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Comparison of Mammary Serum Antigen (MSA) with β_2-Microglobulin (β_2M) and Carcinoembryonic Antigen (CEA) Assays in Patients with Breast Cancer

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Abstract—*Serum levels of mammary serum antigen (MSA), β_2-microglobulin (β_2M) and carcinoembryonic antigen (CEA) were evaluated in* 186 *subjects to assess their use in the diagnosis and monitoring of breast cancer, either singly or in combination. Raised MSA levels* (>300 I.U.) *were detected in* 79% *of patients with Stage I/II breast cancer, compared with* 25% *for β_2M* (>2000 μg/l) *and* 12% *for CEA* (>5 ng/ml) *levels respectively. Of* 53 *patients with Stage III/IV breast cancer,* 98% (*MSA*), 55% (β_2M) *and* 64% (*CEA*) *had raised levels. In* 25 *patients followed over* 3–9 *months, the changes in MSA levels correlated with the clinical course of the disease in* 23/25 (92%), *compared with* 7/25 (28%) *using β_2M, and* 9/25 (36%) *using CEA assays.*

The overall sensitivity, specificity and accuracy in detecting breast cancer were 88%, 95% *and* 99% *for MSA;* 39%, 90% *and* 96% *for β_2M; and* 38%, 95% *and* 98% *for CEA, respectively. MSA and β_2M assays in combination enhanced the sensitivity in the detection of breast cancer* (93%) *especially early breast cancer, while maintaining specificity* (90%). *MSA seems to be superior to β_2M or CEA as a tumour marker in breast cancer and its levels seem to correlate with tumour burden. While it appears that β_2M or CEA measurements used alone are of little value in the current management of breast cancer, β_2M may be a helpful adjunct to enhance the sensitivity of MSA assay especially in early breast cancer.*

INTRODUCTION

CURRENTLY little is known of the natural history of untreated 'early' carcinoma of the breast and there has been little change in the 10-year survival rate over the past 40 years despite many innovations in therapy [1]. However, the fact that mammographic screening of asymptomatic women will result in a 30% decrease in the mortality due to breast cancer implies that strategies which address early detection of the disease and prompt treatment are important [2]. Many tumour markers have been shown to be elevated in association with breast cancer, including hormones (calcitonin and βHCG); enzymes (sialyl transferases and placental alkaline phosphatases), nucleotides, polyamines, acute phase proteins; and high molecular weight glycoproteins such as CA 15.3. Most of these have not gained widespread clinical use, mainly because of limitations in their sensitivity in detecting localized breast cancer [3–5]. Carcinoembryonic antigen (CEA) and β_2-microglobulins (β_2M) have been suggested as useful in the management of patients with breast cancer [6, 7]. We have previously described the murine monoclonal antibody 3E1.2 which identifies the mammary serum antigen (MSA) in breast cancer tissue sections and an enzyme immunoassay was developed for detection of MSA in the serum of patients with breast cancer [8, 9]. 3E1.2 defines a determinant present on a high molecular weight glycoprotein (M_r > 300 KD), which has been isolated and purified from human serum but is absent from high molecular weight glycoproteins of milk and human milk fat globule membrane [8, 9].

The aim of the present study was to assess the individual and combined value of MSA, β_2-microglobulin and CEA tests in patients with breast cancer.

