Eur J Cancer, Vol. 29A, No. 12, pp. 1691–1696, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Poor Prognosis Associated with Elevated Serum CA 19-9 Level in Advanced Colorectal Carcinoma, Independent of DNA Ploidy or SPF

Mauri Kouri, Stig Nordling, Pentti Kuusela and Seppo Pyrhönen

DNA ploidy, S-phase fraction (SPF) for the tumours, serum tumour markers such as carcinoembryonic antigen (CEA) and serum CA 19-9 and major clinical parameters were analysed as prognostic factors in 105 patients with advanced colorectal carcinoma. All 105 were treated with a three-drug schedule including low dose epirubicin and sequential methotrexate, 5-fluorouracil, followed by leucovorin rescue. In univariate analysis, gender, Karnofsky index, extent of metastases, presence of abdominal metastases, CEA and CA 19-9 correlated with survival. Age, presence of liver or of lung metastases, DNA ploidy or SPF were not significantly associated with survival. In stepwise multivariate analysis an elevated serum CA 19-9 level, a poor Karnofsky index and multiple sites of metastases were independent adverse prognostic factors. Based on the multivariate analysis, patients were grouped in three categories. Group 1 consisted of 32 patients with Karnofsky ≥ 80, with a normal serum CA 19-9 level and a single site of metastases. Group 2 consisted of 48 patients with Karnofsky ≥ 80 and with an elevated serum CA 19-9 level or multiple sites of metastases. Group 3 consisted of 14 patients with Karnofsky ≤ 70. This classification gave a highly significant correlation with survival ($\chi^2 = 45.52$, $P < 0.001$, log rank test). The median survival in group 1, group 2 and group 3 was 30.1 months, 13.5 months and 3.9 months, respectively. Based on these results we suggest that trials involving advanced colorectal cancer should include the measurement of serum CA 19-9 levels as one of the most important prognostic factors, but also include documentation of other independent prognostic factors.
Eur J Cancer, Vol. 29A, No. 12, pp. 1691–1696, 1993.

INTRODUCTION

PRESENTLY THERE is no uniform prognostic classification system for patients with advanced colorectal carcinoma. An increasing number of studies assessing the prognosis of colorectal carcinomas analyse the value of flow cytometric DNA measurement [1–6]. Most studies have shown that, in patients with operable colorectal carcinoma, aneuploidy is associated with a worse prognosis [1, 2, 4–6]. However, when a detailed histological analysis is included in a multivariate analysis, the independent value of DNA ploidy as a prognostic sign becomes controversial [4, 7]. In metastatic colorectal carcinoma DNA ploidy does not correlate with prognosis [5, 8]. Some studies suggest that an increased fraction of cells in S-phase (SPF) in diploid tumours in operable colorectal carcinoma associates with poor prognosis [1, 6], while others have not observed such a correlation [3, 5].

DNA aneuploidy is associated with preoperatively elevated serum carcinoembryonic antigen (CEA) levels in patients with colorectal carcinoma [9–11], although this has not been a constant observation [1]. Recently, we analysed the value of serial CEA and CA 19-9 measurements in sera of patients with advanced colorectal carcinoma treated with chemotherapy [12]. The aim of the present study was to analyse the relationship between DNA ploidy and SPF as well as serum tumour markers CEA and CA 19-9 in predicting the prognosis of patients with advanced colorectal carcinoma.

Correspondence to M. Kouri.
M. Kouri and S. Pyrhönen are at the Department of Radiotherapy and Oncology, Helsinki University Central Hospital, Haartmaninkatu 4, SF-00290 Helsinki, Finland; P. Kuusela is at the Department of Bacteriology and Immunology; and S. Nordling is at the Department of Pathology, Helsinki University, Helsinki, Finland.
Received 14 Sep. 1992; accepted 9 Mar. 1993.

PATIENTS AND METHODS

105 patients with advanced histologically verified colorectal carcinoma were analysed. Their characteristics are presented in Table 1. Our series of 91 patients participating in a phase II chemotherapy study from October 1984 to July 1990 has recently been reported [13]. A three-drug schedule: epirubicin (20 mg/m^2) and then, sequential methotrexate (150 mg/m^2) 5-fluorouracil (600 mg/m^2) at a 1-h interval (EMF) was given once a week for successive three weeks followed by a rest period of 2–3 weeks. Folinic acid (leucovorin) rescue (15 mg every 6 h, eight times) was initiated 24 h after methotrexate administration. The treatment was continued until the tumour progressed. The responses to chemotherapy were evaluated according to the UICC criteria [14].

