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Original Paper

Assessment of Five Serum Marker Assays in Patients with Advanced Breast Cancer Treated with Medroxyprogesterone Acetate

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This study concerns five different tumour marker assays examined in the context of 94 patients with advanced breast cancer treated in a prospectively randomised trial of different doses of medroxyprogesterone acetate (MPA). MPA was administered at doses of 500 or 1000 mg daily and clinical evaluation of patients was carried out according to UICC criteria. Carcinoembryonic antigen (CEA) was selected as a standard marker, with three assays for MUC1 mucins (epithelial mucin core antigens (EMCA), EMCA2 and BR-MA immunoradiometric assay) differing in antibody specificities for different mucin epitopes. An additional novel assay for soluble cytokeratin was also evaluated as an example of an independent marker with a different nature and biology. Sensitivity of individual assays ranged between 44 (EMCA2) and 69% (cytokeratin) and the use of two assays in combination led to sensitivities as high as 84% (cytokeratin + BR-MA). The proportion of patients found to be assessable by each assay ranged between 51 (EMCA2) and 76% (cytokeratin). Of those patients whose marker changes were assessable, those receiving the higher dose of MPA displayed significant falls in marker levels after 12 weeks of treatment. This effect was not observed in patients receiving 500 mg. The change in cytokeratin levels in patients undergoing high dose MPA therapy proved to be most marked. Using the cytokeratin assay, 91% (of 23 patients) of patients with progressive disease showed at least a 25% rise in serum marker levels. Of these, 66% showed increases before disease progression was detected clinically with a mean lead time of 14 weeks. There was very little difference between the responses of the five tumour marker assays in patients with stable or responding disease, the proportion of these patients with stable or falling tumour marker levels ranging between 58% (CEA) and 77% (EMCA). We conclude that the cytokeratin assay has an application in monitoring response to therapy and predicting tumour progression in advanced breast cancer patients with assessable tumour marker profiles, especially if used in combination with a MUC1 mucin assay.

Key words: advanced breast cancer, serum marker assays, MUC1 mucins, CEA, cytokeratin
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INTRODUCTION

THE USE of serum tumour markers in the management of patients with malignancy has been recently reviewed [1–3]. Markers which accurately reflect the status of disease in a patient with known malignancy could be extremely valuable in overall management.

Clinical studies of sufficient homogeneity in which to assess the relative efficiency of different serum markers are rare. Ideally, the markers should be assessed in patients with well defined clinical conditions, and in response to a single treatment. However, clinical decisions do not always make that an easy goal to achieve. Moreover, statistical evaluation of different efficiencies of markers is hampered by study designs whose main indicators of outcome examine treatment rather than marker efficacy. This is entirely appropriate, which makes it especially important to exploit any study which offers the opportunity for an "explanatory" rather than "pragmatic" evaluation of different tumour markers [4].

The present report concerns five different tumour marker assays examined in the context of patients with advanced breast cancer treated in a prospectively randomised trial of two different doses of medroxyprogesterone acetate (MPA). Carcinoembryonic antigen (CEA) was chosen as the standard marker, with

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three assays for MUC1 mucins differing in antibody specificities for different mucin epitopes. An additional, novel assay for soluble cytokeratins was also chosen to measure a marker with a completely different molecular nature and biology, and which has not previously been evaluated in this clinical context [5–7].

The aim of the study was to assess the performance of these five tumour marker assays with respect to their reliability, sensitivity, ability to reflect the patients' responses to treatment and to monitor disease progression. A major objective of the investigation was to find an assay, or combination of assays, which would have clinical value in the management of patients suffering from advanced breast cancer.

PATIENTS AND METHODS

Patients and treatment

A total of 94 women were enrolled in the study at Queen Elizabeth Hospital, Birmingham, U.K., from July 1989 to December 1992. All were diagnosed as having advanced breast cancer according to UICC guidelines [8], with locally recurrent or metastatic disease, or both. The patients were randomised to receive either 500 or 1000 mg daily of MPA (Farmitalia) taken orally after a loading dose of 1000 mg 6 hourly for 48 h. The patients were then seen at intervals for periods up to 40 months, ending in June 1993.

At follow-up visits, patients were assessed for response at each metastatic site and overall response according to UICC criteria. An assessment was also made of the patients' general quality of life and well-being. Blood samples were collected at the initial and follow-up visits for tumour marker measurements. Serum was separated, aliquoted and stored at −20°C.

