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Utility of Serum Activity of Angiotensin-Converting Enzyme as a Tumor Marker

Key Words
Angiotensin-converting enzyme (ACE)
Seric angiotensin-converting enzyme (SACE)
Cancer marker

Abstract
The objective of this study was to evaluate the diagnostic utility of the measurement of the serum activity of angiotensin-converting enzyme (SACE) as a cancer marker. This case-control study included 135 patients with cancer of different sites, confirmed histologically, and 145 controls (107 normal individuals plus 38 chronically ill patients with nontumoral diseases). Determination of SACE activity was done by a spectophotometric method using as substrate the synthetic tripeptide N-(3-[2-furyl]acryloyl)-*L*-phenylalanylglycine. There were no sex- or age-related variations in SACE activity. Mean SACE activity in 107 normal controls was 51.6 U/l (95% C.I., 50.1–53.1); in 145 nontumoral individuals, including 38 chronic nonmalignant diseases plus 107 normal controls, 51.5 (50.1–53.1) and in malignant tumors 35.7 (32.8–38.5). There was no statistically significant difference between chronic diseases and normal controls ($p > 0.05$); but there was one between cancer patients and nontumoral individuals, normals and chronic nontumoral diseases. The mean of SACE activity values by tumoral site are (U/l; 95% C.I.): breast, 41.3 (36.2–46.5); gastrointestinal, 31.5 (24.3–38.8); head and neck, 32.3 (26.7–37.8), and lung 27.6 (21.6–33.6) ($p < 0.001$). The means by clinical stage are: complete remission, 58.0 (53.7–62.3), significantly higher than in normal controls ($p < 0.001$); local disease, 40.56 (34.5–46.5); locoregional disease, 35.09 (30.7–39.4); metastatic disease, 23.04 (19.5–26.5), and in relapse at diverse stages, 30.86 (25.1–36.5). In clinical active cases, there is a statistically significant decrease of SACE activity, especially in metastatic disease ($p < 0.001$). The calculated cutoff value, excluding complete remission cases, is 40.7 U/l, with sensitivity of 69.5% and specificity of 91.6%. We conclude that there is a decrease of SACE activity in cases of clinically active cancer and an increase in clinical complete remission.

Introduction

Serum angiotensin-converting enzyme (SACE) activity has been described to be increased in pulmonary sarcoidosis and pulmonary embolism and decreased in lung cancer [1]. It is a protease of pulmonary endothelial origin, and Watanabe et al. [2] have shown that the decrease in serum of the ACE activity during endothelial injury reflects the impairment of vascular endothelial cells. Calabro et al. [3] have investigated the increase of

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ACE activity in silicotuberculosis and concluded that this increase might have been caused by a numerical or functional enhancement of the macrophages, which produce ACE and play an important role in the pathogenesis of diseases. For Sandstrom et al. [4], it is just an inflammatory mediator. Nussberger et al. [5] have shown the limited value of conventional ACE activity measurements in vitro, since plasma ACE activity is not correlated to the fall in blood pressure induced by ACE inhibitors being related to the substrate employed [5]. Gorski et al. [6] have concluded that the measurement of ACE activity in vitro with N-(3-[2-furyl]acrolyl)-*L*-phenylalanyl-glycine (FAPGG) as substrate provides a reliable measure of changes in the conversion of angiotensin I to angiotensin II in vivo during therapy with converting enzyme inhibitors.

The objective of the current investigation is to evaluate the diagnostic utility of the determination of levels of SACE for positive diagnosis and the monitoring of malignant tumors, as a cancer marker.

Materials and Methods

A sample of 135 patients, with cancer of different sites, and 145 controls (107 normal individuals plus 38 chronically ill patients with nontumoral diseases) was evaluated. The calculated minimum sample size for a fiability of 95% are 15 cases, and this figure was the limit for stratification analysis.

Details of cancer and noncancer patients included in this study are summarized in table 1. Included in the current investigation were only those cancer patients with histological verification of diagnosis who had received neither treatment during the last year or ever for their cancer nor ACE inhibitors. The consecutive sampling of cancer patients resulted in that the cases included in this study at random were in different clinical stages. The evaluation of the clinical stage of the disease was made with physical examination, CT scan, ultrasound, nuclear imaging and surgical examinations, and all cases were classified as follows: (1) local disease: the tumoral mass was limited to the organ of origin with only local involvement with neither nodal nor vascular spread; (2) localized regional disease: there was local spread of tumor into surrounding tissue, fixation to deeper structures, bone invasion and first-stage lymph nodes; (3) metastatic disease: evidence of distant metastases beyond the local site or organ; (4) complete remission: no evidence of tumor; and (5) relapse: clinical relapse of disease after months or years of complete remission.

