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Specialist Interest Articles

Clinical Value of a Mucin-like Carcinoma-associated Antigen in Monitoring Breast Cancer Patients in Comparison with CA 15-3

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A new tumour marker, mucin-like carcinoma-associated antigen (MCA), was evaluated in 176 breast cancer patients classified either as free of tumour (NED, $n = 141$) or as having metastases (PD, $n = 35$). During the 5 year follow-up, 842 measurements of MCA and 363 measurements of CA 15-3 were done. MCA levels were significantly increased in the PD group ($P = 0.0001$) but not in the NED group. The sensitivities of the MCA and the CA 15-3 assays were 84% and 78% and the specificities were 81% and 78%, respectively. The negative predictive value of 97% for MCA was significantly higher ($P = 0.0001$) than the 88% for CA 15-3. Thus the MCA enzyme immunoassay is at least equivalent to the CA 15-3 test and is recommended in the assessment of metastatic spread or tumour recurrence in breast cancer patients.
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INTRODUCTION

BREAST CANCER causes 20–25% of all cancer deaths and affects about 7% of all women in the western world [1, 2]. Thus reliable markers for breast cancer are of great clinical interest. A good marker should be synthesised by the primary tumour and its metastases but not by benign cells, even if they are affected by other non-malignant diseases; it should reflect the tumour mass; and it should be measurable in serum by reproducible methods [3]. Since no tumour marker fulfils all these criteria, the interpretation of the values should be based on the curve from several consecutive measurements [4].

Breast carcinoma cells in culture produce mucin-like carcinoma-associated antigen (MCA), which has a long protein backbone with many carbohydrate side-chains linked to threonine and serine. The carbohydrate chains contain negatively charged terminal sialic acids [5]. Intracytoplasmic MCA was immunohistochemically demonstrated in all of 122 mammary tumours investigated by Zenklusen *et al.* [6]. MCA was detected even in carcinoma cells of lymph node metastases. Furthermore, the staining pattern of metastases and the respective primary tumours was identical.

We have monitored levels of MCA and another breast carcinoma antigen, CA 15-3, in consecutive serum levels from breast cancer patients.

MATERIALS AND METHODS

176 patients with histologically confirmed carcinoma of the breast were examined during follow-up up to 5 years. In these patients, 842 measurements of MCA, and in 119 patients, 363 measurements of CA 15-3 were recorded. To determine the presence of the primary tumour or of metastases, assessors were blinded. Requirements for the study were operation reports, histological examinations of the primary tumours, complete clinical documentation including flow sheets of the treating radio-oncologists, and skeletal scintigraphy. The bone scans were re-evaluated without clinical information by two radiologists. In most cases, further information existed (ultrasonic and computer tomographic investigations for the assessment of questionable soft-tissue metastases and X-rays to confirm skeletal metastases). Patients presenting further neoplasms were excluded. The patients were classified in two groups: those without evidence of metastases or local recurrence (no evidence of disease [NED]) and those developing metastases (progressive disease [PD]) during follow-up. MCA was measured with a two-step enzyme immunoassay (MCA EIA; Hoffmann–La Roche) with the mouse monoclonal antibody b-12 for capturing and quantifying MCA [7]. In the first step, blood samples were incubated with beads coated with the antibody. After washing, peroxidase-conjugated b-12 antibodies were added (second step). The enzymatic reaction was started by incubation with *o*-phenylenediamine, which is converted to 2,2′-diaminoazobenzene. The intensity of the colour by spectrophotometry is proportional to the MCA concentration in the patient's serum.

CA 15-3 was measured immunoradiometrically with the monoclonal antibodies 115 D-8 and DF-3 (solid-phase RIA CA 15-3; CIS International). The test tubes are coated with 115 D-8 to fix CA 15-3 to the solid phase. DF-3, directed against another antigenic determinant of CA 15-3, is labelled with ^{125}I. The count rate, measured in a gamma counter, represents the concentration of CA 15-3 in the sample.