CEA and CA 19-9 *determination*

CEA was measured in the sera of 102 of the 105 (97%) patients. Serum CEA levels were measured either by a double antibody assay [15] employing commercially available CEA-antisera (Dakopats A/S Copenhagen, Denmark) as the first antibody, or by the Abbot-CEA-RIA Diagnostic Kit (Abbot, Wiesbahn, West Germany). In 100 samples with CEA concentrations ranging from normal up to 60 500 μg/l [16], the two assays showed a good correlation ($r^2 = 0.9997$). A cut-off value of 5 μg/l was used for the CEA assay.

CA 19-9 measurements were not included in the follow-up scheme during the first year of the study, and therefore CA 19-9 values were unavailable for 11 of 105 patients. The CA 19-9

Table 1. Univariate analysis of relationship of pretreatment characteristics to survival in 105 patients with advanced colorectal cancer (using product limit survival analysis and log rank test)

Variable	n	Median survival (months)	Observed/ expected	χ^2	P
Age					
< 55 years	56	12.0	1.09		
≥ 55 years	49	15.2	0.91	0.72	n.s.
Sex					
Male	42	12.5	1.29		
Female	63	14.1	0.86	4.08	0.043
Karnofsky index					
80–100	87	15.5	0.86		
60–70	18	4.4	3.29	31.64	< 0.001
Site of primary tumour					
Right colon	32	7.9	1.28		
Left colon	25	17.9	0.83		
Rectum	48	13.8	0.97	2.52	n.s.
Extent of metastases					
One site	65	15.6	0.79		
Multiple sites	40	11.3	1.58	12.15	< 0.001
Primary tumour					
Resected	97	13.8	1.00		
Unresectable	8	8.5	1.05	0.02	n.s.
Abdominal/pelvic metastases					
Absent	54	16.2	0.81		
Present	51	11.7	1.30	5.49	0.019
Liver metastases					
Absent	47	15.6	0.84		
Present	58	12.5	1.16	2.46	n.s.
Lung metastases					
Absent	75	13.5	1.03		
Present	30	14.1	0.93	0.23	n.s.
CEA					
< 5 μg/l	30	23.0	0.62		
≥ 5 μg/l	72	11.6	1.28	10.23	0.001
CEA					
< 10 μg/l	39	19.8	0.75		
≥ 10 μg/l	63	12.8	1.22	5.17	0.023
CEA					
< 20 μg/l	52	18.3	0.77		
≥ 20 μg/l	50	12.0	1.37	7.83	0.005
CA19-9					
< 37 U/ml	51	21.1	0.68		
≥ 37 U/ml	43	10.2	1.84	24.87	< 0.001
Ploidy					
Diploid	21	13.5	1.00		
Aneuploid	61	14.1	1.00	0.00	n.s.
SPF					
< median	39	15.3	0.88		
≥ median	38	11.3	1.16	1.34	n.s.

n.s. = not significant.

antigen was measured by a solid phase radioimmunoassay (Centocor, Melvern, Pennsylvania, U.S.A.) using a cut-off value of 37 U/ml.

Serum specimens for CEA and CA 19-9 determinations were obtained prior to initiation of chemotherapy, and subsequent specimens were drawn at intervals of 4–6 weeks.

DNA analysis

DNA measurements from paraffin blocks were performed as described by Hedley [17], but with slight modifications. Briefly, 100 μm sections were cut by microtome. The sections were deparaffinised, rehydrated and digested for 1 h at 37°C with 0.4 mg/ml Proteinase K (Merck, Darmstadt, Germany) in a phosphate buffered saline. The cells were stained with ethidium bromide 50 μg/ml (Sigma) in Tris–HCl buffer containing RNase I 100 μg/ml (Sigma) and 1 mmol/l Na-EDTA (Titriplex, Merck, Darmstadt, F.R.G.) and a 0.3% non-ionic detergent, Nonidet P40 (BDH Chemical Ltd., Poole, U.K.). Immediately before analysis the samples were filtered through a 50-μm nylon mesh.

DNA analysis was performed with a FACScan flow cytometer (Becton Dickinson) equipped with a 15 mW argon-ion laser. Excitation of ethidium bromide occurred at 488 nm, and the fluorescent emission was measured above 590 nm. A minimum of 10 000 nuclei from each specimen were analysed.

A total of 324 paraffin blocks were recovered from 84 of the 105 patients. Thirteen of 324 histograms were uninterpretable. An evaluable DNA histogram was observed for 82 of the 84 patients. The mean coefficient of variation of the 311 samples was 5.5% ± 2.4 (± S.D., range 1.1–14.2).

