Prognostic Value of Plasma Prostate Specific Antigen after Megestrol Acetate Treatment in Patients with Metastatic Breast Carcinoma

Eleftherios P. Diamandis, M.D.[1]
Svein I. Helle, M.D.[2]
He Yu, M.D.[3]
Dimitrios N. Melegos, B.Sc.[1]
Steinar Lundgren, M.D.[4]
Per E. Lonning, M.D.[2]

[1] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

[2] Department of Oncology, Haukeland Hospital, University of Bergen, Bergen, Norway.

[3] Department of Medicine, Section for Cancer Prevention, Louisiana State University Medical School, Shreveport, Louisiana.

[4] Department of Oncology, Trondheim University Hospital, Trondheim, Norway.

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Address for reprints: Eleftherios P. Diamandis, M.D., Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5.

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BACKGROUND. Prostate specific antigen (PSA) is an established tumor marker of prostate adenocarcinoma that recently also was found in breast tumors. Minute amounts of PSA are found in female plasma. It is known from cell culture studies that PSA expression can be up-regulated by androgens and progestins but not estrogens. In this study, the authors examined whether plasma PSA in women with breast carcinoma changes after the therapeutic administration of the progestin megestrol acetate (MA) and whether these changes have any prognostic value.

METHODS. Plasma PSA was measured by a highly sensitive immunofluorometric procedure that can measure within 1 ng/L of PSA. Serial plasma levels from women with metastatic breast carcinoma who received either MA (N = 52) or other treatments (N = 24) were evaluated. PSA changes in plasma were correlated with patient outcomes.

RESULTS. The study found that approximately 50% of the patients receiving MA had a significant increase in their plasma PSA concentration after the treatment and that this increase was rapid (starting within 1 week) and dose-dependent. PSA levels declined when treatment was withdrawn. Further comparisons with similar groups of patients receiving tamoxifen or doxorubicin have shown that the plasma PSA increases are specific to the MA treatment. The plasma PSA increases reflect the stimulation of the tumor by MA to produce PSA and the secretion of PSA into the general circulation. There is a statistically significant association between the plasma PSA changes after MA treatment and overall patient survival; patients with increased plasma PSA have an approximate threefold increase in their relative risk of death during the follow-up period. Multivariate analysis has shown that the increased risk of death in this group is associated, at least in part, with the frequent presence of distant metastasis.

CONCLUSIONS. The measurement of plasma PSA after treatment with MA allows for patient classification into two groups. The group that did not demonstrate any changes in their plasma PSA level after MA treatment (approximately 50% of the patients) had a significantly better prognosis. The group that did demonstrate an increase in their plasma PSA level after MA treatment represented a subset of patients who may benefit more from MA withdrawal and the initiation of alternative regimens. However, these data need further confirmation with a larger pool of patients. ***Cancer* 1999;85:891–8.** *© 1999 American Cancer Society.*

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Prostate specific antigen (PSA) is a 33-kilodalton glycoprotein produced by the prostate gland and found in seminal plasma as well as in the serum of normal males.[1] Because of its tissue specificity, serum PSA is a valuable biochemical marker for the detection and

management of patients with prostate carcinoma.[2] Until recently PSA was believed to be produced specifically by the epithelial cells of the prostate,[3] but new data have shown that PSA is found in a number of female organs and body fluids.[4] The breast is one of the female organs capable of producing this protein. PSA is detected in breast milk,[5] breast cyst fluid,[6] breast nipple aspirate fluid,[7] and cytosolic extracts of normal breast tissues as well as tissues from fibroadenomas and breast carcinoma.[8,9] Similar to the regulation in the prostate, the expression of the PSA gene in the breast is up-regulated by androgens via androgen receptors.[10,11] Moreover, PSA gene expression in the breast also is under the influence of progestins and estrogens. Estrogens could impair the production of PSA stimulated by androgens. Progestins are able to up-regulate the production of PSA through progesterone receptors.[10,11]

