previously [25]. An elevated AFP did not correlate with survival on both univariate and multivariate analysis and thus is not a useful prognostic marker in gastric cancer.

HCG is a glycoprotein hormone consisting of two subunits, $\alpha$ and $\beta$. Serum $\beta$HGC is secreted by syncytiotrophoblasts of the placenta and by neoplastic cells in tumours of trophoblastic origin where it is a valuable tumour marker. Elevated serum $\beta$HCG levels have been demonstrated in various nontrophoblastic neoplasms and $\beta$HCG-immunoreactive cells have been identified in the tissues of these neoplasms.

We found an elevated serum $\beta$HCG in 21% of patients compared to previous reports of 16–23% [26–29]. Positive tissue staining for $\beta$HCG was found in 14% in this study with a previous reported range of 7–53% [3–5, 30–32]. This discrepancy between reports is probably due to variations in staining and reporting techniques. Our results show no correlation between serum and immunohistochemical staining suggesting that the biopsied tumour is not responsible for raised serum levels. It has been reported that an elevated serum $\beta$HCG or tissue-staining positivity tended to be associated with poorer survival [3–5] although this was in the setting of relapse following surgery rather than in advanced disease. Our findings confirm that an elevated serum $\beta$HCG is a prognostic marker of poor survival on univariate and multivariate analysis (HR 1.7) at the lower cut-off value but not at the higher value. However, $\beta$HCG tissue staining did not correlate with survival and has no prognostic significance which is in accordance with one previous study of 92 cases [30].

CA125 is an antigenic determinant expressed by epithelial ovarian carcinomas that can be detected by a monoclonal antibody. An elevated CA125 is detectable in 80–90% of patients with ovarian cancer and the true half-life has been estimated to be 4.8 days [33]. In ovarian cancer, CA125 correlates well with tumour staging and disease monitoring but has a low specificity with elevations in breast and gastrointestinal malignancies. An elevated CA125 has been reported in 34–55% cases [17, 34, 35] in gastric cancer and our result of 55% falls within that range. The role of CA125 as a prognostic factor has not previously been reported and our findings indicate that an elevated CA125 at both the higher and lower cut-off values is a poor prognostic factor on univariate analysis but only the higher cut-off is significant on multivariate analysis. These results may just reflect a higher tumour burden but significance in the multivariate analysis, which contains indicators of tumour burden, suggests more aggressive tumour biology. Therefore, serum CA125 is probably a useful prognostic marker in gastric cancer.

The tumour marker CA19-9 is an oncofetal antigen and was developed by raising a monoclonal antibody against a human colorectal cancer line. An elevated serum CA19-9 has been found in a number of gastrointestinal malignancies and also in breast cancer. However, only in pancreatic cancer has the sensitivity and specificity been found to be better than CEA [36]. In gastric cancer, an elevated serum CA19-9 has been reported in 28–70% [18, 35–38] and our result of 55% is within this reported range. The prognostic significance of CA19-9 in gastric cancer has not previously been reported. Our results showed that both liver metastases and metastases in general were correlated with an elevated CA19-9, but on univariate and multivariate analysis there was no survival difference. Consequently, our recommendations would be that CA19-9 should not be used as a marker of prognostic significance in gastric cancer.

The *C-ERBB-2* gene is localised on chromosome 17 at q21 and encodes a 185 kDa cell surface glycoprotein. In breast cancer, amplification of the *C-ERBB-2* oncogene (detected by DNA hybridisation analysis) and enhanced expression of C-erbB-2 protein (detected by immunohistochemistry) have been shown to be associated with poor prognosis independent of TNM factors and clinical stage [9, 39–42]. In gastric cancer, C-erbB-2 protein overexpression has been reported in 8–14% of cases [6–8, 43, 44], which included only membrane-positive staining which correlates with *C-ERBB-2* gene amplification in gastric cancer [6]. Our results of 8% staining correlate well with these results of previous publications. It has been reported that there is a lower instance of C-erbB-2 positivity in the poorly differentiated histology type [7, 43]. There are conflicting reports about the correlation between C-erbB-2 positivity and survival. There are three papers reporting overexpression of C-erbB-2 protein as an adverse prognostic factor [6–8], one paper in which C-erbB-2-positive tumours had a better prognosis [43] and another paper which concluded that C-erbB-2 protein expression was not a useful prognostic indicator [44]. Our results indicate that immunohistochemical staining of C-erbB-2 protein expression is not a useful prognostic indicator in gastric cancer.

Although heterogeneous chemotherapy regimens were used, the majority received one regimen, ECF. However, any degree of heterogeneity may cloud the precision of the correlation. The correlation between pretreatment tumour markers and

subsequent responses to chemotherapy has not been reported in gastric cancer. Our results demonstrated a correlation with CA125 ($P = 0.04$) only, but this result would need further confirmation.