Analytical methods

At the completion of the clinical study, samples were thawed and analysed together in five assays. CEA was measured by ELISA (EURO/DPC, Llanberis, Gwynedd, U.K.) over the range 0–100 ng/ml. For all assays, samples with values greater than that of the top calibrator were diluted to obtain a result within the dynamic range of the assay. The 95th centile of a normal population was determined to be 5 ng/ml.

Epithelial mucin core antigen (EMCA) (EURO/DPC, Llanberis, Gwynedd, U.K.) was measured as previously described [9], using an ELISA technique; the working range of the assay was 0–300 kU/l. A modification of the assay (EMCA2) was also used, in which the sample was incubated with a buffer containing neuraminidase to remove sialic acid residues and expose more of the core protein epitope consisting of the amino acids Arg–Pro–Ala–Pro [10]; this assay gave a much larger signal, and the test was desensitised to provide numerical values which approximated to those of other mucin assays, with a working range of 0–250 kU/l. BR-MA (EURO/DPC, Llanberis, Gwynedd, U.K.) is a double determinant, murine monoclonal sandwich IRMA (immunoradiometric assay) in which one antibody is directed at the protein core and the other at a carbohydrate epitope [11]; it has been shown to be essentially equivalent to CA 15-3 (data not shown) and the working range was up to 500 kU/l. Standardisation of the mucin assays was with cell culture supernatant, and the 95th centile of a normal population was determined to be 25 kU/l for EMCA, and 37 kU/l for EMCA2 and BR-MA assays.

Soluble cytokeratins were measured in an ELISA assay using a pair of murine monoclonal antibodies shown by immunoblotting to be specific for cytokeratins 18 and 8 (Birgit Lane, **Medical Sciences Institute, University of Dundee, U.K.,** personal communication). Cross-reactivity studies showed that cytokeratin 18 was the predominant immunoreactive form. Standardisation of the cytokeratin assay was with cell culture supernatants, and the 95th centile of a normal population was determined to be 100 kU/l; the working range of the assay was 0–2500 kU/l.

Statistical methods

Results obtained over the first 12 weeks of treatment were used when analysing the sensitivity of each tumour marker assay, changes in marker levels in response to treatment and when assessing the differences between the marker assays. The data sets for 500 and 1000 mg per day doses of MPA were analysed separately, unless stated otherwise. Data sets from patients in which the tumour markers were assessable (that is, where values were above the 95th centile for a normal population at any time during the 12 week period) were analysed separately from those in which the tumour markers were not assessable (where values never rose above the 95th centile at any time).

Over the first 12 weeks of treatment, changes between pretreatment values and later values were assessed using the Wilcoxon signed-rank test on raw data. The significance of differences between the proportions of patients assessable with each marker was tested using the Chi-square test with Yates' correction.

When assessing the correlation of clinical tumour response with marker movement, analysis was performed only where there was a minimum of 8 weeks of data. Patients' reponses were divided into stable/responder or progressive disease according to UICC criteria.

For progressive disease, the date of clinical annotation of progression was noted, and the nadir of any marker seen previously was also noted. Any increase from this nadir of 25% or more was deemed to be a "biochemical progression", and the lead time in weeks between biochemical and clinical progression (if any) was noted. In the case of the group of patients whose disease was stable or responding to treatment, marker levels were compared with pretreatment values. Levels were judged to be rising if they subsequently increased by at least 25% of the pretreatment value or if they moved from below the cut-off value to above it. An increase of 25% or more was selected since this value represents twice the largest interassay coefficient of variation found for any of the assays.

Statistical analysis was with the Mann–Whitney U test and Chi-square test as appropriate. Statistical significance was taken to be $P < 0.05$ with a two-tailed test throughout. Statistical analysis was with the C-Stat package from Cherwell Scientific Publishing (Oxford, U.K.).

RESULTS

Patients

There were 94 patients in the study with a median age of 65.4 years (range 37–87 years) at the outset of the study and a median disease duration of 3.5 years (range 2 months to 19.4 years). Patients' subjective assessment of well-being was 1 (good) or 2 (moderate) in all but one case, and the ECOG (Eastern Cooperative Oncology Group) performance scores were generally 2 (bed/chair rest for less than 50% of the day) or better in more than 90% of cases. 65 patients fulfilled the inclusion criteria for analysis of the correlation of marker levels with clinical tumour response; 40 stable/responders and 25 with progressive disease.