Early in the morning, fasting blood samples were collected from the three groups of individuals (cancer patients, normal controls and controls of other chronic diseases). The sera were kept at –20 °C until analyzed.

To eliminate differential bias, the values of SACE activity were determined by the observer with blinded duplicates of cases and controls. The SACE activity was determined twice in a single sample, and its value always refers to the mean of the two measurements in that clinical stage.

Table 1. Sample characteristics

	Total n	Males n	Females n	Mean age (range)
Controls				
Normal individuals	107	23	84	45 (13–72)
Chronic diseases	38	26	12	49 (23–83)
COPD	18	13	5	
Peptic ulcer	11	9	2	
Chronic hepatopathy	9	4	5	
Cancer patients	135	70	65	47 (20–87)
Breast	40	0	40	
Lung	24	24	0	
Head and neck	25	24	1	
Gastrointestinal	19	9	10	

COPD = Chronic obstructive pulmonary disease.

Determination of SACE activity was done by a spectrophotometric method using as substrate the synthetic tripeptide FAPGG, supplied by Sigma Laboratories [7]. Statistical methods used to evaluate quantitative results were Student's t test, the Mann-Whitney U test and Pearson's correlation test [8]. Estimation and confidence intervals were calculated following the guidelines of Gardner and Altman [9]. The normal limits of a serum value were established by their mean values in normal controls ±2 SD and were considered a cancer-positive SACE test in cases with values below mean –2 SD. For dichotomous results (positive/negative test), sensitivity, specificity, diagnostic weights and the receiver operating characteristics (ROC) curves were used [10]. A cutoff value for disease confirmation was estimated by the procedure described by Strike [11], with the criteria of a prior probability of disease of 0.50. The sensitivity and specificity were calculated by a more exact approach that uses the normal distribution functions fitted to the healthy and disease sample data [11].

Results

Results by Sex and Age. In normal controls, there was no statistically significant difference between males and females ($p > 0.05$) (table 2).

Regarding age, there was no significant correlation between age and levels of SACE activity in either normal controls ($r = 0.04$) or in cancer patients ($r = 0.05$).

SACE Activity. The mean values of SACE activity in cancer patients and controls (normal individuals and chronic nontumoral patients) are shown in table 2. The difference between the means of serum values in 107 normal controls (51.6) and in 38 chronic nonmalignant diseases (51.4) is not statistically significant ($p > 0.05$); but the difference between the mean of each one and the mean

Table 2. SACE activity by cancer site

	n	Mean values SD ± U/l	C.I.
Cancer patients	135	35.7 ± 16.7	32.8–38.5
Breast	40	41.3 ± 16.7	36.2–46.5
Head and Neck	25	32.3 ± 14.7	26.7–37.8
Gastrointestinal	19	31.5 ± 16.1	24.3–38.8
Lung	24	27.6 ± 14.9	21.6–33.6
Chronic nontumoral diseases	38	51.4 ± 11.3	47.8–55.0
Pulmonary chronic disease	18	49.2 ± 11.4	43.9–54.4
Peptic ulcer	11	55.9 ± 11	49.4–62.4
Liver chronic disease	9	51.6 ± 10.9	44.3–58.7
Controls			
Normal individuals	107	51.6 ± 7.91	50.1–53.1
Males	23	53.8	50.7–56.8
Females	84	50.4	48.9–51.9

Table 3. SACE activity by clinical stage

Clinical stage	n	Trend	Mean values SD ± U/l	C.I.
Normal controls	107	→	51.6 ± 7.91	50.1–53.1
Local disease	24	↓	40.5 ± 14.9	34.5–46.5
Locoregional disease	37	↓	35.0 ± 13.3	30.7–39.4
Metastatic disease	39	↓	23.0 ± 11.1	19.5–26.5
Complete remission	21	↑	58.0 ± 10.0	53.7–62.3
Relapse	14	↓	30.8 ± 10.9	25.1–36.3
Clinically active cancers	114		31.5 ± 17.9	

of both as a whole (51.5) with the mean of malignant diseases (35.7) is statistically significant ($p < 0.001$).