The MCA cut-off was established in a control group of 231 healthy individuals. The CA 15-3 cut-off was based on a

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subgroup of 55 of these volunteers. Both cut-offs were defined by the 95th percentiles.

The overall differences of the MCA and CA 15-3 mean values between NED and PD groups and comparisons in 2 month periods were tested by two-tailed *t*-test. An increase or decrease over the whole follow-up was investigated by one-way, and differences between groups by two-way repeated-measures analysis of variance (ANOVA). The calculations of the sensitivity, specificity, and positive and negative predictive values were based on MCA and CA 15-3 cut-offs of 11 and 23 U/ml, respectively. 259 MCA and CA 15-3 values from identical blood samples were correlated. With the resulting equation of regression, the CA 15-3 values were rescaled and the course of the mean values of the two tumour markers in the PD groups were compared in periods of 8 months.

RESULTS

In 159 of 176 patients (90.3%) breast conserving therapy was used. 17 patients (9.7%) had modified radical mastectomy: 9 combined with radiotherapy and 8 without radiotherapy. 82 of the 176 (46.6%) received adjuvant chemotherapy. Most patients were histopathologically classified as $pT_1pN_0cM_0$ (71, 40.3%), $pT_1pN_1cM_0$ (32, 18.2%), $pT_2pN_0cM_0$ (29, 16.5%) or $pT_2pN_1cM_0$ (34, 19.3%). 3 patients were classified as $pT_2pN_2cM_0$, 1 each as $pT_2pN_2cM_1$, $pT_3pN_1cM_0$, $pT_3pN_1cM_1$, $pT_4pN_1cM_0$, or $pT_4pN_1cM_1$ and 2 as $pT_4pN_0cM_0$.

141 (80.1%) of the 176 investigated patients with breast cancer (682 MCA and 240 CA 15-3 measurements) were in the NED group. 35 patients (19.9%; 160 MCA and 123 CA 15-3 measurements) were in the PD. The frequency distributions of the MCA and CA 15-3 values of the controls and in the NED and PD groups are shown in Figs 1 and 2, respectively. The mean of the MCA control group was 6.30 (S.D. 2.33) U/ml and 95th percentile (cut-off) was 11 U/ml. For CA 15-3, the mean control value was 14.02 (4.49) and the cut-off was 23 U/ml. The overall MCA mean values were 7.59 (4.78) U/ml in the NED group and 21.74 (19.28) U/ml in the PD group. The means of CA 15-3 in the NED and PD groups were 18.43 (7.79) and 93.96 (161.01) U/ml, respectively. For both tumour markers, differences between the overall mean values of the NED and PD groups were significant (Fig. 3).

For MCA, comparison of mean values between the two groups in 2 month periods showed no differences between the third and the tenth month after removal of the primary tumour. However, the differences achieved statistical significance in the 1st and 2nd month and in the 11th and 12th month onwards (Table 1 and Fig. 4). The increase of MCA concentrations during follow-up was significant in patients in the PD group ($P = 0.0001$). There was no elevation of MCA levels in patients in the NED group. The interaction between the groups was also significant ($P = 0.0001$). In the NED group, the mean values month by month were always lower than the cut-off, whereas in the PD group, the means were higher than the cut-off from the 1st to the 4th month and from the 9th month onwards.