Assay performance

Between-assay variability was determined by testing control samples in each assay. The results for all five assays are shown in

*Table 1. Between-assay variability**

Assay	Units	Control 1 Reference value	Control 1 Mean	Control 1 CV%	Control 2 Reference value	Control 2 Mean	Control 2 CV%
EMCA	kU/l	24	24	8.5	124	134	12.3
EMCA2	kU/l	10	12	12.5	30	31	2.9
BR-MA	kU/l	30	31	4.2	200	205	5.6
CEA	μg/l	3	3.6	4.5	60	58	9.8
Cytokeratin	kU/l	100	82	8.2	500	473	10.2

*Results are the mean of 12 assays using individual control pools made for each assay.

Table 1; between-assay coefficients of variation were generally below 10%.

The assay performance characteristics were employed to define objective criteria for measuring marker levels in sequential samples. Sequential measurements showing changes of greater than 25% were taken as indicating significant changes in marker levels whether they represented a rise or a fall. The value of 25% represents twice the highest value for the interassay coefficient of variation in any of the assay systems (Table 1), and was thus taken as a reliable and objective indicator of tumour marker alterations.

Sensitivity of markers

The sensitivity of the five tumour markers was assessed using all the pretreatment values, both singly and in combinations of two (Table 2) and three tumour markers. For single markers, the cytokeratin assay at 69% and BR-MA at 63% were better than CEA (55%), EMCA (54%) and EMCA2 (44%). Using combinations of two markers, the highest sensitivities were with cytokeratin in combination with mucins (BR-MA or EMCA at 84% and 81%, respectively) or CEA (82%). Sensitivities obtained using three serum markers in combination were not significantly higher than those obtained using two markers (data not shown).

Not all patients were assessable—as defined by having a concentration above the 95th centile for a normal population at any time during the study (Table 3). Overall, 76% of patients were assessable with cytokeratin and 71% with BR-MA. The proportion of assessable patients was lower for EMCA (61%), CEA (56%) and EMCA2 (51%). Using the Chi-square test, the proportion of assessable patients with cytokeratin was significantly higher than with EMCA, EMCA2 and CEA, but not significantly better than BR-MA. The proportion of assessable patients using BR-MA was significantly higher than with EMCA2 and CEA.

Table 2. Sensitivity of individual or combinations of tumour marker assays to advanced breast cancer in pretreatment serum samples

Assay	EMCA	EMCA2	BR-MA	CEA	Cytokeratin
EMCA	54%*	58%	70%	72%	81%
EMCA2	–	44%	69%	66%	68%
BR-MA	–	–	63%	73%	84%
CEA	–	–	–	55%	82%
Cytokeratin	–	–	–	–	69%

*Values are percentage sensitivity for tumour markers calculated using the pretreatment sample for each patient.

Table 3. Patients assessable with each tumour marker

Assay*	MPA dose (mg/day)	Assessable (*n*)	Not assessable† (*n*)	Assessable overall (%)
EMCA	1000	29	20	61
	500	28	17	
EMCA2	1000	27	22	51
	500	20	24	
BR-MA	1000	31	16	71†
	500	33	10	
CEA	1000	17	17	56
	500	25	14	
Cytokeratin	1000	23	11	76‡
	500	33	7	

*There are less than 94 patients' results in all assays except EMCA due to insufficient samples; †Patients were defined as being "biochemically not assessable" for any marker if the level of that marker never rose above normal cut-off values at any time during the study. The proportion of assessable patients with cytokeratin‡ was significantly ($P < 0.05$, Chi-square test) greater than with EMCA, EMCA2 and CEA, but not significantly different from BR-MA†. The proportion of assessable patients with BR-MA was significantly better than EMCA2 and CEA.

Change in tumour marker values with treatment

Testing serum tumour marker concentrations after 2, 6 and 12 weeks of treatment against pretreatment concentration in assessable patients at doses of 500 or 1000 mg MPA per day using the Wilcoxon signed-rank test showed some significant changes, and these results are shown in Table 4. Assessable patients on 500 mg showed significant increases of EMCA and EMCA2 at 2 weeks and of CEA at 2 and 6 weeks. In contrast, at a dose of 1000 mg per day, assessable patients showed no significant increase in tumour marker levels, and BR-MA, EMCA and EMCA2 showed significant decreases at 12 weeks of treatment. Cytokeratin showed significant decreases at 2, 6 and 12 weeks of treatment.