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MATERIALS AND METHODS

Serum samples

One hundred and forty serum samples were obtained from patients presenting at the Breast Clinic of the Royal Melbourne Hospital or at the Melbourne Private Consulting Rooms. They comprise 109 patients with breast cancer and 31 patients with benign breast disease which include benign mammary dysplasia (20), simple cysts (9) and fibro-adenoma (2). A further 21 specimens were obtained from normal volunteer females. The criteria for staging and disease status is in accordance with accepted definitions [10, 11], and pathological staging of mastectomy and axillary dissection specimens was performed on all cases who did not have evidence of disseminated disease (Stage I, II) on clinical examination or standard investigations. Serum samples were also obtained at regular intervals (over 3–9 months) from 25 patients with advanced breast cancer (Stage III, IV) undergoing treatment at the Peter MacCallum Cancer Institute, Melbourne, Australia; the blood was drawn with the patient's consent and at the time of each clinical evaluation over the study period. The frequency of patient follow-up and the extent of investigation such as chest X-ray, bone scan and liver ultrasound was left to the discretion of the treating oncologists. The MSA, β_2M and CEA levels on entry into the study were documented as the baseline levels and the levels at the time of changes in disease status were then compared with the baseline levels. In the absence of changes in disease status, MSA, β_2M or CEA level which changed more than 25% from the original value, or its value at the conclusion of the study (if changes <25%) was documented and compared with the initial baseline level. Serum samples were collected from clotted blood, aliquoted and stored at −70°C until use. Information on disease status was documented by the treating oncologist and was not obtained by the Research Centre until all the MSA, β_2 and CEA levels had been determined.

MSA, β_2-microglobulin and CEA assays

MSA levels were determined in a competitive inhibition assay using purified 3E1.2 monoclonal antibody [8]. In brief, MSA present on a solid phase immunoadsorbant was used to bind 3E1.2 previously reacted with a 1/32 dilution of patients' sera at room temperature for 3 h. After an overnight incubation at 4°C, excess serum and antibody was washed away and sheep anti-mouse horse-radish peroxidase conjugate (Amersham International, U.K.) added and incubated for 3 h at 37°C. Assays were developed using a 2,2-azino-di-[3-ethylbenzthiazoline]-sulphonate (ABTS) substrate system. An arbitrary system of inhibition units (I.U.) was

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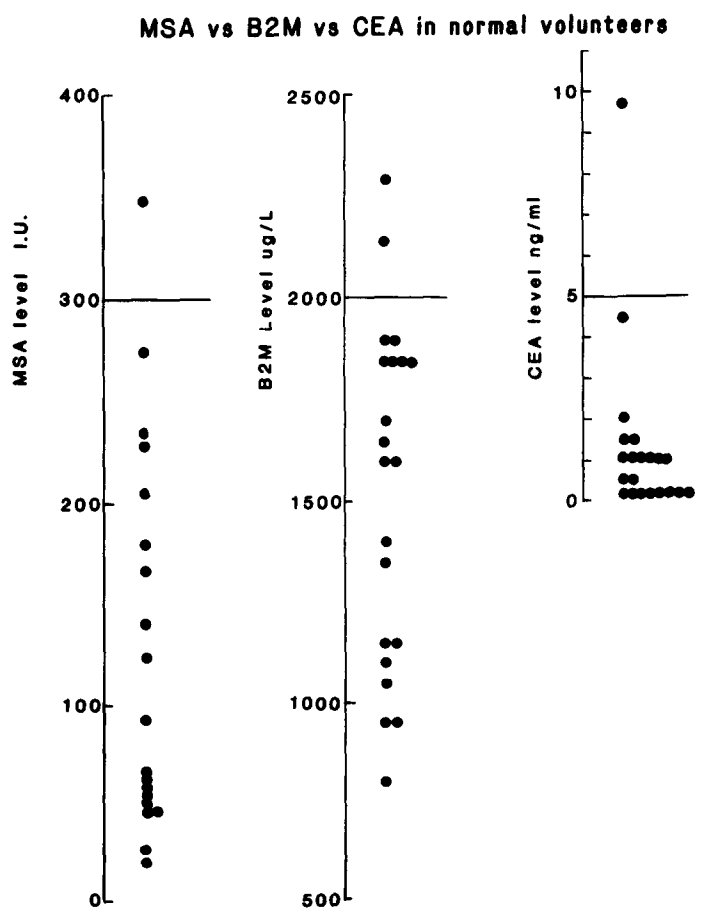