Tumors by Site. There is a statistically significant decrease of SACE activity in all tumoral sites compared with that in nontumoral individuals ($p < 0.001$); with the values of SACE activity being significantly lower in lung cancer than in the other tumors investigated ($p < 0.001$).

Clinical Stage of Tumor. The mean values of SACE activity by the different clinical stages of tumor are shown in table 3. The mean values observed in complete clinical remission are significantly higher than those observed in normal controls ($p < 0.001$). In patients with clinically active cancer, there is in all stages a decrease of the SACE activity mean values that is significantly lower than that in normal controls, and, in metastatic disease, it is statistically lower than that in any other stage ($p < 0.001$) (fig. 1).

ROC Curve. The ROC curce of diagnostic performance of SACE activity is shown in figure 2, and it is a curve that fits, after Weinstein et al. [10], with the pattern of a good diagnostic test.

Cutoff Value. In the complete sample of cancer patients, we estimated a cutoff value of 41.5 U/l for predicting cancer. Using normal distribution functions fitted to the healthy and disease sample data, the sensitivity of the test is 63.1%, the specificity 89.8%, the false-positive rate 36.9%, the false-negative rate 10.2% [11], the positive diagnostic weight (W+) 1.83 and the negative diagnostic weight (W–) –0.89.

When the cases of complete remission are excluded, the new cutoff value calculated is 40.7 U/l, in which case the sensitivity is 69.6%, the specificity 91.6%, the false-positive rate 30.4%, the false-negative rate 8.4%, W+ 2.11 and W– 1.10.

Discussion

In normal controls, there were no sex- or age-related variations of SACE activity, as Salzer-Muhar et al. [12] have previously described, and, in cancer patients, the variations by sex are related to tumor site.

The decreased values of SACE in malignant tumors discriminate between cancer and noncancer patients, including normal controls and chronically ill patients. It can be a useful cancer marker in solid tumors with an acceptable sensitivity and a very good specificity. The actual sensitivity and specificity are those calculated with the normal distribution functions fitted to the healthy and disease sample data, excluding from the evaluation the cases of clinical complete remission, because of their specific differences with the clinically active cancer, and, in this case, the true cutoff value predictor of cancer diagnosis is 40.7 U/l and the actual sensitivity is 69.6% and the specificity 91.6%.

This cutoff value discriminates well the mean (± C.I.) of cases with either locoregional disease, metastases or relapses and the entire sample of cancer patients that are below the cutoff value. It does the same with the normal controls and the cases of clinical complete remission that are over the cutoff, but it is of limited value in local disease (fig. 3). However, there is a statistically significant difference between the means of normal controls and cancer patients in all clinical stages, including local disease.

The ROC curve shows that the determination of SACE activity as a cancer marker has a good diagnostic performance [10].

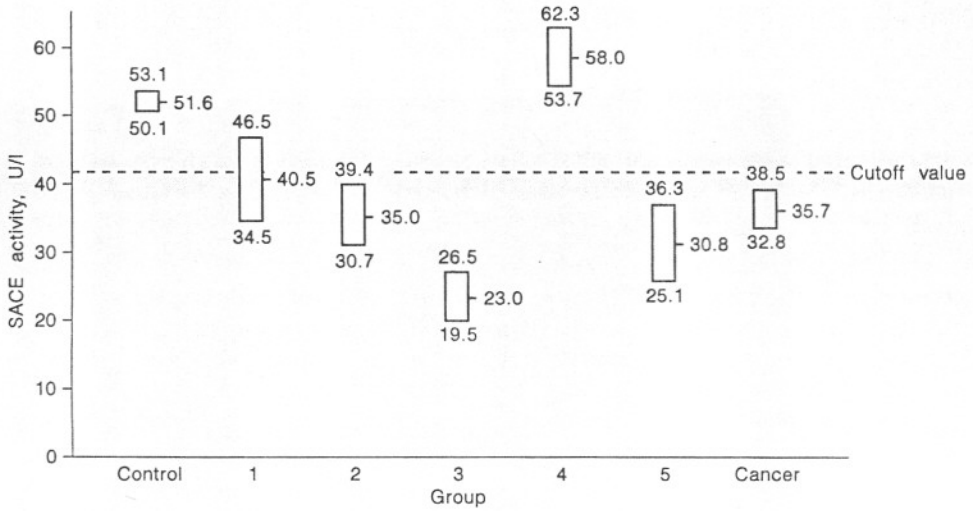

Fig. 1. SACE activity by clinical stage (mean ± C.I.). Groups: Control: disease: 1 = local; 2 = locoregional; 3 = metastatic; 4 = complete remission; 5 = relapse; cancer.