Using conventional PSA assay methods, it frequently is impossible to detect PSA in female serum. PSA levels similar to those in male serum were detected in $< 5\%$ of female sera.[1,12] However, if a highly sensitive PSA assay is used, up to 50% of female sera are shown to contain trace amounts of PSA.[13] Due to the apparent lack of clinical implication, PSA in female serum has not been studied extensively. Little is known regarding the source of PSA in female serum. We studied PSA levels in breast carcinoma tissues in association with PSA concentrations in matched presurgical and postsurgical sera of breast carcinoma patients and did not find any correlation between serum PSA and breast tissue PSA.[14]

Although the source of PSA in female serum remains to be determined, accumulated evidence suggests that PSA levels in female serum are under the influence of steroids, especially androgens and progestins. One study shows that serum PSA levels are elevated in hirsute women, a manifestation of the presence of excess androgen.[15] Serum PSA levels also are increased in pregnant women[16] and vary during the menstrual cycle after the changes of progesterone.[17] These observations are in agreement with findings from cell culture experiments indicating that PSA production in breast cells is up-regulated by androgens or progestins.[10]

Megestrol acetate (MA) is a synthetic progestin used in hormonal therapy regimens for the treatment of breast carcinoma patients with recurrent or metastatic disease.[18] In this study, we measured PSA levels in the plasma of breast carcinoma patients before, during, and after MA treatment and examined whether plasma PSA levels undergo changes under the influence of treatment. For comparison purposes, we also included breast carcinoma patients treated with tamoxifen or doxorubicin. We then examined whether the changes in plasma PSA levels after MA treatment were associated with patient survival.

MATERIALS AND METHODS

Breast Carcinoma Patients and Blood Specimens

All patients enrolled in this study had locally advanced or metastatic breast carcinoma. During this trial, all women were treated only with one medication at a time.

To test the hypothesis that treatment with MA may affect plasma PSA levels in women with breast carcinoma, blood samples were obtained from 52 patients before the initiation of and during treatment with MA (Groups 1, 2, and 3). In addition, another three groups of patients also were included for comparison.

Group 1

A total of 16 patients received MA in escalating doses from 40–160 mg daily as part of a study evaluating the influence of MA, administered in different doses, on endocrine parameters.[18] These patients received MA in doses of 40, 80, 120, and 160 mg daily for 4 weeks per dose and then continued on MA, 160 mg daily, until the time of progressive disease. At this time, 8 patients had a dose of MA further escalated to 320 mg and, after 4 weeks, to 800 mg daily. Blood samples were obtained prior to initiation of therapy and at the end of each 4-week dose treatment period.

Group 2

Fourteen patients received MA, 160 mg daily, from the initiation of therapy as part of a study protocol evaluating time-dependence of alteration in insulin-like growth factor parameters in relation to MA treatment. These patients had blood samples obtained prior to the initiation of treatment and after 3 days, 1 week, 2 weeks, 1 month, and 2 months of treatment.

Group 3

Twenty-two patients treated routinely with MA, 160 mg daily, had blood samples obtained prior to and after different time intervals of therapy. To evaluate the influence of MA treatment on PSA levels, samples obtained during treatment were compared with pretreatment values. In addition, serial samples obtained in Groups 1 and 2 allowed evaluation of the influence of time on treatment and MA dose on PSA.

Group 4

To test the reversibility of possible changes in PSA during treatment, 14 patients progressing while receiving MA treatment had fasting blood samples ob-

tained on the last day of therapy and subsequently ≥ 4 weeks after terminating MA treatment, before the initiation of new systemic therapy.

Group 5

To test for possible influences of other types of hormonal treatment on PSA levels, 14 patients had blood samples obtained before and during treatment with tamoxifen, 30 mg daily.

Group 6

Ten patients with locally advanced breast carcinoma who were treated with the cytotoxic anthracycline doxorubicin were analyzed for plasma PSA before and after 14–16 weeks of therapy.

All blood samples were obtained in heparinized vials between 8 am and 10 am after an overnight fast. Plasma was separated by centrifugation and stored at −20°C until time of analysis.

PSA Determination

A highly sensitive, in-house PSA assay was used in this study. The detection limit was approximately 1 ng/L. The method was a time-resolved immunofluorometric assay utilizing two monoclonal antibodies against PSA. The assay has been described in detail and its performance evaluated elsewhere.[19] All plasma samples were measured in triplicate.