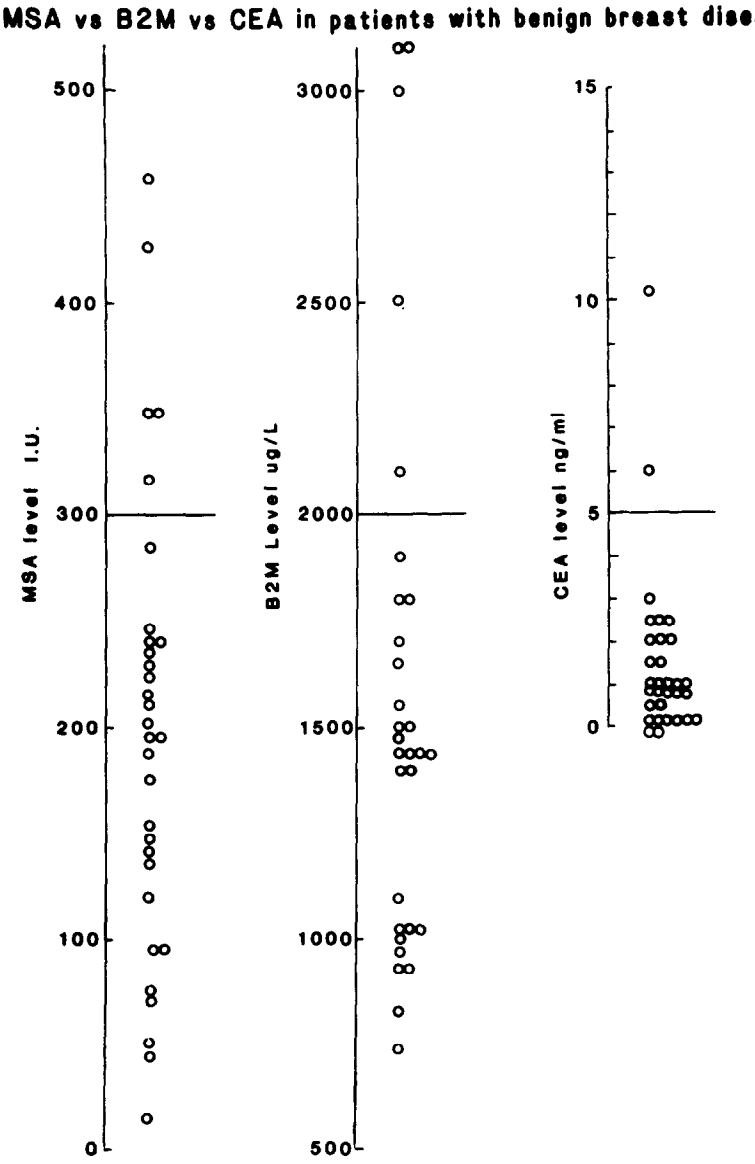


Fig. 1. Circulating levels of MSA, β_2M and CEA in normal individuals (A) and patients with benign breast disease (B). Each point represents an individual. Horizontal lines indicating the respective cut off values of 300 I.U. *(MSA),* 2000 μg/l *(β_2M) and* 5 ng/ml *(CEA) are shown.*

used to express the level of MSA in serum. A standard dilution of 3E1.2 and reference normal sera were used for calculation of units. Levels of MSA vary between 1 and 10,000 I.U., the latter indicating a high level of MSA present. In the normal population, 98% have serum levels <300 I.U. which has been predetermined as the upper limit of normal, based on a previous study of over 2400 normal subjects [9]. β_2-Microglobulin and CEA levels were determined by means of radioimmunoassay kits (β_2-microglobulin RIA and CEA-RIA monoclonal; Abbott, North Chicago) according to the manufacturer's instructions. The upper limit of normal of β_2-microglobulin was 2000 μg/l (mean ± 2 S.D.) and as some of the patients studied were smokers, 5 ng/ml was used as the upper limit of the CEA assay in this study [12, 13]. MSA, β_2M and CEA determinations were performed, in triplicate, simultaneously on freshly thawed serum samples.

Statistical analysis

For statistical inference about differences in the sensitivity for the detection of breast cancer, specificity and correlative data (MSA assay vs. β_2-microglobulin or CEA assay), McNemar's test for correlated proportions was applied [14]. The 'exact', binomial version of the test was used. A difference in assay parameters was considered to be statistically significant if $P < 0.05$.