We have observed a great difference among the mean values of SACE activity in different tumors by site; but, in our opinion, this difference is more apparent than real. The mean value of SACE activity in breast carcinoma has a minimal decrease from normal values due to the disturbing effect of including cases of complete remission (with values over the normal range) and also due to the fact that many cases are cases of early local disease. The cases of lung cancer were all in advanced clinical stages (metastatic), and not one of them was in an early stage. The head and neck and gastrointestinal tumors in our sample also were in a more advanced stage than the cases of breast carcinoma. The clinical stage of the disease is more important than the anatomical site of the tumor in the variations of SACE activity.

Schweisfurth et al. [1] have described a decrease of SACE activity in lung cancer and pulmonary metastases, but not in lymphomas; for this reason, they believe that SACE determinations seem to be of help in the differential diagnosis of lymphomas and lung cancer. Bakan et al. [13] observed the same decrease of SACE activity in lung cancer patients; but the specific activities of ACE were higher in cancerous than in normal lung tissues.

The mean values of SACE activity observed by Schweisfurth et al. [1] were lower than our values due to the use of a different substrate; but the relative variations have the same trend in both cases. Gorski et al. [16] have

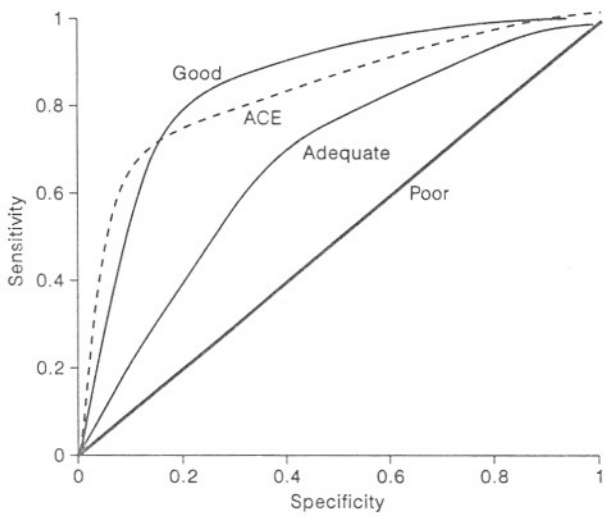

Fig. 2. ROC curve for ACE.

done a comparative evaluation of the procedure using FAPGG as substrate and concluded that it was the best one.

In chronic inflammatory diseases (e.g. pulmonary sarcoidosis, silicotuberculosis), there is an increase of SACE activity [1] that can be interpreted by its possible