Tumours were classified as aneuploid, if a second G_1 peak occurred in addition to the diploid G_1 peak [18]. The DNA index was calculated as the ratio of the aneuploid G_1 peak channel to the diploid G_1 peak channel. The peak with the smallest DNA content was considered to represent diploid cells. Tumours with a DNA index between 1.9 and 2.1 were classified as tetraploid. There were only three tetraploid tumours and in addition six tumours with multiple aneuploid populations including a tetraploid population. Because of the small number of cases, tetraploid tumours were not analysed separately. When DNA indices of aneuploid populations in separate samples differed by more than 10%, they were considered to be separate aneuploid populations.

The SPF was calculated as described previously [19], and samples with a coefficient of variation greater than 8.0% were excluded from cell cycle analysis. Cell cycle analysis was possible in 77 of 82 cases. When multiple samples were available, both the mean and the highest value for the SPF were analysed. The grouping of SPF values below or higher than the median was calculated using the median SPF of each ploidy group. The proliferative index (PI) was calculated as the sum of SPF and fraction of cells in G_2M-phase.

Statistical description and analysis

Differences between frequencies were analysed using contingency tables.

For calculation of overall survival from the beginning of treatment, product limit survival analysis was performed using the BMDP 1L computer program [20]. Calculations of significance of observed differences were performed using the log rank test (Mantel-Cox). None of the 105 patients were lost from the follow-up. Thus far, 95 of the 105 patients have died.

The relative prognostic importance of all parameters was investigated using Cox's regression model and the BMDP 2L computer program [20]. A prognostic variable with two or more categories of outcome is represented by a number of variables and parameters equal to the number of its categories minus one. The reference category was not included as a variable.

RESULTS

Flow cytometric findings

Overall, 61 of 82 (74%) patients had an aneuploid tumour. Of 63 patients with multiple samples, 24 (38%) had a heterogeneous tumour with both diploid and aneuploid areas. An additional 9

(14%) tumours had multiple aneuploid populations. The samples for flow cytometry were taken from the primary tumour in 37 patients, from metastases in 13 and from both primary tumour and metastases in 32. The percentage of aneuploidy was: 70% in primary tumours (n = 69), 47% in lymph node metastases (n = 15), 77% in intra-abdominal metastases (n = 13), 88% in ovarian, uterine or vaginal metastases (n = 8), 57% in liver metastases (n = 14), and 100% in pulmonary metastases (n = 3), and in addition one brain metastasis was aneuploid.

When the primary tumours and all analysed metastases were compared, a similar ploidy was observed in 30 out of 40 cases (75%). In 5 cases the metastatic tumour was aneuploid, whereas the primary tumour was diploid, and in 5 cases *vice versa*. When compared by site the ploidy of primary tumours and metastases was similar in nine of 13 lymph node metastases, nine of 12 intra-abdominal (including gynaecological) metastases, 10 of 12 liver metastases, and in two of two pulmonary metastases as well as in one brain metastasis.

There were no significant differences between the ploidy of the primary tumour (n = 69) and sites of metastases. The percentages of different metastatic sites for aneuploid and diploid primary tumours, respectively, were: multiple sites of metastases 44 vs. 38%, intra-abdominal metastases 44 vs. 48%, liver metastases 60 vs. 62% and pulmonary metastases 35 vs. 24%. Fifty-two per cent of tumours in the right colon, 75% of tumours in the left colon and 80% of tumours in the rectum were aneuploid (not significant).

The median SPF of all cases was 19.6% (n = 77; range 3.3–41.9%) and the median PI 23.1% (n = 77; range 5.1–43.8). Aneuploid tumours had a significantly higher SPF than did diploid tumours (22.8 vs. 9.5%, $P < 0.001$). In tumours with multiple samples (n = 57), a 2.6-fold variation (median) of SPF was found in different parts of the tumour. When SPF of the tumours was calculated by using the highest rather than the mean value of each tumour, the grouping according to median SPF differed in 7 of 57 (12%) patients.

There were no significant differences between aneuploid and diploid tumours by age, gender or Karnofsky index. SPF or PI did not correlate with any of these three parameters.

In patients with measurable metastatic disease, DNA ploidy, SPF or PI did not correlate with response to chemotherapy (Table 2). In cases with multiple DNA samples the use of the highest rather than the mean value of SPF did not alter the results.