Statistical Analysis

Primarily, the statistical analysis was applied in two sets of comparisons. The first set compared changes in PSA between patients who were treated with MA (Groups 1–4) and those treated with tamoxifen (Group 5) or chemotherapy (Group 6). The second set focused on comparisons between patients with and without increased plasma PSA during MA treatment (Groups 1–3). A significant plasma PSA increase was defined arbitrarily as ≥ 5 ng/L above baseline value. This is a clear elevation, considering that the majority of patients had baseline values < 3 ng/L and that the assay can recognize changes ≥ 1 ng/L.

The significance of the difference between the means or medians of the variables of interest was examined using the analysis of variance or the Wilcoxon rank sum test, respectively. The differences in percentages were compared using either the chi-square test or Fisher's exact test. The Cox proportional hazards regression model was utilized to evaluate the strength of the association between serum PSA changes and the risk of death.[20] The model was developed at both univariate and multivariate levels. The difference in overall survival between patients with and without changes in plasma PSA also was demonstrated with use of Kaplan–Meier survival curves.[21]

RESULTS

We first examined whether the plasma PSA concentration changed significantly in patients who were treated with MA. Considering the detection limit of the assay used (1 ng/L), we defined that any PSA concentration > 5 ng/L over baseline clearly was a significant elevation that could not be attributed to the analytic variability of the assay. Among the 52 patients who were receiving MA and for whom we had pretreatment values, 26 had a plasma PSA elevation and 26 had no elevation after the administration of MA. Of the 26 patients who were treated with MA and had increased plasma PSA during treatment, 3 (12%) had a PSA level increase above baseline > 1,000 ng/L, 7 (27%) had a level > 100 ng/L, 10 (38%) had a level > 10 ng/L, and 6 (23%) had a level between 5–10 ng/L. Considering that the baseline values of these patients were quite low (< 1 ng/L in 68% of the patients), the plasma PSA changes observed after MA treatment were quite substantial. Using the one sample sign test we verified that the PSA changes after MA treatment were statistically highly significant ($P < 0.0001$). Using the same test, we also verified that the PSA changes after MA treatment were statistically highly significant in the subgroup of patients (N = 16) who had a pretreatment PSA level > 1 ng/L ($P < 0.01$).

In the second MA treated group (Group 2), who were given a constant dose of MA at 160 mg daily, 14 patients provided plasma samples at a very early date (2–9 days after the treatment was initiated). Of these 14 patients, 8 had increased plasma PSA and 3 of the increases were demonstrated during the first week of treatment. Figure 1 shows representative examples of patients whose plasma PSA increased with time during the treatment. In general, plasma PSA increases relatively quickly (> 30 days) and reaches a plateau that is sustained for many months. There also were 16 patients who received variable dosages, gradually increasing from 40 to 800 mg daily during the MA treatment (Group 1). To a certain extent, the PSA level in the plasma increases with increasing dosage, but the elevation was saturable with dosages ≥ 160 mg daily. Examples of patients whose plasma PSA level changed with the MA dosage are shown in Figure 2.

Group 5 was comprised of 14 patients who were treated with tamoxifen and plasma samples were collected before and after treatment. The pretreatment plasma PSA was < 1 ng/L in 64% of the patients; this value was similar to the corresponding value of Group 1. None of these patients had an increase in their plasma PSA > 5 ng/L during tamoxifen treatment. The