Differences between tumour markers

The percentages of patients receiving 500 and 1000 mg MPA, who responded with a decrease of at least 25% in marker level at 2, 6 and 12 weeks compared with the pretreatment sample, are shown in Figure 1a and b, respectively. A greater proportion of patients receiving 1000 mg MPA responded with a significant fall (a decrease of 25% or greater) in marker values (Figure 1b). Nearly 90% of patients receiving 1000 mg MPA showed reduction of cytokeratin levels at 12 weeks (Figure 1b), compared with 60% of patients receiving the lower dose (Figure 1a). The mucin markers and CEA all behaved similarly and it is clear that cytokeratin levels were more responsive in more patients, and from an earlier time than any of the other marker determinations. Chi-square analysis of the combined patient groups confirmed that a greater number of patients had decreases in cytokeratin levels compared with the number of patients with marker reductions in any of the other assays at 2, 6 and 12 weeks of treatment ($P < 0.01$).

Correlation of clinical tumour response with serum marker changes in stable/responding patients

Of the 40 stable/responding patients, 16 were receiving 500 mg MPA/day and 24 were receiving 1000 mg/day. The median follow-up period in this group was 27 weeks (mean 33, range

Table 4. Results of Wilcoxon signed-rank test

	Marker changes in patients treated with MPA*					
	500 mg/day			1000 mg/day		
Assay	2 weeks	6 weeks	12 weeks	2 weeks	6 weeks	12 weeks
EMCA	↑ †P = 0.018	NS	NS	NS	NS	↓ P = 0.012
EMCA2	↑ P = 0.004	NS	NS	NS	NS	↓ P = 0.012
BR-MA	NS	NS	NS	NS	NS	↓ P = 0.008
Cytokeratin	NS	NS	NS	↓ P <0.001	↓ P <0.001	↓ P <0.001
CEA	↑ P = 0.018	↑ P = 0.019	NS	NS	NS	NS

*The assessable data sets obtained from samples taken after 2, 6 and 12 weeks of treatment were compared with those of the pretreatment samples using the Wilcoxon signed-rank test; †Statistically significant rises in tumour marker levels and statistically significant falls in tumour marker levels are indicated by ↑ and ↓, respectively; NS indicates no significant difference between marker level and pretreatment level.