RESULTS

A. *Reproducibility of MSA, β_2-microglobulin (β_2M) and carcinoembryonic antigen (CEA) assay*

MSA, β_2M and CEA assays have intra-assay variabilities of 12, 9 and 10.1% respectively; and inter-assay variability of 18, 11.1 and 12.2% respectively. These three assays have a reproducibility which is comparable and acceptable for this comparative study.

B. *MSA, β_2M and CEA levels in serum samples*

1. *Normal individuals* (Fig. 1A). It was established, in this study, that the serum level for all three markers (MSA, β_2M and CEA) for apparently normal females were of the expected low levels—the means ± standard errors of the mean (S.E.) were 129 ± 20 I.U. for MSA, 1529 ± 94 μg/l for β_2M, and 1.3 ± 0.5 ng/ml for CEA. Of the 21 normal subjects, 5, 10 and 5% respectively had elevated MSA (>300 I.U.), β_2M (>2000 μg/l) and CEA levels (>5 ng/ml). None of the individuals with elevated MSA, β_2M or CEA levels had clinical evidence of breast cancer, although one female with both a raised MSA and β_2M level had a history of recurrent cystitis, the relevance of which is not known.

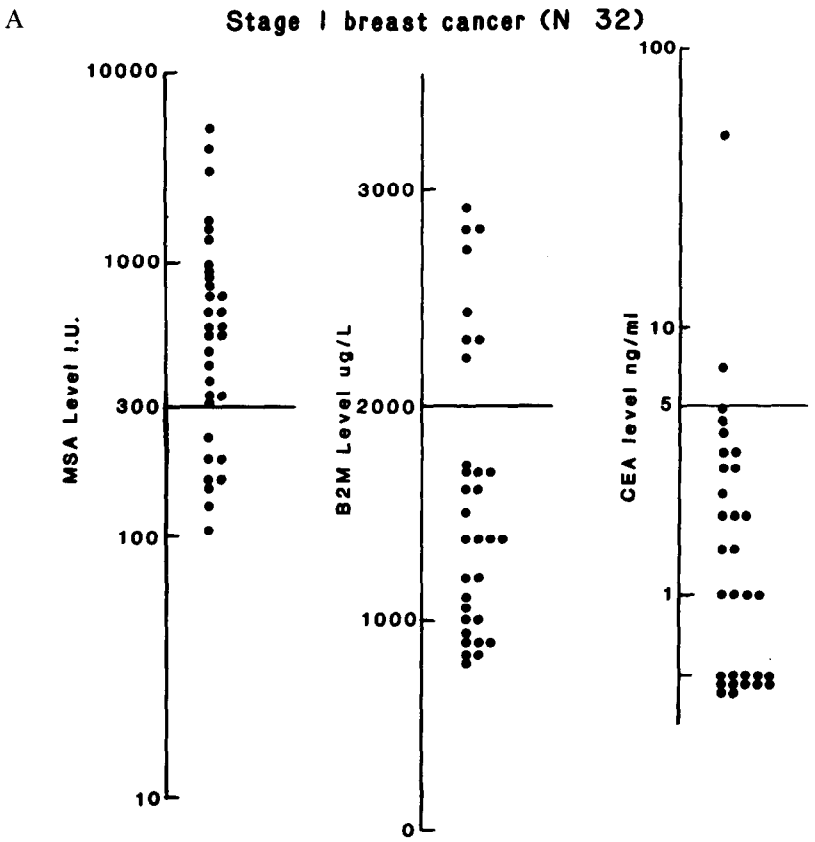
2. *Benign breast disease* (Fig. 1B). Of the 31 patients with histologically proven benign breast disease, 16, 16 and 6% respectively had elevated MSA (>300 I.U.), β_2M (>2000 μg/l) and CEA levels (>5 ng/ml). The mean (± S.E.) levels were low with 201 ± 34 I.U. for MSA, 1600 ± 130 μg/l for β_2M and 1.5 ± 0.4 ng/ml for CEA respectively. Interestingly, the mean levels in the group of patients with benign breast disease were slightly higher than the mean levels of the normal subjects as measured by each of the three assays.