Comparison of the mean values of CA 15-3 between the NED and the PD groups in 4 month periods revealed significant differences between the 1st and 4th month, the 9th and 12th, the 17th and 48th, and the 53rd and 56th month after initial treatment (Table 2 and Fig. 5). CA 15-3 values in the PD group increased significantly during follow-up ($P = 0.0001$), but there was no elevation in the NED group. The interaction between the groups was also significant ($P = 0.0001$). In the PD group, the mean values were elevated at all times, whereas the means

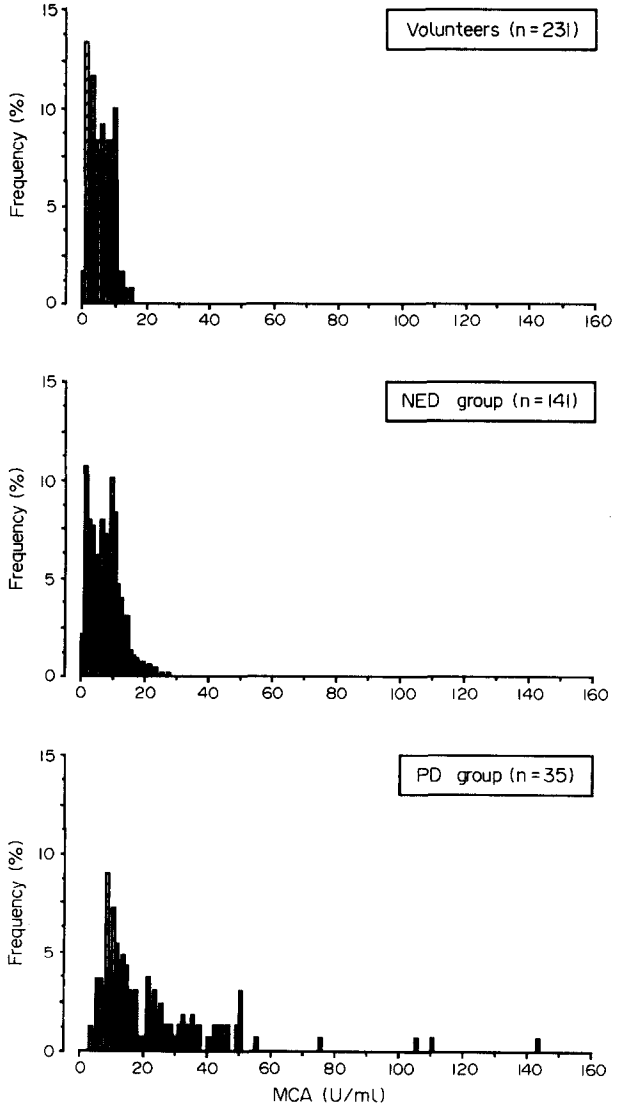

Fig. 1. Frequency distributions of MCA. Shaded bars for control group = 95% of measurement.

in the NED group were lower than the cut-off, except from the 45th to 48th and from the 57th to 60th month.

There was a significant difference in the positive ($P = 0.001$) and negative predictive values ($P = 0.0001$) between the two markers. The differences in sensitivity and specificity were not significant (Table 3). There was a slight but not significant increase in sensitivity with the combination of MCA and CA 15-3 compared with MCA or CA 15-3 alone (84.7% vs. 84.3% or 77.8%, respectively). However, a significant loss of specificity was found with the combined test (66.9% vs. 81.4%, $P = 0.0001$; 66.9% vs. 77.6%, $P = 0.008$).

The correlation between 259 MCA and CA 15-3 values was significant ($r = 0.842$, $P = 0.0001$). Linear regression revealed $y = 2.437x + 1.730$ and $x = 0.291y + 2.945$ (Fig. 6). No significant difference was detected between the two PD groups (MCA and CA 15-3) during follow-up (two-way ANOVA, $P = 0.057$). The means of the two PD groups initially decreased for both tumour markers, and MCA levels were higher than but not significantly different from rescaled CA 15-3 concentrations between the 22nd and the 37th month. After the 38th month,