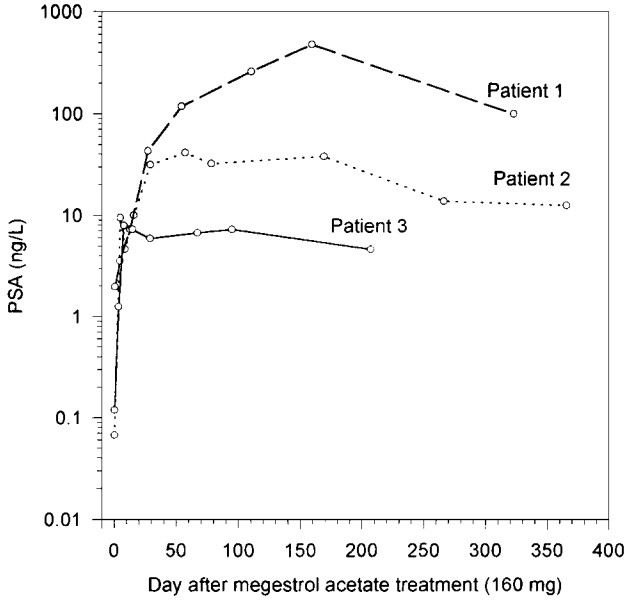

FIGURE 1. Serum prostate specific antigen in three representative patients who were treated with 160 mg daily of megestrol acetate over a period up to 1 year. Zero time represents serum PSA before the treatment was initiated. Significant PSA changes in the serum can be observed within the first week of treatment and a plateau was reached within approximately 50 days after treatment.

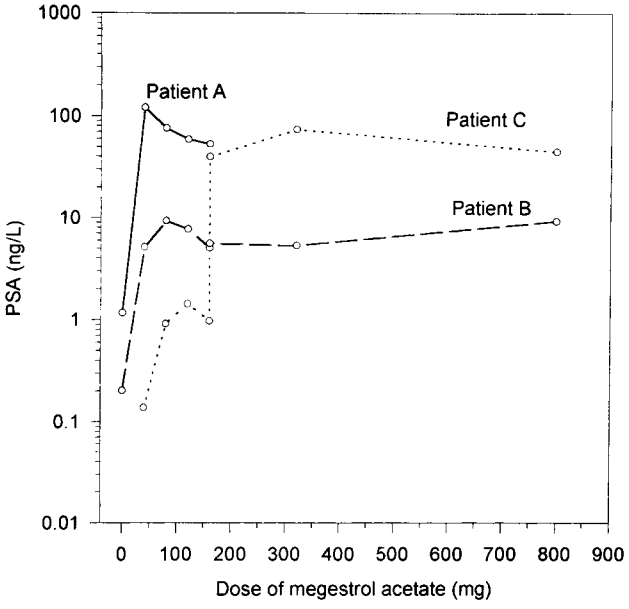

FIGURE 2. Serum prostate specific antigen (PSA) in three representative patients who were treated with progressively increasing doses of megestrol acetate daily.

difference in response between Groups 5 and 1, 2, and 3 combined was statistically highly significant ($P < 0.01$).

Of the 10 patients treated with doxorubicin (Group 6), 7 had no detectable PSA in their initial sera and 3 had initial PSA levels between 1–3 ng/L. None of the patients in this group had an increase in their serum PSA during the treatment, similar to the data with tamoxifen.

TABLE 1
Plasma PSA Levels (ng/L) during Therapy (First Sample) and after MA Withdrawal (Second Sample)

	Patient Group 4	
Patient	First sample[a]	Second sample[b]
1	38	2
2	29	8
3	0	0
4	27	27
5	1	1
6	12	1
7	4	2
8	100	47
9	0	0
10	15	0
11	1	1
12	0	0
13	5	0
14	0	0

PSA: prostate specific antigen; MA: megestrol acetate.
[a] During treatment with megestrol acetate.
[b] After treatment with megestrol acetate at 27–121 days.

To examine whether the PSA induction by MA was reversible after MA withdrawal, we measured plasma PSA in 14 patients (Group 4) who gave 1 sample during the treatment and 1 sample 27–121 days after the termination of treatment (Table 1). Of the 8 patients who had a PSA level > 1 ng/L during treatment, 7 demonstrated a $\geq 50\%$ reduction in PSA levels in their second specimen. No patient had a higher PSA level in their second specimen compared with the first sample. The trend for PSA reduction after MA withdrawal was highly significant ($P < 0.01$ by the Wilcoxon rank sum test).