Table 2. Response to chemotherapy in patients with measurable disease by serum CA 19-9 level, serum CEA level, and DNA ploidy and SPF of tumours

Group	n	CR + PR	%	NC + PD	%
Serum CA 19-9 level					
< 37 U/ml	40	14	35%	26	65%
> 37 U/ml	35	9	26%	26	74%
Serum CEA level					
< 5 μg/l	23	6	26%	17	74%
≥ 5 μg/l	60	19	32%	41	68%
DNA ploidy					
Diploid	18	4	22%	14	78%
Aneuploid	49	16	33%	33	67%
SPF					
< median	31	10	32%	21	68%
≥ median	32	7	22%	25	78%

Table 3. Summary of stepwise results from Cox's regression model for prognostic factors. See text for details

Variable	Coefficient	Standard error	Hazard ratio
CA19-9 ≥ 37 U/ml	0.81	0.29	2.25
Karnofsky index 60–70	1.75	0.35	5.73
Extent of disease	0.81	0.24	2.26
CEA ≥ 5 μg/ml	0.52	0.31	1.69

Serum CEA and CA 19-9 levels

Serum CEA levels were elevated in 72 of 102 patients (71%); range 5.7–37 600 μg/l; median 60 μg/l. CEA level was elevated in 83% of patients with liver metastases (n = 58) and in 55% of those without liver metastases (n = 44, P = 0.002). Eighty-two per cent of the patients with metastases at multiple sites (n = 39) had an elevated CEA level, and 63% with metastases at a single site (n = 63; P = 0.046). Of 19 patients with diploid tumours, 14 (74%) and of 60 patients with aneuploid tumours 45 (75%) had an elevated CEA level. Serum CA 19-9 values were elevated in 43 of 94 patients (46%); range 52–633 900 U/ml; median 360 U/ml. Three patients had an elevated CA 19-9 but a normal CEA value. Similarly to CEA, CA 19-9 was elevated significantly more often in patients with liver metastases (n = 55) than in the others (n = 39), 58 vs. 28%, respectively (P = 0.004). Thirty-two per cent of patients with tumours originating from the rectum, 52% of those from the left colon and 60% of those from the right colon had an elevated CA 19-9 level (P = 0.048). Of 17 patients with diploid tumours, 8 (47%) and of 55 patients with aneuploid tumours 26 (47%) had elevated CA 19-9 levels.

Prognostic factors

In univariate analysis, sex, Karnofsky index, extent of metastases, presence of abdominal metastases, CEA and CA 19-9 correlated with survival (Table 1). Age, presence of liver or of lung metastases, DNA ploidy, SPF or PI were not significantly associated with survival.

By stepwise multivariate analysis of the 93 complete cases an elevated CA 19-9 level, a poor Karnofsky index and multiple sites of metastases were independent adverse prognostic factors (Table 3). An elevated CEA level was not a statistically significant prognostic factor by multivariate analysis. The median survival of patients with a normal CA 19-9 value was 21.1 months (16.4–30.0 months, 95% confidence interval), and the median survival of those with an elevated CA 19-9 level was 10.2 months (7.0–12.6 months, Fig. 1).

Based on the multivariate analysis the 94 patients with a measured CA 19-9 level were grouped in three categories according to Karnofsky, serum CA 19-9 level and extent of metastases. Group 1 consisted of 32 patients with a Karnofsky index of ≥ 80 and with a normal CA 19-9 level and single site of metastases. Group 2 consisted of 48 patients with a Karnofsky index of ≥ 80 and with an elevated CA 19-9 or multiple sites of metastases. Group 3 consisted of 14 patients with a Karnofsky index of ≤ 70. This classification gave a highly significant correlation with survival (χ^2 = 45.52, $P < 0.001$, log rank test, Fig. 2). The median survival in group 1, group 2 and group 3 was 30.1 months, 13.5 months and 3.9 months, respectively (Table 4).

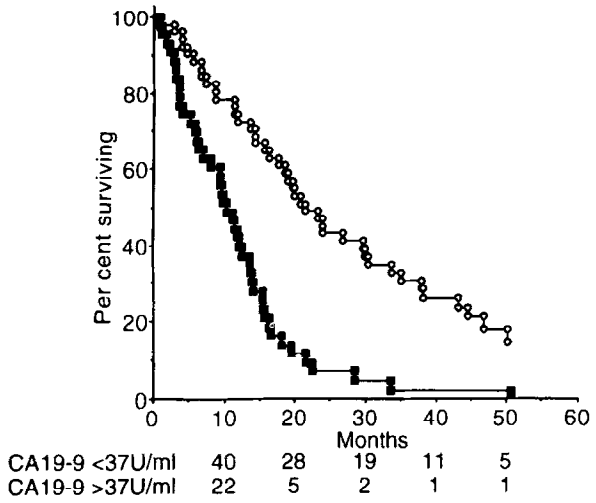

Fig. 1. Per cent of patients surviving by CA 19-9 level. Symbols used: open circle = CA 19-9 < 37 U/ml (n = 51), solid square = CA 19-9 > 37 U/ml (n = 43). Difference statistically significant (χ^2 = 24.87, P < 0.001, log rank test). Number of patients at risk shown below figure.