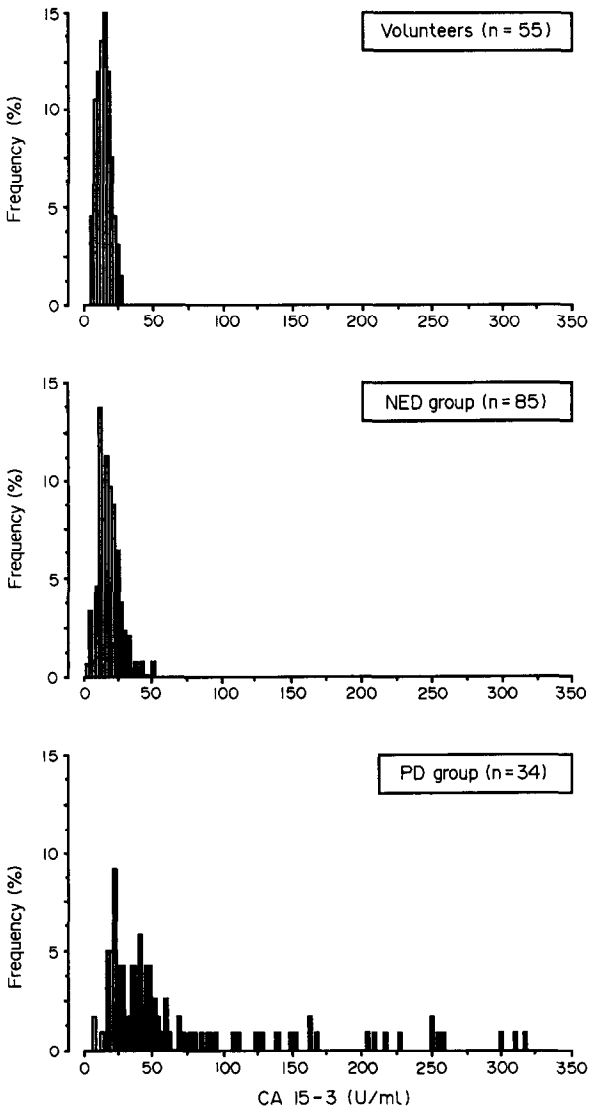

Fig. 2. Frequency distributions of CA 15-3.

the CA 15-3 means exceeded the MCA mean concentrations (Fig. 7).

DISCUSSION

Hitherto, numerous markers applicable in the assessment of breast cancer have been reported: prognostic markers, such as oncogenes (*c-erb*B-2/HER-2/*neu*, *ras*, *c-myc*, and *int*-2 [8–10]), low nm23RNA levels [11], loss of heterozygosity on chromosome 1q [12], Ki-67 scores [9], receptor proteins (oestrogen, progesterone, and epidermal growth factor receptors [8, 9]), enzymes (cathepsin D-related protein, plasminogen activators [8]), and

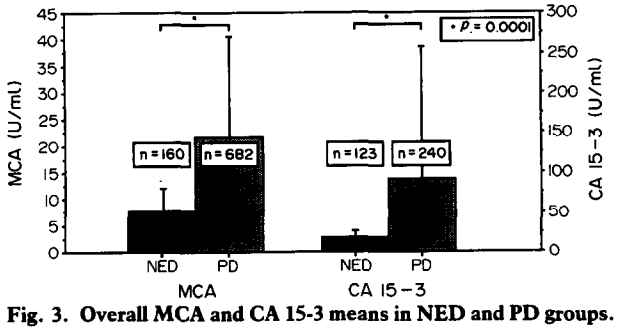

Fig. 3. Overall MCA and CA 15-3 means in NED and PD groups.