Patient Survival and Increased Plasma PSA after MA Treatment

Among the 52 patients who were treated with MA (Groups 1, 2, and 3), 26 did not show any substantial changes in their plasma PSA whereas the remaining 26 did. We compared some clinical features between the patients who did or did not have increased plasma PSA during the MA treatment. The 2 subgroups did not show any significant difference in their mean ages (58 years vs. 68 years; $P = 0.96$), median days of serum collection during treatment (171 days vs. 184 days; $P = 0.92$), and the median follow-up (18 months vs. 24 months; $P = 0.06$). There also was no difference in

TABLE 2
Association between Death and Features of Patients Treated with Megestrol Acetate

Features	No.	RR[a]	95% CI	*P* value
Serum PSA increase after treatment (> 5 ng/L)				
No	26	1.00		
Yes	26	2.89	1.13–7.37	0.026
Initial serum PSA				
< 1 ng/L	35	1.00		
≥ 1 ng/L	16	1.22	0.47–3.12	0.69
Age group				
< 70 yrs	27	1.00		
≥ 70 yrs	24	0.82	0.34–1.97	0.66
Days of serum collection after treatment				
< 150	24	1.00		
≥ 150	27	0.59	0.25–1.39	0.27
ER status				
< 10 fmol/mg	12	1.00		
≥ 10 fmol/mg	30	2.07	0.59–7.22	0.25
PR status				
< 10 fmol/mg	22	1.00		
≥ 10 fmol/mg	20	1.12	0.44–2.85	0.81
Metastatic sites				
Local	23	1.00		
Distant	15	1.68	0.61–4.62	0.32
Both	12	2.18	0.76–6.24	0.15
Response to treatment				
Stable disease	33	1.00		
Partial response	2	5.57	0.65–47.9	0.12
Progressive disease	14	6.04	2.41–15.2	< 0.001

RR: relative risk; 95% CI: 95% confidence interval; PSA: prostate specific antigen; ER: estrogen receptor; PR: progesterone receptor.
[a] The relative risk was calculated using the Cox proportional hazards regression model.

steroid receptor positivity between the two subgroups (80% vs. 65% [$P = 0.33$] for ER and 57% vs. 46% [$P = 0.55$] for PR).

More patients in the increased PSA group had distant metastases or both distant metastases and local recurrence compared with the stable PSA group, although the difference did not reach statistical significance (69% vs. 44%; $P = 0.07$). Patients in the increased PSA group also tended to respond poorly to the MA treatment compared with those in the stable PSA group, but the difference was not statistically significant (33% vs. 19%; $P = 0.13$). However, the death rates between the 2 subgroups were significantly different (58% in the increased PSA group vs. 28% in the stable PSA group; $P = 0.03$).

Table 2 shows the results of the survival analysis with the use of the univariate Cox proportional hazards regression model. Patients with increased serum PSA during treatment had a significantly increased risk of death compared with patients with stable serum PSA (relative risk [RR] = 2.89; $P = 0.03$). As expected, patients responding poorly to the treatment had a higher risk of death (RR = 6.04; $P < 0.001$). The risk of death was not affected by the following variables: patient age, initial PSA levels, days serum collected after the treatment, metastatic site, and ER and PR status. The difference in overall survival between patients with and without increased serum PSA after MA treatment is shown in Figure 3.

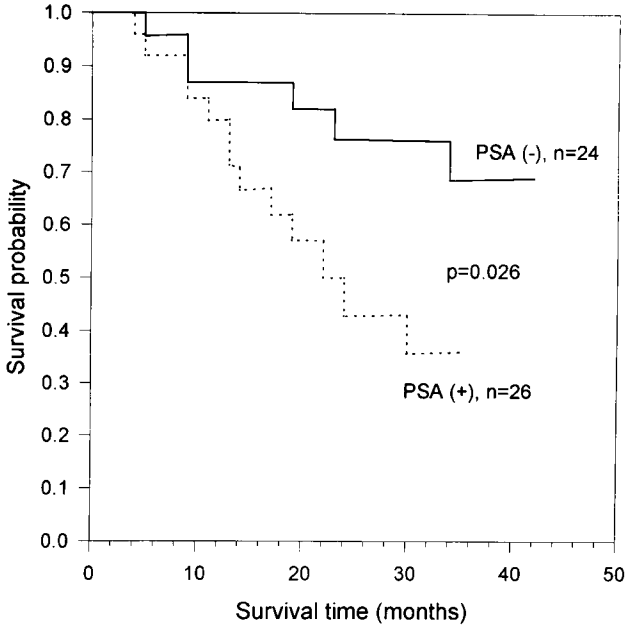

FIGURE 3. Kaplan–Meier overall survival curves in patients who did [PSA (+)] or did not [PSA (−)] have elevations in their serum prostate specific antigen (PSA) levels after megestrol acetate treatment. PSA (−) patients had improved survival.