3. *Early breast cancer (Stages I and II)*. Raised MSA levels were detected in a greater number of patients with early breast cancer than either β_2M or CEA. Analysis of 32 patients with Stage I breast cancer (Fig. 2A) revealed raised MSA level (>300 I.U.) in 75% of patients, compared with 25% with raised β_2M (>2000 μg/l) and 6% with raised CEA level (>5 ng/ml). The mean (± S.E.) levels for this group were 759 ± 129 I.U. for MSA, 1533 ± 154 μg/l for β_2M and 5 ± 3 ng/ml for CEA assay respectively.

In Stage II breast cancer (Fig. 2B), the mean (± S.E.) levels were higher—1226 ± 260 I.U., 1598 ± 127 μg/l and 6.0 ± 2.5 ng/ml respectively for MSA, β_2M and CEA assays. Analysis of 24 patients with Stage II breast cancer revealed raised MSA levels in 83% whereas β_2M and CEA levels were elevated in only 25 and 21% respectively.

The mean MSA level of either group of early breast cancer was therefore substantially raised when compared to that of the normal population; in contrast, the mean β_2M or CEA level was either not raised or only slightly raised when compared to that of the normal population. Thus the MSA assay is much more sensitive than β_2M or CEA in detecting localized breast cancer.

4. *Advanced breast cancer (Stages III and IV)* (Fig. 2C). There is an increase in the proportion of patients with raised levels of MSA, β_2M and CEA and the levels were, in general, higher than those found with localized breast cancer. However, significantly more patients with advanced breast can-