DISCUSSION

By analysing DNA ploidy and SPF as well as of serum CEA and CA 19-9 for patients with advanced colorectal carcinoma we have shown, by multivariate analysis, a Karnofsky index of ≤ 70, an elevated CA 19-9 level and multiple sites of metastases as independent adverse prognostic factors. Based on this, patients could be classified into three prognostic groups: 59% of patients with a normal CA 19-9 level and a single site of metastases (group 1) were alive at 2 years; 13% of patients with an elevated CA 19-9 or multiple sites of metastases (group 2) were alive at 2 years; while none of those with a Karnofsky index of ≤ 70 (group 3) was alive at 2 years.

The prognostic significance of serum CEA in advanced colorectal carcinoma has been analysed [21–24], whereas serum CA 19-9 as a prognostic factor has not. In patients with hepatic metastases from colorectal cancer, CEA has not been an independent prognostic factor [22, 24]. Arends reported that uniform immunoreactivity of CA 19-9 in colorectal carcinoma is associated with a worse prognosis, although his figures did not reach statistical significance [25]. Recently, we reported that an elevated serum CA 19-9 level is one of the most important prognostic factors in advanced colorectal carcinoma [12].

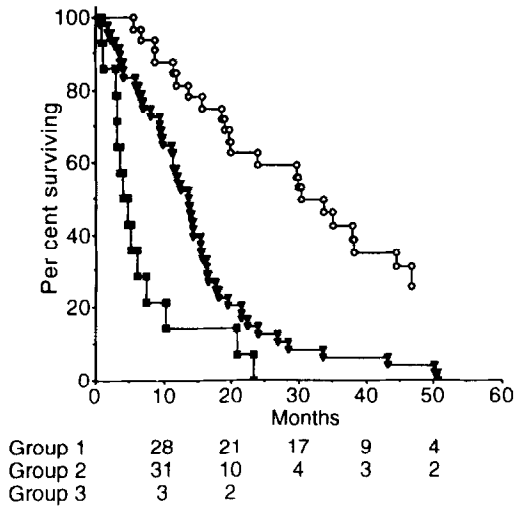

Fig. 2. Per cent of patients surviving by three prognostic groups. Group 1 = Karnofsky ≥ 80 and normal serum CA 19-9 level and single site of metastases, group 2 = Karnofsky ≥ 80 and elevated serum CA 19-9 level or multiple sites of metastases and group 3 = Karnofsky ≤ 70. Symbols used: open circle = group 1 (n = 32), solid triangle = group 2 (n = 48), solid square = group 3 (n = 14). Difference statistically significant (χ^2 = 45.52, P < 0.001, log rank test). Number of patients at risk shown below figure.

Currently we wanted to analyse whether the serum CEA or CA 19-9 levels in advanced colorectal carcinoma are related to DNA ploidy or SPF. Previously, we observed that at the time of primary diagnosis of colorectal cancer, CEA level was elevated significantly more often in patients with aneuploid tumours than in those with diploid or tetraploid tumours [9]. Furthermore, elevated CEA levels correlated with tumour stage in patients with DNA aneuploid tumours, but not in patients with DNA diploid tumours.

However, in these inoperable patients there was no significant correlation between DNA ploidy or SPF and marker levels. In the current series all the patients had metastatic disease and were treated with chemotherapy, while in the previous study we analysed patients operated on for colorectal carcinoma. This finding probably indicates that the difference between diploid and aneuploid tumours in serum CEA levels is dependent also on total tumour mass. Serum CEA levels, normal at the time of diagnosis of recurrent tumour, may become elevated later as the tumours grow and total tumour mass increases. The importance of CEA in follow-up of surgical patients is, however, the detection of recurrent carcinoma at a stage when a curative operation is still possible. So while DNA ploidy determination may be helpful in planning the postoperative CEA follow-up program, it seems to be less useful in advanced metastatic disease.