The association between increased serum PSA posttreatment and a higher risk of death was examined further in the multivariate analysis (Table 3). It is interesting to note that, the effect of PSA on death was not statistically significant when the model was adjusted for patient age, days of serum collection after treatment, initial serum PSA, ER and PR status, and type of metastases (RR = 2.19; $P = 0.25$). This finding suggested that the association of PSA with the risk of death was affected by some of the variables in the model. By examining each variable separately, we found that the type of metastases (local vs. distant) in the model could influence the significance of the association between PSA and the risk of death. After adjusting the Cox proportional hazards regression model with this variable, either individually or in combination with other variables, the RR for PSA increase was no longer significant. However, the RR for PSA increase still was significant if the model was adjusted

TABLE 3
Association between Death and Characteristics of Patients Treated with Megestrol Acetate

Features	No.	RR[a]	95% CI	*P* value
Serum PSA increase after treatment (> 5 ng/L)[b]				
No	19	1.00		
Yes	20	2.19	0.58–8.36	0.25
Serum PSA increase after treatment (> 5 ng/L)[c]				
No	19	1.00		
Yes	20	3.44	1.08–10.99	0.037
Serum PSA increase after treatment (> 5 ng/L)[d]				
No	19	1.00		
Yes	20	2.46	0.78–7.80	0.13

RR: relative risk; 95% CI: 95% confidence interval; PSA: prostate specific antigen.

[a] The relative risk was calculated using the Cox proportional hazards regression model.

[b] The model was adjusted for patient age, days of serum collection after treatment, initial serum prostate specific antigen, estrogen receptor and progesterone receptor status, and the presence of distant metastases.

[c] The model was adjusted for patient age, days of serum collection after treatment, initial serum prostate specific antigen, and estrogen receptor and progesterone receptor status.

[d] The model was adjusted for the presence of distant metastases.

with all available variables except the type of metastases (Table 3).

We recently reported on the presence of PSA subfractions in the serum of women with breast carcinoma.[22] Four serum samples with increased PSA were fractionated on a gel filtration column using high performance liquid chromatography as previously described[19] to resolve the PSA subfractions. The predominant molecular form of PSA in these sera was PSA bound to α_1-antichymotrypsin but free PSA also was detectable at a smaller concentration in all plasmas (Fig. 4).

DISCUSSION

The objectives of this study were 1) to examine whether plasma PSA concentration changes during the treatment of patients with the synthetic progestin MA and 2) to examine whether these changes are related to patient outcomes. With regard to our first objective, we were able to provide strong evidence that the plasma PSA concentration does change substantially in a subgroup (50%) of patients who received MA. The specificity of MA to stimulate PSA production, as reflected by the elevation of plasma PSA, was supported by the following observations. 1) PSA levels in plasma increased within a few days after the initiation of treatment (Fig. 1) and were dose-dependent (Fig. 2). 2) After MA withdrawal, plasma PSA levels declined (Table 1). Although the number of patients in these groups was relatively small, the changes were quite substantial. 3) None of the patients who were treated for a similar disease but with different regimens (e.g., tamoxifen, an antiestrogen compound, or doxorubicin, an antibiotic) demonstrated any plasma PSA changes. 4) Our in vivo data are in accord with results of tissue culture experiments that have shown that synthetic progestins are strong stimulators of PSA production by breast carcinoma cell lines.[11] The presented data allow us to speculate that MA induces breast tumor tissue to produce PSA, which then is secreted into the general circulation. In some patients, the stimulation increased plasma PSA by factors as high as 1000-fold over baseline and the concentration reached levels observed in healthy males. These PSA levels persisted for months if therapy was continued.