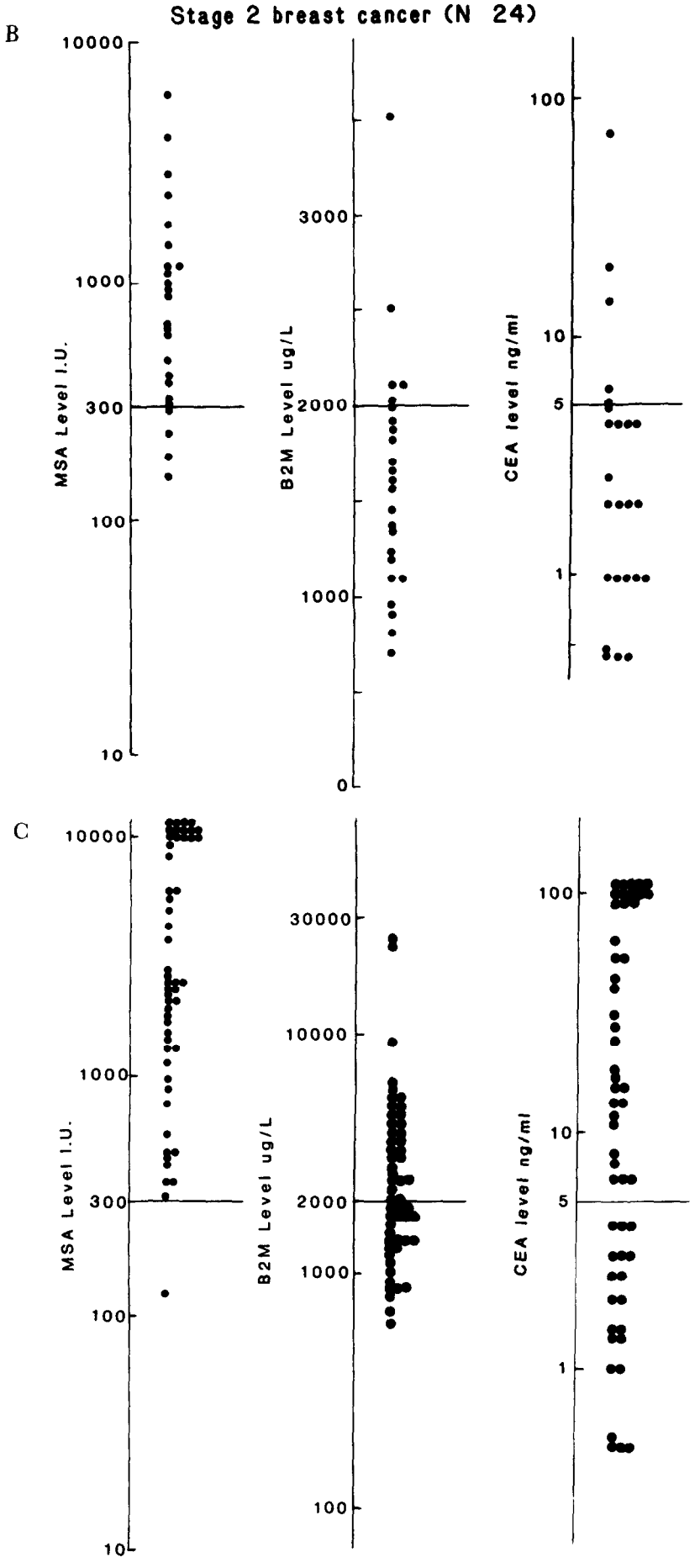


Fig. 2. Circulating levels of MSA, β_2M and CEA in patients with Stage I (A), Stage II (B) and Stage III/IV (C) breast cancer. Each point represents an individual. Horizontal lines indicating the respective cut off values of 300 I.U. *(MSA),* 2000 μg/l (β_2M) *and* 5 ng/ml *(CEA) are shown.*

Table 1. Comparison of MSA, β_2M and CEA assay in breast cancer

Assay parameter*†	Percentage accuracy MSA	β_2M	CEA	MSA + β_2M
Specificity	95	90	95	90
Sensitivity	88	39	38	93
Predictive value of a positive test	99	96	98	98

*Assay parameters have been calculated using the data on patients with breast cancer (n = 109) and compared with the group of normal volunteers (n = 21).
†Specificity = TN/(TN + FP); sensitivity = TP/(TP + FN); predictive value of a positive test = TP/(TP + FP); TP: true positive; FP: false positive; TN: true negative;

cer had elevated levels of MSA (98%) than β_2M (55%) or CEA (64%).

5. *Assay parameters: individual and combined tests.* The assay parameters of the population studied were determined as shown in Table 1. Specificity is defined as the fraction of a non-cancer population not positive at the selected cut-off level, whereas sensitivity is the fraction of a cancer population positive at that value. The predictive value of a positive test is the fraction of a population with a positive test result who have breast cancer (Stages I–IV) [15]. The specificity of the MSA assay is comparable with the β_2M (P = 1.0) and CEA assay in the normal population studied using the respective cut-off levels. The false positive rates of the MSA assay (16%) in patients with benign breast disease is also comparable with the β_2M (P = 1.0) or CEA assay (P = 0.38). The sensitivities of the MSA, β_2M and CEA assays were 88, 39 and 38% respectively. The differences in sensitivity of the MSA assay from the β_2M and CEA assays were highly significant in all groups (Stages I, II, III/IV) of patients with breast cancer ($P \leq 0.002$). Four of 32 patients with Stage I breast cancer and 1/24 patients with Stage II breast cancer had a raised β_2M level but normal MSA level. Hence, by combining β_2M with the MSA assay, there is an improvement in the detection rate (sensitivity) of Stage I breast cancer from 75% (24/32) with MSA alone to 88% (28/32) with MSA and β_2M in combination; in Stage II breast cancer from 83% (20/24) to 88% (21/24). At the same time, using the same criteria, 2/21 (10%) of normal subjects studied will have either an elevated MSA or β_2M level, compared with a false positive rate of 1/21 (5%) by MSA alone. There does not appear to be any advantage in combining β_2M and MSA in patients with advanced breast cancer. CEA does not confer any additional advantage to either MSA or MSA and β_2M in combination.

Hence, in comparison with MSA alone, MSA and β_2M assays in combination will improve the sensitivity in detecting early breast cancer from 79 to 88% and all breast cancers (Stage I–IV) from 88 to 93% but with a reduction in the specificity from 95 to 90%.