Table 1. Mean MCA values of NED and PD groups during follow-up

Month after initial treatment	Mean (U/ml) NED	PD	*P*
1– 2	6.88	13.20	0.031
3– 4	7.84	12.07	0.083
5– 6	7.89	10.73	0.184
7– 8	8.09	10.91	0.168
9–10	8.11	11.28	0.108
11–12	7.75	13.72	0.003
13–14	7.79	15.33	0.0008
15–16	7.88	15.18	0.0002
17–18	8.01	14.18	0.0003
19–20	7.86	13.36	0.0002
21–22	7.94	13.79	0.0001
23–24	7.72	15.04	0.0001
25–26	6.99	16.12	0.0001
27–28	6.94	14.58	0.0001
29–30	7.22	15.93	0.0001
31–32	7.47	16.24	0.0001
33–34	7.55	20.14	0.0001
35–36	7.39	21.97	0.0001
37–38	7.77	21.61	0.0001
39–40	7.93	22.37	0.0001
41–42	8.53	25.22	0.0001
43–44	8.80	21.32	0.0001
45–46	9.40	21.12	0.0001
47–48	10.11	23.25	0.0001
49–50	9.34	23.95	0.0001
51–52	8.57	24.87	0.0002
53–54	8.59	27.91	0.0001
55–56	8.78	29.01	0.01
57–58	8.26	33.87	0.038
59–60	8.00	34.24	0.008

haptoglobin-related protein [13], as well as markers which are frequently found associated with breast carcinomas, such as carcinoembryonic antigen (CEA [10]), tissue polypeptide antigen [10], and high molecular weight epithelial mucins (CA 15-3 [10, 14], CA 549 [10], tumour-associated glycoprotein 72 [10], and MCA [19–23]).

CEA and CA 15-3 are established tumour markers that have

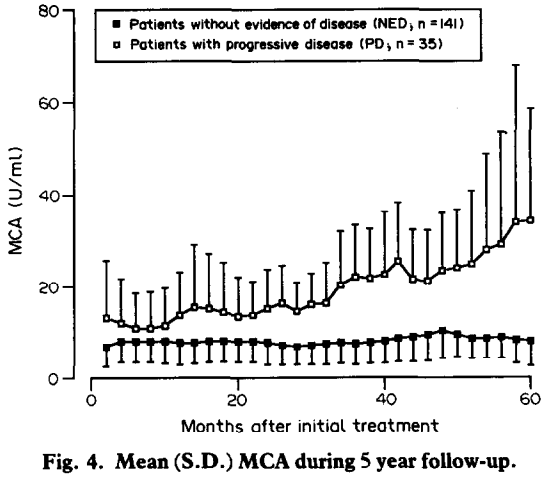

Fig. 4. Mean (S.D.) MCA during 5 year follow-up.

Table 2. Mean CA 15-3 values of NED and PD groups during follow-up

Month after initial treatment	Mean (U/ml)		P
	NED	PD	
1– 4	17.46	134.03	0.023
5– 8	18.78	72.35	0.108
9–12	19.56	82.52	0.016
13–16	19.41	27.14	0.055
17–20	16.47	35.90	0.0001
21–24	16.49	46.96	0.0002
25–28	17.47	45.38	0.003
29–32	17.69	44.80	0.0001
33–36	19.58	56.89	0.0004
37–40	18.54	46.42	0.004
41–44	20.54	83.65	0.04
45–48	24.44	133.43	0.035
49–52	21.63	90.28	0.113
53–56	17.14	108.86	0.037
57–60	23.60	188.72	0.325

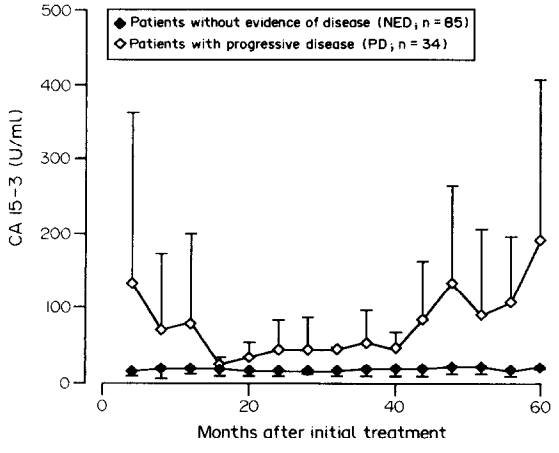

Fig. 5. Mean CA 15-3 during follow-up.