In the present study DNA ploidy and SPF did not correlate with prognosis, which is in line with the results of Finan *et al.* [8] as well as with our previous prospective study, in which DNA ploidy correlated with prognosis in patients with operable disease, but not in metastatic disease [5]. Furthermore, the metastatic pattern did not differ by DNA ploidy or SPF level, which accords with the results of Rübe *et al.* [26]. Evidently, factors that predict a high risk of recurrence in surgically treated colorectal carcinoma are different from those that predict prognosis in metastatic disease.

The heterogenity of ploidy is a well-known feature of colorectal carcinomas [27]. In the present study 38% of patients with multiple samples had a heterogenous tumour with both diploid and aneuploid areas. In addition, 14% of tumours had multiple aneuploid populations. Furthermore, when the primary tumours and all analysed metastases were compared, a similar ploidy was observed in 30 of 40 cases (75%). In 5 cases the metastatic tumour was aneuploid, whereas the primary tumour was diploid, and vice versa in 5 cases. These results are in line with other studies [28, 29].

No reports on colorectal carcinomas have related DNA content and response to chemotherapy. In the current study we observed no correlation between response rate and ploidy status or SPF. The clinical value of ploidy or SPF in predicting tumour response to therapy is uncertain also in regard to the other common cancers. In breast cancer patients, diploidy has been reported to predict response to hormonal therapy in one study [30], but not in another [31]. Similarly, aneuploid head and neck tumours have been reported to be most responsive to

Table 4. Survival of patients by three prognostic groups ($\chi^2 = 45.52$, $P < 0.001$, *log rank test*)

Group	*n*	Median survival (months)	95% confidence interval	1-year survival	2-year survival
Group 1					
Karnofsky ≥ 80 and normal serum CA 19-9 level and single site of metastases	32	30.1	19.5–37.8	81%	59%
Group 2					
Karnofsky ≥ 80 and elevated serum CA 19-9 or multiple sites of metastases	48	13.5	11.0–15.2	54%	13%
Group 3					
Karnofsky index ≤ 70	14	3.9	3.1–6.0	14%	0%

chemotherapy in one series of patients [32], but not in another [33]. Very low, as well as very high, S-phase fractions of oropharyngeal squamous cell carcinomas have been claimed to be associated with poor response to chemotherapy [34].

In conclusion, we suggest that trials on advanced colorectal cancer should include measurement of serum CA 19-9 level as one of the most important prognostic factors, whereas determination of DNA ploidy and SPF of such tumours seems to be less useful in predicting prognosis or response to chemotherapy. A simple three-stage prognostic classification is offered, which should be tested in larger prospective trials.