PSA production by prostatic and breast tissues and cell lines is a steroid hormone receptor-mediated event. In this series, we did not observe any significant association between PSA in plasma and ER or PR positivity in the breast tumors. Moreover, only 50% of patients responded with a plasma PSA increase and among the patients who responded, some were ER negative and PR negative. We have observed such discrepancies between receptor levels and PSA production in both breast carcinoma cell lines and breast tumors.[11,23] We speculate that the steroid hormone receptor system in such cell lines and breast tumors is severely deranged and we recently provided evidence that among the possible explanations of this phenomenon is the high mutability of the 5′-promoter region of the PSA gene, including the androgen response element.[24,25]

Our clinical correlations have indicated that the group of patients who had increases in their plasma PSA while receiving MA treatment have an unfavorable outcome and shortened overall survival (Tables 2

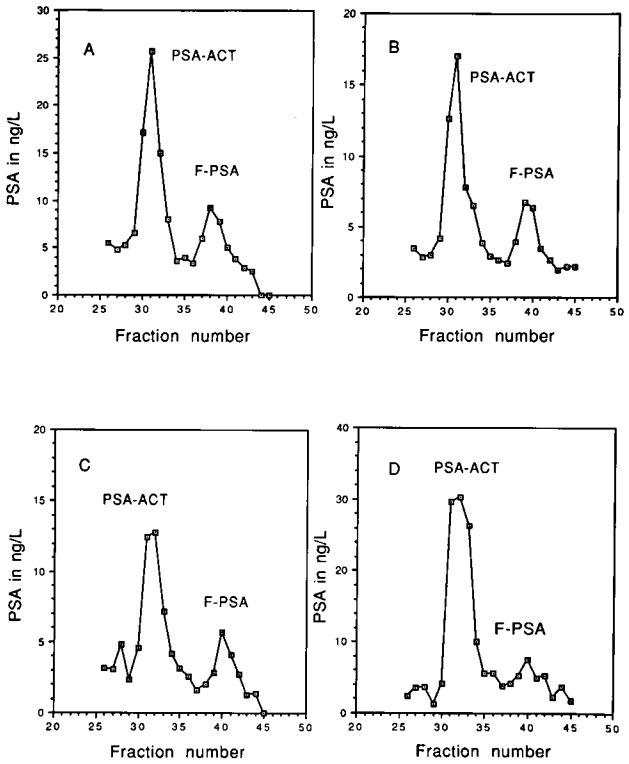

FIGURE 4. Molecular forms of prostate specific antigen (PSA) in serum of four patients who received MA treatment. PSA-ACT: PSA bound to α_1-antichymotrypsin (approximately 100,000 Mr). F-PSA: free PSA (approximately 30,000 Mr).

and 3) (Fig. 3). Multivariate analysis has suggested that, at least in part, this finding relates to the higher incidence of distant metastases in the patients who had an increase in their PSA. Also, it is possible that other factors that are co-up-regulated by the synthetic progestin in addition to PSA (e.g., other proteolytic enzymes or growth factors), may affect patient outcome adversely. A further speculation includes PSA-mediated activation of cytokines such as transforming growth factor-β[26] or PSA-mediated proteolysis on insulin-like growth factor binding proteins[27] for release of mitogenic growth factors. However, these suggestions need further investigation.

Our findings may have value in assessing whether MA treatment in these patients is worthwhile. If appears that the subgroup of patients whose plasma PSA increases after MA treatment are at increased risk of death and that an alternative treatment may be more beneficial in these patients.

With regard to the nature of the PSA subfractions in the plasma of these women, it is clear that the majority of PSA is bound to the proteinase inhibitor α_1-antichymotrypsin, whereas a small proportion is free PSA. These subfractions are similar to those found in male serum.

We demonstrated that MA treatment induces PSA production in approximately 50% of breast carcinoma patients and that this induction, identified by measuring plasma PSA, is associated with increased patient mortality. The mechanism by which PSA stimulation is associated with increased risk for death in these patients currently is obscure. Because our study is based on a small number of patients, larger studies are necessary before this original observation can be used to predict the therapeutic success of MA treatment.

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