

LUMINOIMMUNOMETRIC ASSAY OF TISSUE POLYPEPTIDE ANTIGEN (TPA) AND CANCER ANTIGEN 125 (CA-125) IN BREAST CANCER PATIENTS

O. EL-AHMADY, A.-B. HALIM AND S. EISSA*

*Tumor Marker Oncology Research Unit, Al-Azhar University and *Pathology Department, NCI, Cairo, Egypt.*

SUMMARY

Serum TPA and CA-125 were determined in 86 individuals (66 with breast cancer representing the different stages and grades of the disease and 20 normal healthy controls). TPA and CA-125 were estimated using the LIA reagents supplied by BYK Sangtec.

TPA showed sensitivity rates of 31.8%, 42.4% and 51.5% while CA-125 showed sensitivities of 16.3%, 18.6% and 25.6% at specificity levels of 100%, 95% and 90% respectively. Combined determination of the two markers resulted in some improvement in sensitivity. For follow-up of breast cancer patients after surgery both markers were of value and showed near-identical patterns.

KEY WORDS Breast cancer TPA CA-125

INTRODUCTION

Breast cancer is the most common female malignancy of mid-life and is a leading cause of deaths among women (Omar and Contesso, 1988). Detection of localised breast cancer with negative nodes leads to 87% five years survival rate while, according to the data from the American Cancer Society (1984), if nodes are involved survival rate drops to 47% or less. No single physical technique for detecting metastasis such as radiology, ultrasound or isotope scanning proved to be 100% accurate. Attempts have been made to evaluate biological markers in blood and tissues in order to follow the course of disease during therapy, detect recurrence and possibly serve as a clue in early diagnosis (Shaarawy *et al.*, 1990)

TPA is an unbranched peptide chain with an apparent molecular weight of 4.3×10^4 with an electrophoretic mobility close to that of beta-2-globulin, IP of 4.5 and sedimentation constant of 4.5S (Björklund and Björklund, 1983). CA-125 is an antigenic determinant on a high-molecular-weight glycoprotein recognized by a monoclonal antibody which was raised using an ovarian cancer cell line as immunogen (Jacobs and Bast, 1989). It has been reported that TPA is the marker with the highest applicability in malignancies in general (Lüthgens and Schlegel, 1987). CA-125 has been established as a useful tool in diagnosis and monitoring of patients with ovarian carcinoma (Bast *et al.*, 1983). It has also been evaluated in patients with non-gynecological cancer including

Correspondence to: Omar El-Ahmady, Ph.D., Head of Tumor Marker Oncology Research Unit, Al-Azhar University, 2-Roshdy Street, Safeer Square, Heliopolis, Cairo, Egypt.

carcinoma of breast, pancreas, colon and lung but little attention has been given to its value in breast cancer (Canney *et al.*, 1984; Kawahara *et al.*, 1986). The lack of individual cancer marker tests with satisfactory sensitivity and specificity has led to investigation of the combined discriminatory power of several cancer markers (Pohl, 1992).

Luminometric assay (LIA) has been introduced recently into the field of clinical biochemistry. The availability of simple, reliable instrumentation for chemiluminescence measurement together with a wide range of assays offers a logical alternative to traditional radio-immunoassay (RIA) which has the drawbacks of short shelf-life and disposal problems (Woodhead and Weeks, 1989; Dudley, 1990).

This study was conducted to evaluate concurrent determination of CA-125 and TPA in breast cancer by the LIA technique.

MATERIALS AND METHODS

Eighty six females were included in the present study classified into two groups:

- I- Breast cancer group: Sixty six females aging 30-70 years were diagnosed at National Cancer Institute (NCI) in Cairo. Histopathological examination of the tumor tissues revealed the following classes:
 - 1- Type: Invasive ductal carcinoma (IDC) (n = 62), invasive lobular carcinoma (ILC) (n = 2), intraduct papillary carcinoma (n = 1) and adenosquamous carcinoma (n = 1).
 - 2- Stage: T1 (n = 2) TII (n = 19), TIII (n = 35) and TIV (n = 10).
 - 3- Grade: G1 (n = 3), GII (n = 41), GIII (n = 22).
- II- Normal healthy control group: Twenty females with the same range of ages as the cancer group.