been available for several years. Various studies showed a higher accuracy of CA 15-3 compared with CEA in carcinoma of the breast [15–18]. For this study, we simultaneously measured MCA and CA 15-3 serum levels. Mean MCA in the PD-group exceeded the cut-off. This group of patients with evident metastases had significantly higher MCA values compared with the NED group. However, in 6 of the 35 patients of this group, at least 1 MCA level was not elevated. More detailed analysis revealed that 4 of these patients were receiving cytostatic therapy when MCA was first assayed. Subsequent tests were all positive. The other 2 with intially low MCA concentrations were in the

Table 3. Sensitivity, Specificity and Predictive Value

	MCA	CA 15-3
Sensitivity	84.3%	77.8%
Specificity	81.4%	77.6%
Positive predictive value	44.6%	62.3%
Negative predictive value	96.7%	88.0%

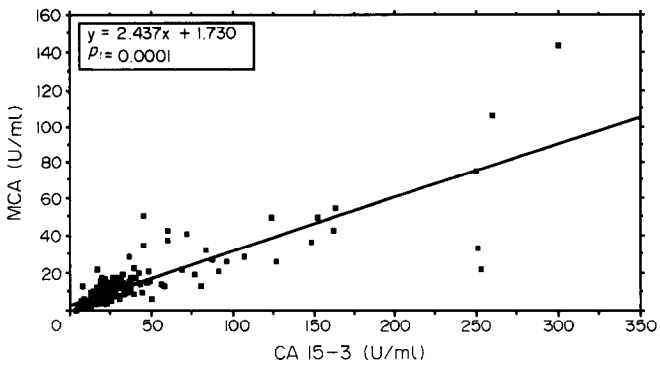

Fig. 6. Correlation between 259 MCA and CA 15-3 measurements.

PD group since they developed skin metastases (2 of 4 patients with skin metastases). As described by others [17], the levels of other tumour markers, such as CA 15-3, tend to be lower in breast cancer patients with skin metastases than in patients with visceral or bone metastases. Because MCA values of the NED and PD groups were (due to removal of primary tumour) low after initial treatment and did not differ significantly until the 10th month, the increase of serum MCA in patients with progressive disease might be the result of the growth of cells producing the tumour marker. MCA is therefore especially released when the MCA-producing malignant cells infiltrate the tissue far from their initial organ. At this advanced stage, there are probably many MCA-producing foci and less barriers to blood. This suggestion was supported by ANOVA which revealed a significant elevation of the MCA in the PD group but not in the NED group. The interaction was larger than would be expected by chance. Hence, an increase of MCA in the course of follow-up was due to a difference between the NED and PD groups and was not caused by variation within a given group. A significant difference between the PD and NED groups and a significant increase in the PD group during follow-up were also revealed for CA 15-3.

Two monoclonal antibodies, 115 D-8 and DF-3, are used in combination in the immunoradiometric assay for CA 15-3. 115 D-8, raised against human milk fat globule membranes,

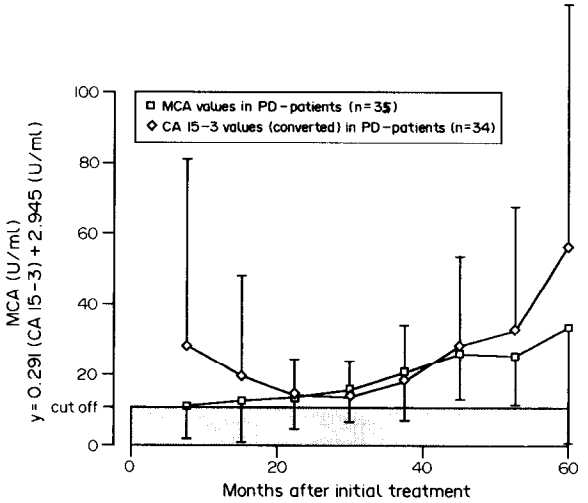

Fig. 7. Mean MCA and rescaled CA 15-3 in patients in PD group during follow-up.