1. Albe X, Vassilakos P, Helfer GK, *et al.* Independent prognostic value of ploidy in colorectal cancer. A prospective study using image cytometry. *Cancer* 1990, **66**,1168–1175.
2. Armitage NC, Ballantyne KC, Sheffield JP, Clarke P, Evans DF, Hardcastle JD. A prospective evaluation of the effect of tumor cell DNA content on recurrence in colorectal cancer. *Cancer* 1991, **67**, 2599–2604.
3. Enker WE, Kimmel M, Cibas ES, Cranor ML, Melamed MR. DNA/RNA content and proliferative fractions of colorectal carcinomas: a five-year prospective study relating flow cytometry to survival. *J Natl Cancer Inst* 1991, **83**, 701–707.
4. Jass JR, Mukawa K, Goh HS, Love SB, Capellaro D. Clinical importance of DNA content in rectal cancer measured by flow cytometry. *J Clin Pathol* 1989, **42**, 254–259.
5. Kouri M, Pyrhönen S, Mecklin JP, *et al.* The prognostic value of DNA-ploidy in colorectal carcinoma: a prospective study. *Br J Cancer* 1990, **62**, 976–981.
6. Witzig TE, Loprinzi CL, Gonchoroff NJ, *et al.* DNA ploidy and cell kinetic measurements as predictors of recurrence and survival in stages B2 and C colorectal adenocarcinoma. *Cancer* 1991, **68**, 879–888.
7. Jass JR. Prognostic factors in colorectal cancer. *Cur Top Pathol* 1990, **81**, 295–322.
8. Finan PJ, Quirke P, Dixon MF, Dyson JED, Giles GR, Bird CC. Is DNA aneuploidy a good prognostic indicator in patients with advanced colorectal cancer? *Br J Cancer* 1986, **54**, 327–330.
9. Kouri M, Pyrhönen S, Mecklin J-P, *et al.* Serum carcinoembryonic antigen and DNA ploidy in colorectal carcinoma. A prospective study. *Scand J Gastroenterol* 1991, **26**, 812–818.
10. Rognum TO. A new approach in carcinoembryonic antigen-guided follow-up of large bowel carcinoma patients. *Scand J Gastroenterol* 1986, **21**, 641–649.
11. Rognum TO, Thorud E, Elgjo K, Brandtzaeg P, Ørjasaeter H, Nygaard K. Large-bowel carcinomas with different ploidy, related to secretory component, IgA, and CEA in epithelium and plasma. *Br J Cancer* 1982, **45**, 921–933.
12. Kouri M, Pyrhönen S, Kuusela P. Elevated CA 19-9 as the most significant prognostic factor in advanced colorectal carcinoma. *J Surg Oncol* 1992, **49**, 78–85.
13. Pyrhönen SO, Kouri MO. Phase II study of epirubicin sequential methotrexate and 5-fluorouracil for advanced colorectal cancer. *Eur J Cancer* 1992, **28A**, 1828–1832.
14. Miller AB, Hoogstraaten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
15. Rutanen EM, Lindgren J, Sipponen P, Stenman UH, Saksela E, Seppälä M. Carcinomaembryonic antigen in malignant and nonmalignant gynecologic tumors. Circulating levels and tissue localization. *Cancer* 1978, **42**, 581–587.
16. Jalanko H, Kuusela P, Roberts P, Sipponen P, Haglund C, Mäkelä O. Comparison of a new tumour marker, CA19-9™, with alfa-fetoprotein and carcinoembryonic antigen in patients with upper gastrointestinal diseases. *J Clin Pathol* 1984, **37**, 218–222.
17. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method for analysis of cellular DNA content of paraffin-embedded material using flow cytometry. *J Histochem Cytochem* 1983, **31**, 1333–1335.
18. Hiddemann W, Schumann J, Andreeff M, *et al.* Convention on nomenclature for DNA cytometry. *Cytometry* 1984, **5**, 445–446.
19. Baisch H, Göhde W, Linden WA. Analysis of PCP-data to determine the fraction of cells in the various phases of the cell cycle. *Radiat Environ Biophys* 1975, **12**, 31–39.
20. Dixon WJ. *BMDP Statistical Software Berkeley*. University of California Press, 1988.
21. Barone C, Astone A, Cassano A, *et al.* Advanced colon cancer: staging and prognosis by CEA test. *Oncology* 1990, **47**, 128–132.
22. Chang AE, Steinberg SM, Culnane M, White DE. Determinants of survival in patients with unresectable colorectal liver metastases. *J Surg Oncol* 1989, **40**, 245–251.
23. Herrera MA, Chu TM, Holyoke ED, Mittelman A. CEA monitoring of palliative treatment for colorectal carcinoma. *Ann Surg* 1977, **185**, 23–30.
24 Kemeny N, Niedzwiecki D, Shurgot B, Oderman P. Prognostic variables in patients with hepatic metastases from colorectal cancer. Importance of medical assessment of liver involvement. *Cancer* 1989, **63**, 742–747.
25. Arends JW, Wiggers T, Verstunen C, Hilgers J, Bosman FT. Gastrointestinal cancer-associated antigen (GICA) immunoreactivity in colorectal carcinoma in relation to patient survival. *Int J Cancer* 1984, **34**, 193–196.
26. Rübe C, Valet G, Eder M. Cellular DNA event and metastasis pattern in colorectal carcinomas. *Virchows Arch A* 1988, **413**, 419–424.
27. Wersto RP, Liblit RL, Deitch D, Koss LG. Variability in DNA measurements in multiple tumor samples of human colonic carcinoma. *Cancer* 1991, **67**, 106–115.
28. Arends JW, Schutte B, Wiggers T, Verstijnen CPHJ, Blijham GH, Bosman FT. Comparison of phenotypic and genotypic features in primary large bowel carcinomas and lymph node metastases. *Cancer Res* 1987, **47**, 4342–4344.
29. Jass JR, Mukawa K, Richman PI, Hall PA. Do aggressive subclones

within primary colorectal cancer give rise to liver metastases? *Int J Colorectal Dis* 1989, **4**, 109–117.
30. Seymour L, Bezwoda WR, Meyer K. Response to second-line hormone treatment for advanced breast cancer. Predictive value of ploidy determination. *Cancer* 1990, **65**, 2720–2724.
31. Stuart-Harris R, Hedley DW, Taylor IW, Levene AL, Smith IE. Tumour ploidy, response and survival in patients receiving endocrine therapy for advanced breast cancer. *Br J Cancer* 1985, **51**, 573–576.
32. Cooke LD, Cooke TG, Bootz F, *et al.* Ploidy as a prognostic indicator in end stage squamous cell carcinoma of the head and neck region treated with cisplatinum. *Br J Cancer* 1990, **61**, 759–762.
33. Campbell BH, Schemmel JC, Hopwood LE, Hoffmann RG. Flow cytometric evaluation of chemosensitive and chemoresistant head and neck tumors. *Am J Surg* 1990, **160**, 424–426.
34. Feichter GE, Maier H, Adler D, *et al.* S-phase fractions and DNA-ploidy of oropharyngeal squamous epithelium carcinomas compared with histological grade, stage, response to chemotherapy and survival. *Acta Otolaryngol (Stockh)* 1987, **104**, 377–384.