Serum TPA and CA-125 were determined using the chemiluminescence reagents supplied by Byk-Sangtec Diagnostica, Germany (LIA-mat). LIA-mat assays are non-competitive techniques using two different monoclonal antibodies: the first one is coated onto the inner surface of the test tube and the other (tracer) is covalently linked to an aminobutylethylisoluminol derivative. After the addition of standards or specimens to be analyzed to the coated tube, incubation and washing to remove unbound antigen (TPA or CA-125), the labelled antibody is added to detect a specific epitope on the antigen. After the second wash, the tube is introduced into the Optocomp I Luminometer which automatically injects alkaline peroxide solution and then the catalyst (microperoxidase) solution. On the addition of the catalyst luminol is oxidized and emits light (Dudley, 1990). The luminescence is measured for 5 seconds and the intensity is proportional to the concentration of antigen.

RESULTS

Both TPA and CA-125 were significantly elevated in the breast cancer group compared to the normal control group (Table 1), but TPA showed higher sensitivity values than CA-125 as a diagnostic tool in breast cancer. It is clear that combined determination of TPA and CA-125 improved sensitivity (Table 2).

Table 1. Serum TPA and CA-125 in breast cancer and control groups.

Markers	TPA (U/l)		CA-125 (IU/ml)	
	Cancer (n=66)	Control (n=20)	Cancer (n=66)	Control (n=20)
Mean \pm SE	*165.5 \pm 54.9	35.1 \pm 5.4	**38.7 \pm 9.1	22 \pm 1.4
Range	37-2380	8-110	17.2-408	9.4-49.4

* Significant difference from control group at $p = 0.001$.** Significant difference from control group at $p = 0.05$

Table 2. Cutt-off values, sensitivity rates of TPA, CA-125 and combined markers at different specificity levels.

Specificity %		100	95	90
TPA:	Cut-off (U/L)	110	70	60
	Sensitivity %	31.8	42.4	51.5
CA-125:	Cut-off (IU/ml)	50	40	30
	Sensitivity %	16.6	18.2	25.8
Sensitivity of combined markers		40.9	47.0	57.6

Table 3. Serum TPA in the different stages and grades of breast cancer.

TPA	Stages				Grades		
	I (n=2)	II (n=19)	III (n=35)	IV (n=10)	I (n=3)	II (n=41)	III (n=22)
Mean \pm SE (U/l)	55.1 \pm 4.2	*87.1 \pm 13.9	222 \pm 115	174 \pm 59	58.8 \pm 0.4	99 \pm 15.7	308 \pm 208
Range (U/l)	50.9-59.2	41-157	36.6-2380	29.7-596	58.4-59.2	41-252	36.6-2380
Sensitivity at 100% specificity	0%	36.8%	40%	50%	0%	34.1%	36%

* Significant difference from T_i at $p = 0.05$.

Table 4. Serum CA-125 in the different stages and grades of breast cancer.

CA-125	Stages				Grades		
	I (n=2)	II (n=19)	III (n=35)	IV (n=10)	I (n=3)	II (n=41)	III (n=22)
Mean \pm SE (IU/ml)	27.6 \pm 0.6	24.6 \pm 3.3	49.7 \pm 19.2	35.9 \pm 6.3	30.5 \pm 3.5	25.1 \pm 2	69.9 \pm 34.2
Range (IU/ml)	27-28.1	17.2-56	16.9-408	19.5-73.3	27-34	16.9-56	18.3-408
Sensitivity at 100% specificity	0%	10.5%	14.3%	30%	0%	4.9%	36%

No correlation was obtained between the mean values of TPA and stages of breast cancer but non-significant correlation was seen with the grades of disease. TPA exhibited high sensitivity in late stages of the disease and none of the two patients with stage I or the three patients with grade I breast cancer showed TPA above the cut-off value (Table 3). Although the mean values of CA-125 showed no correlation with either stages or grades of breast cancer, sensitivity rates correlated well (Table 4).