In advanced gastric cancer, an elevated CA125 (≥ 350 U/ml only) and βHCG (≥ 4 IU/l only) were significant prognostic factors of poor prognosis on both univariate and multivariate analysis. Prognostic factors other than tumour markers on multivariate analysis were poor performance status and the presence of metastatic disease. This study also confirms that serum levels of CEA, AFP and CA19-9, and tissue staining for C-erbB-2 and βHCG have no prognostic role in advanced gastric cancer. Unfortunately, none of the prognostic markers were able to predict convincingly for chemosensitivity. The identification of these tumour markers as independent prognostic factors may allow for patient stratification to appropriate treatment regimens and they raise the possibility that they reflect the tumour biology and not just the tumour burden.


1. HMSO. *Cancer Statistics: Registrations. England and Wales* 1987. HMSO, 1993.
2. HMSO. *Mortality Statistics: Cause. England and Wales* 1991. HMSO, in press.
3. Wittekind Ch, Wachner R, Henke W, *et al.* Localisation of CEA, HCG, lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin in gastric cancer and prognosis. *Virchows Arch (A)* 1986, **409**, 715–724.
4. Ito H, Tahara H. Human chorionic gonadotropin in human gastric carcinoma: a retrospective immunohistochemical study. *Acta Pathol Jpn* 1983, **33**, 287–296.
5. Tomita K, Kuwajima M. Chorionic gonadotropin in gastric cancer tissue, especially its relation to the patients prognosis. *Jpn J Cancer Clin* 1981, **27**, 1281–1282 (in Japanese).
6. Mizutani T, Onda M, Tokunaga A, *et al.* Relationship of C-erbB-2 protein expression and gene amplification to invasion and metastasis in human gastric cancer. *Cancer* 1993, **72**, 2083–2088.
7. Uchino S, Tsuda H, Maruyama K, *et al.* Overexpression of C-erbB-2 protein in gastric cancer. *Cancer* 1993, **72**, 3179–3184.
8. Yonemura Y, Ninomiya I, Ohoyama S, *et al.* Immunoreactivity for C-erbB-2 protein is an independent indicator of poor short term prognosis in patients with gastric carcinoma. *Cancer* 1991, **67**, 2914–2918.
9. Paik S, Hazan R, Risher E, *et al.* Pathologic findings from the national surgical adjuvant breast and bowel project: prognostic significance of C-erbB-2 protein overexpression in primary breast cancer. *J Clin Oncol* 1990, **8**, 103–112.
10. Gold P, Shuster J, Freedman SO. Carcinoembryonic antigen (CEA) in clinical medicine. Historical perspectives, pitfalls and projections. *Cancer* 1978, **42**, 1399–1405.
11. Carcinoembryonic antigen: its role as a marker in the management of cancer. A National Institutes of Health Consensus Development Conference. *Ann Int Med* 1981, **94**, 407–409.
12. Beatty JD, Romero C, Brown PW, *et al.* Clinical value of carcinoembryonic antigen. Diagnosis, prognosis and follow up of patients with cancer. *Arch Surg* 1979, **114**, 563–567.
13. Cooper MJ, Mackie CR, Skinner DB, *et al.* A reappraisal of the value of carcinoembryonic antigen in the management of patients with various neoplasms. *Br J Surg* 1979, **66**, 120–123.
14. Kuusela P, Haglund C, Roberts PJ, *et al.* Comparison of CA-50, a new tumour marker, with carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) in patients with gastrointestinal diseases. *Br J Cancer* 1987, **55**, 673–676.
15. Beatty JD, Romero C, Brown PW, *et al.* Clinical value of carcinoembryonic antigen. *Arch Surg* 1979, **114**, 563–567.
16. Dittrich C, Jakesz R, Havelec L, *et al.* Carcinoembryonic antigen (CEA) plasma level determination in the management of gastric cancer patients. *Cancer Detection and Prevention* 1985, **8**, 181–187.
17. Sakamoto K, Haga Y, Yoshimura R, *et al.* Comparative effectiveness of the tumour diagnostics, CA19-9, CA 125 and carcinoembryonic antigen in patients with diseases of the digestive system. *Gut* 1987, **28**, 323–329.
18. Wobbes T, Thomas CMG, Segers MFG, *et al.* Evaluation of seven tumour markers (CA50, CA 19-9, CA 19-9 TruQuant, CA 72-4, CA 195, carcinoembryonic antigen, and tissue polypeptide antigen) in the pretreatment sera of patients with gastric carcinoma. *Cancer* 1992, **69**, 2036–2041.
19. Tamada R, Hiramoto Y, Tsujitani S, *et al.* Serum CEA levels facilitate detection of recurrences of cancer in patients after gastrectomy. *Jpn J Surg* 1985, **15**, 23–29.