Acknowledgements—This work was supported by the Finnish Cancer Society. The skilful technical assistance of Ms Monica Schoulz in flow cytometric DNA analysis and Ms Sirpa Kuisma and Ms Helena Kauranen in determining the CEA and CA 19-9 levels is gratefully acknowledged.

Eur J Cancer, Vol. 29A, No. 12, pp. 1696–1699, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Carboplatin as Second Line Treatment for Recurrent or Progressive Brain Metastases From Small Cell Lung Cancer

H.J.M. Groen, E.F. Smit, H. Haaxma-Reiche and P.E. Postmus

Patients with brain metastases from small cell lung cancer (SCLC) have a poor prognosis. Although most patients die from metastatic disease outside the central nervous system, this disabling metastatic site often needs treatment to mitigate the signs and symptoms of intracranial disease. The effect of carboplatin (400 mg/m² every 4 weeks) as second line treatment for recurrent or progressive brain metastases was studied in 20 SCLC patients. 19 patients could be evaluated: 16 by contrast enhanced brain computer tomography (CT) scan (2 patients had complete response, 6 partial response, 4 stable disease and 4 progressive disease) and 3 patients clinically, who had progressive disease. The objective response rate in the brain was 40% (95% CI:22–61%). The median response duration was 8 weeks (range 2–29). The median survival was 15 weeks (range 1–44). Previous cranial irradiation appeared to be beneficial for survival. There was only mild haematological and gastrointestinal toxicity. Carboplatin has activity against brain metastases and gives palliation in responding patients.
Eur J Cancer, Vol. 29A, No. 12, pp. 1696–1699, 1993.

INTRODUCTION

COMBINATION CHEMOTHERAPY in small cell lung cancer (SCLC) improves median and long-term survival [1]. The management of brain metastases in SCLC is an important clinical problem. In more than 40% of SCLC patients symptomatic brain metastases will develop [2]. In addition, with prolonged survival, the incidence of brain metastases increases [3].

Measures to reduce the high relapse rate in the brain have been unsuccessful. Although prophylactic cranial irradiation (PCI) reduces the clinical relapse rate from 23 to 6% [2, 4], the value of PCI is controversial, due to limited effect on survival and long-term neurological sequelae [4, 5]. Treatment of brain metastases with whole brain radiotherapy (WBRT) has been standard for decades. For patients with intracranial relapse after radiotherapy (PCI or WBRT), retreatment with radiotherapy is usually unfeasible. For these patients chemotherapy may be a modality of further palliation. In two previous studies from our group it has been shown that brain metastases responded to treatment with podophyllotoxin derivatives in about 40% of the patients [6, 7]. The durations of these radiologically proven responses were comparable to the durations of clinical responses after WBRT [8–10].

In this report we describe the results of carboplatin, one of the most active agents against SCLC, for SCLC patients with progressive brain metastases, after treatment failure with teniposide, etoposide or cranial irradiation.

PATIENTS AND METHODS

Patients

From January 1987 until March 1992, 20 patients have been entered into this prospective study. Criteria for eligibility were: histologically or cytologically proven SCLC, leucocyte count $> 3.0 \times 10^9$/l, platelet count $> 100 \times 10^9$/l, serum creatinine < 150 μmol/l, progressing brain metastases proven with contrast enhanced brain computed tomography (CT) during or shortly after treatment with teniposide, reinduction combination chemotherapy or cranial irradiation for symptomatic brain metastases. Patients, who previously received carboplatin, were not eligible. Informed consent was obtained from all patients. 2

Correspondence to H.J.M. Groen.
H.J.M. Groen, E.F. Smit and P.E. Postmus are at the Department of Pulmonary Diseases; and H. Haaxma-Reiche is at the Department of Neurology, University Hospital Groningen, Oostersingel 59, 9713 EZ Groningen, The Netherlands.
Received 26 Feb. 1993; accepted 5 Mar. 1993.