C. *Correlation of MSA, β_2M and CEA levels with clinical status* (Fig. 3A,B,C)

In a prospective study MSA, β_2M and CEA were determined in the serum samples of 25 patients with advanced breast cancer treated over 3–9 months previously. Figure 3A,B,C show the results of serial MSA, β_2M and CEA testing in relation to different disease states—progressive disease, stable disease and disease regression [11]. A change of 25% in the level of MSA, β_2M or CEA was considered to be significant. In total, 23/25 (92%), 7/25 (28%) and 9/25 (36%) patients had changes in MSA levels, β_2M levels and CEA levels respectively which correlated with progression, stability and regression of disease and the differences in correlation with disease status between MSA assay and β_2M ($P < 0.001$) or CEA assay ($P < 0.001$) are statistically significant.

In nine patients with clinically progressive disease, 8/9 (89%), 1/9 (11%) and 4/9 (44%) respectively had increasing serum levels of MSA, β_2M and CEA. Only one patient with progressive disease had no significant change in MSA levels and in this case there was mainly local recurrent disease. In nine patients with stable disease, 8/9 (89%), 7/9 (78%) and 2/9 (22%) respectively had serial MSA levels, β_2M levels and CEA levels which changed not more than 25% from the original values. One patient with stable disease had a fall in MSA level and the only site of metastasis was the local skin flap recurrence which had remained static in size for 4 years. In seven patients with partial or complete response to therapy, 7/7 (100% for MSA), 0/7 (0% for β_2M) and 3/7 (43% for CEA) had levels which fell by more than 25% from the original values.

DISCUSSION

This study has examined the relative merits of measuring MSA, β_2M and CEA levels in the detection and monitoring of breast cancer. The false

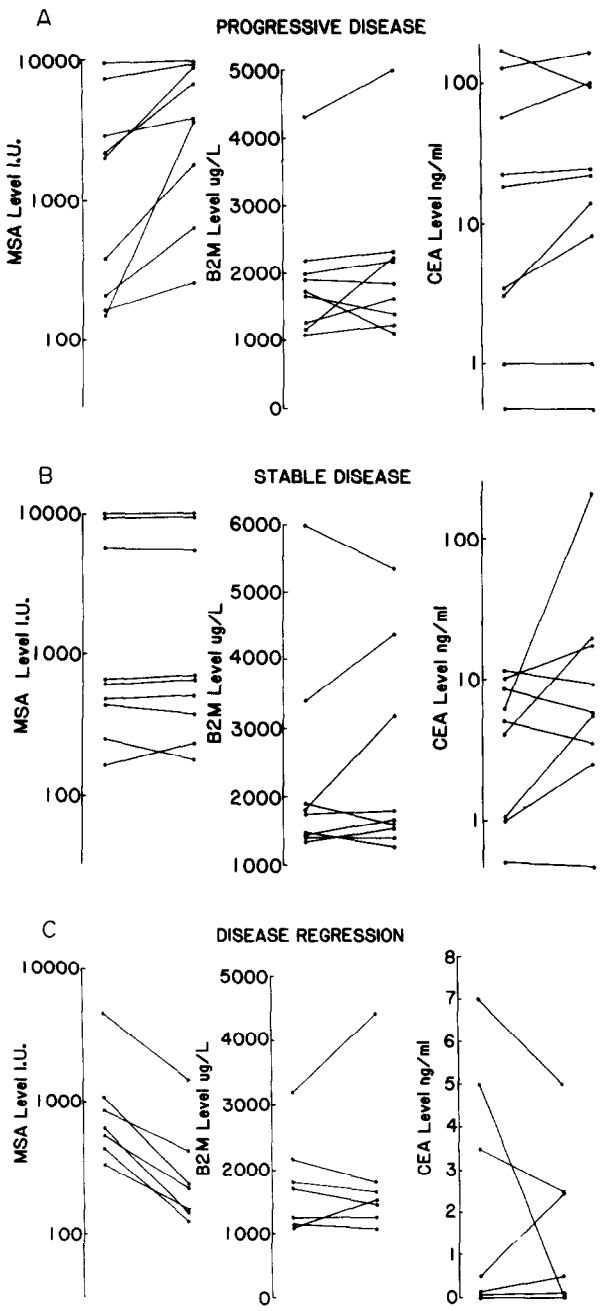


Fig. 3. Graph showing the changes in MSA, β_2M and CEA values over a period of 3–9 months in 25 patients with advanced breast cancer undergoing therapy. The patients were classified clinically as having progressive (A), stable (B) and regressive disease (C).