For the follow-up of breast cancer patients after surgery and during chemotherapy with cyclophosphamide, methotrexate and fluorouracil (CMF), serial determinations of TPA and CA-125 showed similar patterns (figures 1-4). It is noted that TPA changed more rapidly than CA-125. In case (2) TPA levels showed marked reduction shortly after surgery while CA-125 declined slowly. In the same case TPA increased sharply on cessation of chemotherapy but CA-125 showed no change. In case (3) TPA began to increase 4 weeks before CA-125. In case (4) TPA level decreased 2 weeks after surgery while CA-125 showed no change till the 5th week.

DISCUSSION

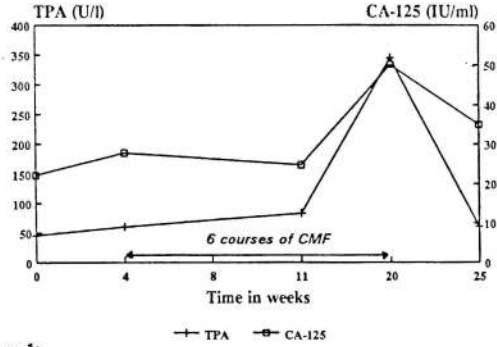
The exposed site of the breast permits a diagnosis of tumor by palpation even at a tumor size of T1, whereas a microcarcinoma is seldom recognized exclusively by mammography. In primary breast cancer, the pre-operative cancer estimation serves for completion of the pathogram which comprises data on tumor size, lymph node involvement and receptor status (Schlegel and Lüthgens, 1989).

The present study revealed sensitivity rates of 31.8, 42.4 and 51.5% for TPA in breast cancer an specificity levels of 100, 95 and 90% respectively. These sensitivity values are similar to those obtained by RIA where Kreienberg *et al.* (1983); Schmidt-Rode and Schulz (1984) recorded sensitivities of 45% and 40% respectively. CA-125 has been shown to be a useful tool in diagnosis and follow-up of patients with ovarian carcinoma. It showed sensitivity as high as 85% (Björklund and Björklund, 1983; Bast *et al.*, 1983; Kabawat *et al.*, 1983). In breast cancer, much lower sensitivity was reported by El-Sayed *et al.* (1991) who found 10% and 40% positivity for tissue and serum CA-125 respectively. In the present work, CA 125 exhibited positivity rates about 50% of those for TPA. Eissa *et al.* in 1992 found that 48.9% of breast cancer patients have serum CA-125 above 31 IU/ml.

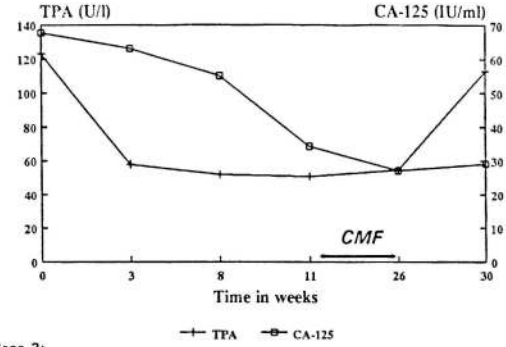
To improve the rather low diagnostic sensitivities of single marker determination, multiple combination of biochemical markers can be used (Pohl, 1990). Our results revealed that combined determination of TPA and CA-125 was superior to the individual marker analysis. At 100% specificity we obtained a sensitivity of 40.9% by combined determination compared to 31.8% and 16.6% for TPA and CA-125 respectively.

Although the mean values of TPA or CA-125 were not found to correlate with stages or grades of breast cancer except the non-significant correlation between TPA and grades, we found that sensitivities of the markers increased as we proceed from low to high stages or grades. In 1988, El-Ahmady and Abou-Shaloc found significant correlation between TPA and stages of breast cancer. Other studies reported that rates of positivity of TPA in breast cancer gradually increase from 0-30% in stage I to 66-90% in stage IV (Schmidt-Rhode and Schulz, 1984; Madeddu *et al.*, 1985; Mross and Bandlow, 1986). El-Sayed *et al.* (1991) found no relation between either tissue CA-125 or serum CA-125 and stages or grades of breast cancer, in agreement with the findings