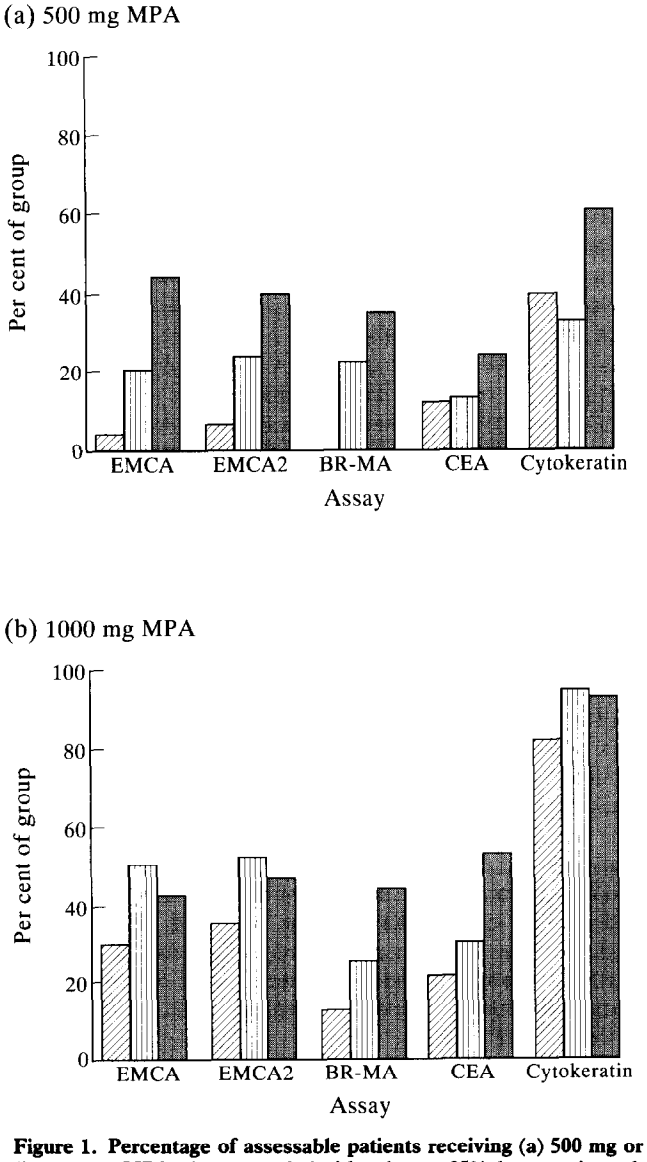

Figure 1. Percentage of assessable patients receiving (a) 500 mg or (b) 1000 mg MPA who responded with at least a 25% decrease in each marker level at 2 weeks (▨), 6 weeks (▥), and 12 weeks (▩) of treatment.

9–90 weeks). The proportions of patients in this group with stable/falling, or with rising tumour marker levels are given in Table 5. The assays which gave the best correlation with clinical tumour response in patients with stable or responding disease were the mucin markers (EMCA, EMCA2 and BR-MA). The proportions of patients with stable/falling mucin marker levels were found to be significantly higher than CEA, but not cytokeratin ($P < 0.05$, Chi-square test).

Correlation of clinical tumour response with serum marker changes in patients with progressive disease

25 patients had progressive disease, 11 of whom were taking 500 mg MPA/day and 14 who were taking 1000 mg/day; these proportions were not significantly different from those for stable/responders ($P < 0.05$, Chi-square test). The median follow-up period was 24 weeks (mean 33, range 8–92 weeks); this was not significantly different from that for stable/responders ($P < 0.05$, Mann–Whitney U test).

The proportion of progressive disease patients with increases in marker levels was highest with the cytokeratin assay (91%) (Table 6). This proportion was significantly higher than with any other marker ($P < 0.05$, Chi-square test). Mean lead times varied between 13 and 17 weeks, and there were no significant differences between the lead times observed with the five assays ($P < 0.05$, Mann–Whitney U test).

DISCUSSION

Polymorphic epithelial mucins are a heterogeneous group of high molecular weight glycoproteins which are released into the circulation of patients with breast cancer [12]. Antibodies directed against different protein core and carbohydrate epitopes have been used in immunoassays to measure concentrations of the mucins in serum and to relate those concentrations to clinical course [13].

Serum mucin assays, possibly in combination with CEA, have been recognised as the most useful of available markers in breast cancer [1]. Multiple mucin assay measurements have not been recommended, as most assays available for breast cancer are considered to recognise different parts of the same mucin superfamily of molecules. Even so, there is evidence suggesting that some anti-MUC1 mucin antibodies, such as SM3, recognise determinants which are preferentially expressed upon mucins derived from the tumour rather than from normal tissues [14].

Table 5. Correlation of marker changes with clinical tumour response in stable and responding patients

Marker changes*	Number of patients showing marker changes				
	EMCA	EMCA2	BR-MA	CEA	Cytokeratin†
Stable/falling	31 (78%)	29 (73%)	29 (73%)	23 (58%)	25 (68%)
Rising	9 (22%)	11 (27%)	11 (27%)	17 (42%)	12 (32%)
Total	40	40	40	40	37

*Marker levels were compared to the pretreatment sample and were judged to be rising if they subsequently increased by at least 25% of the pretreatment value or if they moved from below the cut-off value to above it; † 37 patients were analysed for cytokeratin due to insufficient samples in 3 patients.

Table 6. Correlation of marker changes with clinical tumour response and lead times in patients with progressive disease

Marker changes†	Number of patients showing marker changes*				
	EMCA	EMCA2	BR-MA	CEA	Cytokeratin
Stable/falling	12 (48%)	8 (33%)	11 (44%)	8 (32%)	2 (9%)
Rising	13 (52%)	16 (67%)	14 (56%)	17 (68%)	21 (91%)
Mean lead time‡ (weeks)	16	17	17	13	14

*Note that only patients with at least 8 weeks of data were included in this analysis; †Marker levels were judged to be rising if they increased by at least 25% of the value observed at the nadir of marker levels measured before clinical detection of progression; ‡Lead time was defined as the time (in weeks) from the first rise to detection of progression.

Measuring tumour-associated molecules with different chemical properties is, however, justified [1].