recognises an epitope of a 400 kD glycoprotein, called MAM-6. DF-3, which binds to a membrane-enriched fraction of a human breast carcinoma metastatic to the liver, detects another epitope on two different glycoprotein molecules of 330 and 450 kD [19, 20]. The antibody used in the MCA enzyme-immunoassay (b-12) reacts strongly with a mucinous 350 kD glycoprotein. Although MCA molecules also seem to present binding sites for 115 D-8 and DF-3, the molecules detected by the CA 15-3 and the MCA assay are probably not identical [19, 21, 22]. CA 15-3 was correlated with MCA taken from the same blood samples. This result is in accordance with other studies [19, 20, 22, 23].

Since both tumour markers identify different molecules associated with breast carcinoma, the expression of the two antigens might be similar in most patients. Because of the strong correlation, we suggest that the assay of MCA reflects tumour dissemination as well as the measurement of CA 15-3. As Fig. 7 shows, in the most reliable part of the two curves between the 22nd and the 37th month with the smallest standard deviations, the MCA means were slightly higher than the CA 15-3 means. However, despite the higher sensitivity of the MCA assay, the presumption that MCA levels might increase at an earlier stage was not confirmed statistically, neither by repeated measures ANOVA nor by comparison of the sensitivities.

Since the negative predictive value for MCA was significantly higher than that for CA 15-3, the MCA result enables us to avoid further diagnostic procedures with a higher degree of safety than the CA 15-3 assay when a given value is not elevated. Combination of the MCA assay with the CA 15-3 test revealed only a modest increase in sensitivity. Since this gain in sensitivity was at the expense of a significant loss of specificity, combined investigation with both tests is not recommended.

Steger *et al.* [20] calculated a sensitivity of 80% and a specificity of 87% of the MCA enzyme immunoassay (cut-off 14 U/ml) and a sensitivity of 87% and specificity 89% for CA 15-3 (cut-off 26 U/ml). The combination of the CA 15-3 and MCA assays showed no significant benefit. However, serial measurements exceeding two months and statistical analysis of differences between the sensitivity-specificity diagrams of the tumour markers were not carried out. In accordance with Fuith *et al.* [21], who revealed a higher pretherapeutic sensitivity (23.7% vs. 21.2%), a superior specificity (97% vs. 94%) and positive predictive value (90% vs. 80%) of the MCA enzyme immunoassay (cut-off 14 U/ml) compared with CA 15-3 (cut-off 30 U/m), and with Gozdz *et al.* [19] (overall pretreatment sensitivity of 21.7% vs. 16.3%) we suggest that the MCA enzyme immunoassay is at least equivalent to the CA 15-3 test in monitoring breast cancer patients.

Following Bayes' theorem, with respect to the low incidence of metastases in breast cancer patients with stage I and II (in the first year after surgery 3–5% [24]) and the high percentage of patients with these stages (in our study 94.3%), clinical diagnosis particularly of progressive disease is difficult. Since MCA continuously increases in the PD group from the 5th month after initial cancer treatment, serial measurements of the tumour marker are an important tool to improve the reliability of the diagnosis.

Both MCA and CA 15-3 measurements enable us to discriminate patients with a low risk of local recurrence or metastases from high-risk patients. Because MCA increases as the cancer spreads and because of its high negative predictive value, the MCA enzyme immunoassay is a powerful tool in monitoring breast cancer patients. From the 5th month after the initial cancer treatment, serial MCA assays are recommended. In our experience, in cases of clinically doubtful metastic spread, the combination of serial MCA measurements and simultaneous bone scans allows unequivocal diagnosis in almost all investigated patients.