positive rates of MSA, β_2M and CEA assays in normal subjects were 5, 10 and 5% respectively with β_2M having the highest false positive rates although the differences are not statistically significant ($P = 1.0$) from MSA assay. Patients with benign breast disease had mean serum MSA level, β_2M level and CEA level that were only slightly higher than those of normal controls but still well within the normal range. In all three assays, however, benign breast disease resulted in low but significant elevation in serum MSA, β_2M and CEA levels in a proportion of patients (which were 16, 16 and 6% respectively).

The study also demonstrated that while the serum MSA, β_2M and CEA levels were related to the stage of the disease, the MSA assay is significantly more sensitive than either the β_2M ($P \leq 0.002$) or CEA assay ($P < 0.001$) in the evaluation of patients with both early (79, 25 and 12% respectively) and advanced breast cancer (98, 55 and 64% respectively). It was noted that combining results of MSA and β_2M assays enhanced the sensitivity of detecting early breast cancer (from 79 to 88%) with a small loss in specificity (from 95 to 90%) compared with MSA assay used alone. Thus the use of MSA and β_2M assays are complementary and may be useful in a population screening programme for breast cancer because in this context it is essential to have simple tests with a high sensitivity of detecting early breast cancer with acceptable specificity although this is not yet proven. As the MSA assay is so sensitive in advanced breast cancer, combination with β_2M and/or CEA did not offer any advantage. Furthermore, addition of CEA either to the MSA assay or the combination of MSA and β_2M did not confer any further advantage in the detection of early breast cancer.

Overall, the sensitivity of MSA determinations alone (88%) was greater than β_2M (39%) or CEA (38%) in detecting breast cancer and when MSA and β_2M were combined, the sensitivity increased to 93%. Furthermore, all tests had good specificity (Table 1). The predictive value of a positive test result of MSA or MSA and β_2M combined are high (99 and 98% respectively) and are comparable with β_2M or CEA (96 and 98% respectively) in the study. Although this is only a limited comparative study, the early results gathered thus far have favoured MSA as a useful marker in the management of breast cancer. The finding that a significant proportion of patients with early breast cancer have an elevated MSA level (79%) and an elevated MSA or β_2M level (88%) renders it of potential value as an adjunct in diagnosis and mass screening.

Previously it was shown that serial determinations of MSA level correlated with changes in disease status (progression, stability and regression) in 95% of cases (38/40) during a period of monitoring of patients post-mastectomy [9]. A prospective study was carried out here to compare the correlation of MSA, β_2M and CEA levels with changes in disease status (progression, stability and regression). A good correlation of changes in MSA levels with changes in disease status was found in 92% of patients in accordance with previous studies [9]. However, in only 28 and 36% of patients were there changes in β_2M and CEA levels respectively which correlated with changes in disease status. An important question to address is whether or not

these changes of disease status could have been detected clinically using other commonly used techniques, and furthermore, whether monitoring with MSA and β_2M in combination provided any new information to aid in patient management beyond that available from conventional methods of follow-up. Appropriate studies are now in progress to resolve these issues.

In conclusion, it is clear that MSA is a useful diagnostic aid and appears to be more sensitive than the measurements of either β_2M or CEA in the management of breast cancer. The MSA levels correlate with the tumour burden and in general the MSA assays were found to be simple, reproducible and sensitive. While it appears that individual measurements of β_2M or CEA on their own is of little value for diagnosis, mass screening or monitoring changes in disease status because of the low sensitivity, β_2M may be a helpful adjunct to enhance the sensitivity of the MSA assay in early breast cancer.

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