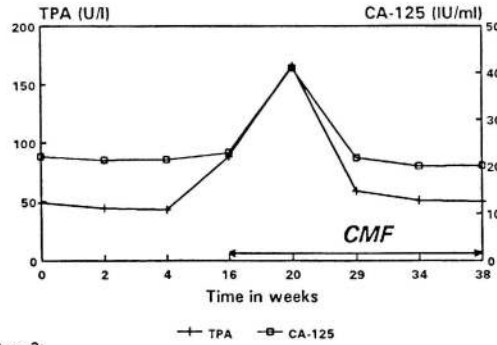
TPA & CA 125 in the follow-up of breast cancer patients after surgery



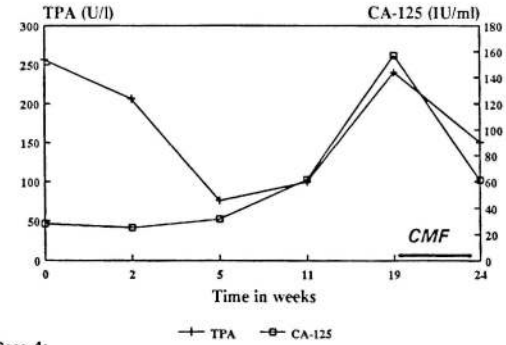
Case 1:
48 y old with IDC, T2, G3, L. N. 0



Case 2:
55 y old with IDC, T3, G3, L. N. 10/14



Case 3:
45 y old with IDC, T3, G2, L. N. 4/5



Case 4:
30 y old with ILC, T3, G2, L. N. 10/14

of other workers (Eissa *et al.*, 1992). In ovarian cancer, no significant correlation was found between serum CA-125 and stage of the disease but stages II, III and IV showed sensitivity over 90% while stage I showed sensitivity of only 50% (Björklund and Björklund, 1983). Zanaboni *et al.* (1987) recorded elevation of serum CA-125 in 42.9%, 93.1% and 83.3% in grades I, II and III ovarian carcinoma respectively.

Mammary carcinoma can metastasize to various regions of the body. Serum TPA shows a sensitivity of 77% for detection of metastases in various organs. Of 114 patients with recurrence of breast cancer, 49% were detected by the patients themselves and confirmed by the physicians, 40.5% were diagnosed by conventional measures such as palpation, X-ray, scintigraphy or CAT scan while the remaining 10.5% were detected only by TPA and CEA (Schlegel and Lüthgens, 1989). Nicolini *et al.* (1985) reported that in metastatic breast cancer patients, constant elevation or progressive increase in serum TPA and/or CEA (carcinoembryonic antigen) levels were the first pathologic findings of relapse. Shaarawy and Omar in 1989 concluded that, in general, TPA increases in progressive disease and falls again following successful therapy. It was suggested that serum CA-125 could be of potential utility in immediate post-surgical monitoring of breast cancer patients (El-Sayed *et al.*, 1991). An accuracy of 67% was obtained for CA-125 in the follow-up of ovarian cancer as indicated by second look laparotomy. It was stated that a persistently rising serum CA-125 level is consistently associated with progression of disease and frequently evident several months prior to clinical evidence of progression (Björklund and Björklund, 1983). Our results indicate that TPA and CA-125 have similar patterns and both are in concordance with the clinical state of the patients. Elevated marker levels decrease after surgery, remain stable in clinically stable cases, begin to rise before clinical detection of metastasis and decline following successful chemotherapy. However TPA was faster in its reaction than CA-125. Bremer *et al.* (1992) showed that TPS (tissue polypeptide specific antigen), which is one of the specific epitope of TPA, reacts faster than cancer antigen 15-3 (CA15-3) and mammary carcinoma antigen (MCA) during monitoring of chemotherapeutic treatment of breast cancer. Other workers (van Dalen, 1992) obtained the similar findings on comparing TPS, CA 15-3 and CEA. These results were explained by the concept that TPS, as a proliferative marker, is related to tumor growth while the other markers reflect tumor mass. Rapid tumor growth is manifested before the tumor mass is affected.

In conclusion, we agree with Schlegel and Lüthgens (1989) that analysis of markers can serve as a valuable non-invasive method supporting other diagnostic measures and in surveillance of the course of the disease.

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