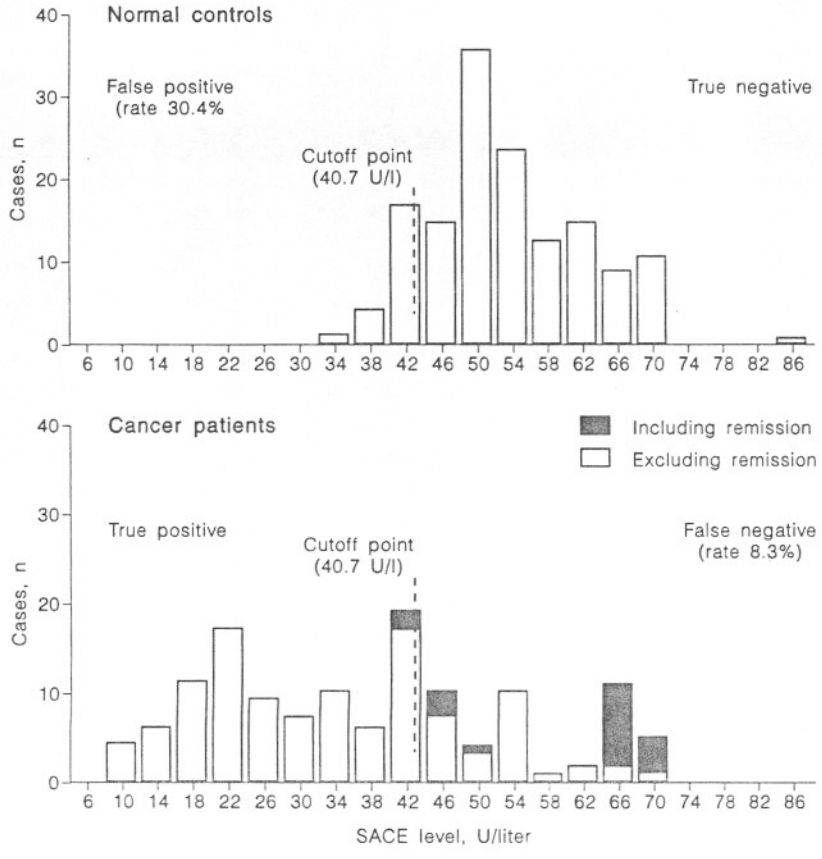

Fig. 3. Frequency distribution by SACE levels in cancer patients and in normal controls.

role as an inflammatory mediator [4], but this explanation is not valid for the decreased activity observed in cancer patients.

Our results support the hypothesis that the decreased SACE activity could be directly related to the clinical progression of the disease, because there is a low mean of SACE activity in advanced clinical stages of the disease: in metastatic disease, there is an extreme decrease (23.04) compared with that in local disease (40.56), and, in clinical relapse (30.8), there is also an extreme decrease compared with complete clinical remission (58). In this cross-sectional study, with a blinded observer, the data strongly support the hypothesis to be tested in the future that the differences among distinct clinical stages may be the result of a clinical evolution. We have analyzed as a single group the values of SACE activity in the cases that at random were in a clinical relapse and were significantly lower than that in normal controls. We believe that this decrease of SACE activity logically occurs as a consequence of the clinical evolution of the disease from complete remission to relapse, and it would indicate that SACE activity predicts relapse. However, this point must be confirmed in a prospective study.

We have no data in our investigation to make any statement on the values of SACE activity and tumoral burden.

It is necessary to remark on the observation that in the cases of clinical complete remission there is a paradoxical higher mean value of SACE activity than in normal controls. We do not have any explanation for this fact; but most of the cases in complete clinical remission were cases of breast carcinoma. If this observation of the elevation of SACE activity in clinical complete remission were confirmed in a prospective study, it would be able to open a new possible utility of SACE activity, as cancer marker, for monitoring clinical cases of complete remission in order to detect early relapses. Magnifying this possible clinical application, the observed divergent deviation from normality in SACE activity can be described as being (1) below normal in clinically active tumors and (2) above normal in cases of complete remission.

The observed high mean of SACE activity in cases of clinical complete remission is not due to patient age or variations of blood pressure, as others have also shown [1]. We do not know the causes of the variations of levels of SACE activity in clinically active tumors or in clinical remission, but Bell et al. [14] have shown that the inhibition of the autocrine angiotensin system with a converting-enzyme inhibitor or a receptor antagonis leads to increased expression of the protooncogene c-*src*, and this mechanism is an interesting point to be investigated in the observed decrease of SACE activity in cancer patients. On the other hand, Bakan et al. [13] observed that the decrease in SACE activity of lung cancer patients is associated with an increase of ACE activity in pulmonary tumoral tissues; if this association occurs in all tumors, it could be the explanation of SACE elevation in clinical complete remission, but this is only an hypothesis and needs to be tested.

There are diverse cancer markers for positive diagnosis of cancer, but very few have the characteristics of SACE: acceptable sensitivity, good specificity, low cost, not dangerous for patients and doctors, very easy and short time of performance, and automated reliability [6].

We have excluded from this investigation all cases previously receiving any type of medical or radiotherapeutic treatment, in order to avoid bias of unexpected secondary effects of any drug on the values of SACE activity; the adverse consequences of this exclusion are that we do not have information about the effect of antineoplastic agents on SACE activity.

In the evaluation of this marker for monitoring complete remission cases, our results have generated expectations that require a further prospective investigation to be confirmed. The present results that show clear differences in the values of SACE among distinct clinical stages (nonevolutive) are the necessary basis to do a prospective study in order to investigate its utility (sensitivity and specificity) in the differential diagnosis of neoplastic disease and for the study of the possibility of using this cancer marker to monitor the clinical evolution of the disease (onset, metastatic diffusion, remission). It is necessary to investigate all the factors associated with increased SACE activity in clinical complete remissions and the possible mechanisms of its decreased level in malignant tumors.

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