20. Bates SE, Longo DL. Use of serum tumour markers in cancer diagnosis and management. *Semin Oncol* 1987, **14**, 102–138.
21. Bloomer JR, Waldmann TA, McIntire KR, *et al.* α-Fetoprotein in non-neoplastic hepatic disorders. *JAMA* 1975, **233**, 38–41.
22. Waldmann TA, McIntire KR. The use of a radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. *Cancer* 1974, **34**, 1510–1515.
23. Chen DS, Sung JL. Serum alphafetoprotein in hepatocellular carcinoma. *Cancer* 1977, **40**, 779–783.
24. McIntire KR, Waldmann TA, Moertel CG, *et al.* Serum α-fetoprotein in patients with neoplasms of the gastrointestinal tract. *Cancer Res* 1975, **35**, 991–996.
25. Takahashi Y, Mai M, Ogino T, *et al.* Clinicopathological study of AFP producing gastric cancer significance of AFP in gastric cancer. 1987, **88**, 696–700.
26. Braunstein GD, Vaitukaitis JL, Carbone PP, *et al.* Ectopic production of human chorionic gonadotrophin by neoplasms. *Ann Intern Med* 1973, **78**, 39–45.
27. Elliott TE, Moertel CG, Wieand HS, *et al.* A phase II study of the combination of etoposide and cisplatin in the therapy of advanced gastric cancer. *Cancer* 1990, **65**, 1491–1494.
28. Gailani S, Chu TM, Nussbaum A, *et al.* Human chorionic gonadotrophins (hCG) in non-trophoblastic neoplasms. *Cancer* 1976, **38**, 1684–1686.
29. Rosen SW, Weintraub BD, Vaitukaitis JL, *et al.* Placental proteins and their subunits as tumour markers. *Ann Intern Med* 1975, **82**, 71–83.
30. Yakeishi Y, Mori M, Enjoji M, *et al.* Distribution of β-human chorionic gonadotropin-positive cells in noncancerous gastric mucosa and in malignant gastric tumours. *Cancer* 1990, **66**, 695–701.
31. Manabe T, Adachi M, Hirao K. Human chorionic gonadotrophin in normal, inflammatory and carcinomatous gastric tissue. *Gastroenterology* 1985, **89**, 1319–1325.
32. Fukayama M, Hayashi Y, Koike M. Human chorionic gonadotropin in gastric carcinoma: an immunohistochemical study suggesting independent regulation of subunits. *Virchows Arch (A)* 1987, **411**, 205–212.
33. Canney PA, Moore M, Wilkinson PM, *et al.* Ovarian cancer antigen CA125: a prospective clinical assessment of its role as a tumour marker. *Br J Cancer* 1984, **50**, 765–769.
34. Haga Y, Sakamoto K, Egami H, *et al.* Clinical significance of serum CA125 values in patients with cancers of the digestive system. *Am J Med Sci* 1986, **292**, 30–34.
35. Jaquinto G, Taccone W, Guarino A, *et al.* Multiparametric tumour marker (CA19 9, TPA, CA125, CEA) analyses of sera in gastric diseases. *Dig Dis Sci New Series* 1985, **31**, 35.
36. Gupta MK, Arciaga R, Bocci L, *et al.* Measurement of a monoclonal antibody defined antigen (CA19-9) in the sera of patients with malignant and non-malignant diseases. *Cancer* 1985, **56**, 277–283.
37. Ritts RE, Del Villano B, Go VLW, *et al.* Initial clinical evaluation of an immunoradiometric assay for CA19-9 using the NCI serum bank. *Int J Cancer* 1984, **33**, 339–345.
38. Jalanko H, Kuusela P, Roberts P, *et al.* Comparison of a new tumour marker CA19-9 with α-fetoprotein and carcinoembryonic antigen in patients with upper gastrointestinal diseases. *J Clin Pathol* 1984, **37**, 218–222.
39. Berger MS, Locher GW, Sauere S, *et al.* Correlation of C-erbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res* 1988, **48**, 1238–1243.
40. Slamon DJ, Godolphin W, Jones LA, *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
41. Wright C, Angus B, Nicholson S, *et al.* Expression of C-erbB-2 oncoprotein: a prognostic indicator in human breast cancer. *Cancer Res* 1989, **49**, 2087–2090.
42. Tsuda H, Hirohashi S, Shimosato Y, *et al.* Correlation between long-term survival in breast cancer patients and amplification of two

putative oncogene coamplification units: hst-1/int-2 and C-erbB-2/ear-1. *Cancer Res* 1989, **49**, 3104–3108.

43. Jain S, Filipe MI, Gullick WJ, *et al.* C-erbB-2 proto-oncogene expression and its relationship to survival in gastric carcinoma: an immunohistochemical study on archival material. *Int J Cancer* 1991, **48**, 668–671.
44. Ohguri T, Sato Y, Koizumi W, *et al.* An immunohistochemical study of C-erbB-2 protein in gastric carcinomas and lymph-node metastases: is the C-erbB-2 protein really a prognostic indicator? *Int J Cancer* 1993, **53**, 75–79.