The cytokeratins are a family of molecules involved in the structure of epithelial cells and are found in malignant tumours [15]. There are at least 20 individual subtypes based on their acidic and basic properties, and cytokeratin intermediate filaments are composed of equimolar amounts of each [16]. Cytokeratins 8 and 18 are among the simplest of the family, and are found widely in normal epithelia and carcinomas, as well as being found in the circulation of patients with cancer at elevated levels [7, 17]. This investigation presented an opportunity to examine the usefulness of serum cytokeratin assays in comparison with the more conventional markers, CEA and MUC1 mucins.

Throughout the study, there were no outstanding differences between the performance of the three mucin assays. BR-MA, however, appeared to have a slightly higher sensitivity at pretreatment, alone and in combination with cytokeratin (Table 2), and a larger proportion of assessable patients (Table 3). BR-MA is similar to the CA15-3 assay [18] in that the capture antibody binds to a carbohydrate determinant, and the tracer antibody reacts with the protein core. The EMCA assay has a single monoclonal antibody (C595) as both capture and reporter molecule. This antibody shows highly specific reactivity with the protein core repeat sequence Arg–Pro–Ala–Pro [19]. The EMCA2 assay was designed to make this more efficient by removing sialic acid residues with neuraminidase. Of all the assays investigated here, this one performed worst with respect to sensitivity (Table 2) and had the lowest proportion of assessable patients (Table 3). It is thought that MUC1 mucin derived from malignant tissue differs from normal mucin in that it has a lower degree of glycosylation [20, 21]. Deglycosylation by the enzyme neuraminidase would, therefore, expose more of the protein core epitopes of the C595 antibody, but would also reduce the differences between the normal and malignant mucin phenotypes.

Similar rises in marker levels to those observed at 2 weeks of treatment with 500 mg MPA using the EMCA, EMCA2 and CEA assays (Table 4) are not uncommon. This may be due to the increased release of MUC1 mucin and CEA into the circulation as the tumour cells begin to be destroyed by the treatment. Then, as the size of the tumour decreases, the levels of these markers begin to fall. This phenomenon was not observed in patients taking 1000 mg MPA, but it may be that it occurred before the first sample was taken.

In breast cancer, no single or combination of serum markers has been shown unequivocally to correspond with or predate clinical change in more than a proportion of patients. This study has demonstrated that, although none of the markers investigated fulfilled this role, the cytokeratin assay showed a remarkably high correlation with clinical disease progression. 21 of the 23 patients who had an episode of progressive disease showed increases in serum cytokeratin levels either prior to or at the time of clinical detection, a much higher proportion than that seen with CEA or mucins. In addition, the average lead time in those patients where increases in cytokeratin levels predated

clinical detection of disease progression was 14 weeks. Early indications of disease progression may have important implications for changes in therapy, with subsequent improvements in survival and, perhaps more importantly, in the quality of life of the patient.

The only assay which was significantly different from the others when assessing the correlation between tumour marker movements and clinical tumour response in patients with stable or responding disease was CEA, which showed a lower proportion of patients with stable or falling marker levels. The mucin assays seemed to perform best in this respect.

Polymorphic epithelial mucin is known to be a marker which reflects tumour burden [9, 22, 23]. The debate continues as to whether serum cytokeratin levels reflect tumour cell proliferation or necrosis. It is of note, however, that although this patient group was judged to have stable or responding breast disease, 58% were removed from the study because their cancer had progressed or because they died. It would seem reasonable, therefore, that if cytokeratin levels reflected tumour cell proliferation they might presage the clinical events which lead to these patients being withdrawn from the study. This may explain the low correlation with clinical evaluation of disease status in this group of patients.

The present study suggests that serum cytokeratin level is the most responsive marker of advanced breast cancer following hormone treatment. The mucin assay, BR-MA, possibly in combination with cytokeratin, would also appear to have an application in increasing the sensitivity to advanced breast cancer and following the disease course in patients with stable or responding disease.