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Breast Size, Handedness and Breast Cancer Risk

C.-c. Hsieh and D. Trichopoulos

Bra cup size and handedness were studied as possible risk factors for breast cancer. Data for 3918 cases and 11 712 controls from 7 centres were used to examine the association of handedness with laterality of breast cancer; data for 2325 cases and 7008 controls from 4 centres were used to assess the relation of bra cup size to breast cancer risk. There was a suggestive (*P* about 0.10) association of handedness with breast cancer laterality: odds ratio of a left-handed (or ambidextrous) woman having a left-sided cancer 1.22 (95% CI 0.96–1.56). Handedness may affect the lateral occurrence of breast cancer, although this tumour is in general more common in the left breast, possibly because this breast is usually slightly larger. Premenopausal women who do not wear bras had half the risk of breast cancer compared with bra users (*P* about 0.09), possibly because they are thinner and likely to have smaller breasts. Among bra users, larger cup size was associated with an increased risk of breast cancer (*P* about 0.026), although the association was found only among postmenopausal women and was accounted for, in part, by obesity. These data suggest that bra cup size (and conceivably mammary gland size) may be a risk factor for breast cancer.
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INTRODUCTION

MOST HYPOTHESES to explain the aetiology of human breast cancer have focused on mammotropic hormones, particularly oestrogens [1–4]. Several investigations have suggested that cell number and mammary gland size (or mass) may be important risk factors for breast cancer [5, 6]. This view is compatible with data from experimental carcinogenesis [6, 7] as well as with epidemiological observations linking breast cancer to diet [8] and to adult height [9, 10]. However, studies that examined the relation of bra cup size (possibly correlated with mammary gland size) to breast cancer risk have given equivocal results. Wynder *et al.* [11], Soini [12] and Hirohata *et al.* [13] found no evidence for an association in their case–control studies, but these studies were not sufficiently large to overcome the sustantial misclassification in the measurement of bra cup size (itself a poor measure correlate of mammary gland size). Katariya *et al.* [14] assessed breast size from mammograms and found no difference between breast cancer cases and controls in a small number of women. In contrast, Deapen *et al.* [15] noted a 43% lower frequency of breast cancer among small-breasted women who had undergone augmentation mammoplasty, and Dupont and Page [16] found that breast size was a risk factor for breast cancer among women with proliferative breast disease.

We have examined the association between bra cup size and breast cancer in a large set of data, collected by MacMahon *et al.* [17, 18] during the late 1960s, in the context of an international multicentre case-control study of breast cancer. The relation between breast cancer laterality and handedness was also assessed.

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SUBJECTS AND METHODS

The international multicentre case-control study was done with an agreed common protocol in seven areas of the world in populations with a low, intermediate and high incidence of breast cancer (Athens, Greece; Boston, USA; Glamorgan, Wales; São Paulo, Brazil; Slovenia, Yugoslavia; Taipei, Taiwan; and Tokyo, Japan). Except in Tokyo, the breast cancer cases included most of the female residents of the study area who were admitted for a first diagnosis of breast cancer during the study period. For each breast cancer patient interviewed, 3 eligible patients in beds closest to the index case were interviewed as controls. A control had to be a resident of the study area, to have never had cancer of the breast, and to be over 35 years of age (except when the index case was under 35, in which case controls were age-matched within 2 years). Details about the original study and collective results have been reported for lactation [17], age at first and at any birth [18, 19] and age at menarche, age at menopause and anthropometric variables [10].

Subjects were excluded from the subsequent analyses when their interviews were judged unreliable or when information was not available for the variables included in the analyses. Among 4395 interviewed women with breast cancer in all seven centres, 443 (10.1%) were excluded from the analysis of laterality of breast cancer and handedness; among the corresponding 12 888 controls, 1176 (9.1%) were excluded. The association between breast size and breast cancer was studied only among women in Athens, Boston, Glamorgan and São Paulo; in the remaining three centres information on bra cup size was not collected (Slovenia, Taipei) or most women were not customarily wearing a bra (Tokyo). Among 2561 interviewed women with