1. Kleist S von, Bombardieri E, Buraggi G, *et al.* Immunodiagnosis of tumours. *Eur J Cancer* 1993, **29A**, 1622–1630.
2. Markman M. The use of serum tumour markers in the management of patients with malignancy. *J Cancer Res Clin Oncol* 1993, **119**, 635–636.
3. Jager W, Ostrowski M, Kramer S, Lang N. Erlangen tumor marker study on breast cancer. *J Tumour Marker Oncol* 1993, **8**, 50.
4. Schwartz D, Lelouch J. Explanatory and pragmatic attitudes in therapeutic trials. *J Chronic Dis* 1967, **20**, 637–648.
5. Leube RE, Bosch FX, Romano V, *et al.* Cytokeratin expression in simple epithelia–III. Detection of mRNAs encoding human cytokeratins nos 8 and 18 in normal and tumor cells by hybridisation with cDNA sequences *in vitro* and *in situ*. *Differentiation* 1986, **33**, 69–85.
6. Mellerick DM, Osborn M, Weber K. On the nature of serological tissue polypeptide antigen (TPA); monoclonal keratin 8, 18 and 19 antibodies react differently with TPA prepared from human cultured carcinoma cells and TPA in human serum. *Oncogene* 1990, **5**, 1007–1017.
7. Pendleton N, Occleston NL, Walshaw MJ, *et al.* Simple cytokeratins in the serum of patients with lung cancer: relationship to cell death. *Eur J Cancer* 1994, **30A**, 93–96.
8. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland. *Cancer* 1977, **39**, 1289–1294.
9. Dixon AR, Price MR, Hand CW, Sibley PEC, Selby C, Blamey RW. Epithelial mucin core antigen (EMCA) in assessing the therapeutic response in advanced breast cancer—a comparison with CA15.3. *Br J Cancer* 1993, **68**, 947–949.
10. O'Sullivan C, Price MR, Baldwin RW. Polymorphic epithelial mucin from the serum of advanced breast cancer patients—isolation and partial characterisation. *Br J Cancer* 1990, **61**, 801–808.
11. Nilsson O, Karlsson B, Petterson A, Lindholm L. Development of a new serological assay for determination of the MUC-1 breast cancer associated antigen. In Klapdor R, ed. *7th Hamburg Symposium on Tumor Markers*. München, W. Zuckschwerdt, 1993, 40.
12. Price MR. High molecular weight epithelial mucins as markers in breast cancer. *Eur J Cancer Clin Oncol* 1988, **24**, 1799–1804.
13. Feller WF. Mucin glycoproteins as tumour markers. In Herberman RB, Mercer DW, eds. *Immunodiagnosis of Cancer*. New York, Marcel Dekker, 1990, 631–672.
14. Girling A, Barkova J, Burchell J, *et al.* A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM3 is selectively exposed in a range of carcinomas. *Int J Cancer* 1989, **43**, 1072–1076.
15. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalogue of human cytokeratins: patterns of expression in normal epithelia, tumours and cultured cells. *Cell* 1982, **31**, 11–24.
16. Quinlan RA, Cohlberg JA, Schiller DL, Hatzfield J, Franke WW. Heterotypic tetramer (A2D2) complexes of non-epidermal keratins isolated from cytoskeletons of rat hepatocytes and hepatoma cells. *J Mol Biol* 1984, **178**, 365–388.
17. Wiklund B, Silen A, Nilsson S, Andresson E-L. Cytokeratin 8 and 18 fragments quantified in serum of healthy individuals and pancreatic cancer patients with a novel monoclonal assay. *J Tumour Marker Oncol* 1993, **8**, 46.
18. Kufe DW, Hayes DF, Abe M, *et al.* Monoclonal antibody assays for breast cancer. In Kupchik HZ, ed. *Cancer Diagnosis* In Vitro *Using Monoclonal Antibodies*. New York, Marcel Dekker, 1988, 67–100.
19. Briggs S, Price MR, Tendler SJB. Fine specificity of antibody recognition of carcinoma-associated epithelial mucins: antibody binding to synthetic peptide epitopes. *Eur J Cancer* 1993, **29A**, 230–237.
20. Gendler SJ, Spicer AP, Lalani EN, *et al.* Structure and biology of carcinoma-associated mucin, MUC1. *Am Rev Respir Dis* 1991, **144**, S42–S47.
21. Burchell J, Taylor-Papadimitriou J. Effect of modification of carbohydrate side chains on the reactivity of antibodies with core protein epitopes of the MUC1 gene product. *Epithel Cell Biol* 1993, **2**, 155–162.
22. Hayes DF, Zurawski VR, Kufe DW. Comparison of circulating CA15-3 and carcinoembryonic antigen in patients with breast cancer. *J Clin Oncol* 1986, **4**, 1542–1550.
23. Pons-Anicet DMF, Krebs BP, Mira R, Namer M. Value of CA15:3 in the follow up of breast cancer patients. *Br J Cancer* 1987, **55**, 567–569.

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