

Serum p53 Antibodies as a Marker for monitoring patients with Breast Cancer

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Abstract:

Objective: To detect serum p53 antibodies in patients with breast cancer, and to assess prognostic significance of these antibodies during and after treatment.

Patients and methods: Sixty patients with breast cancer and 35 patients with non-malignant breast disease were tested for p53 antibodies by enzyme linked immunosorbent assay (ELISA), and for the carbohydrate antigen 15-3 (CA 15-3).

Results

Eight patients (13.3%) with breast cancer and one patient (2.9%) with non-malignant breast disease displayed p53 antibodies. p53-Abs were present in five patients with high CA 15-3 and three patients with normal values of this marker. After tumor resection, there was a fluctuating in p53 antibodies status during follow up period.

Conclusion

Testing for serum p53 antibodies may be constituted as a prognostic biomarker for assessing alterations in p53 to follow up patients with breast cancer.

Keywords: p53 gene, p53 antibodies, breast cancer, carbohydrate antigen 15-3.

Introduction

The p53 gene is located at chromosome 17p13.1, and it encodes a 53-kDa nuclear phosphoprotein [1]. Since its discovery in 1979, many different roles for the tumor suppressor protein p53 in tumorigenesis have been described. Correct p53 function is required for proper regulation of cell division, apoptosis, senescence, and the responses to cellular stresses such as DNA damage and hypoxia. Indeed, mutations in p53 are observed in as many as 50% of human cancers [2].

Because p53 accumulation is the main trigger of humoral response, it was of interest to examine the behavior of these p53-Abs during therapy to see whether there was a relationship between tumor disappearance and a

decrease in p53-Abs. Antibodies against p53 can be detected in sera from patients with cancer and a correlation exists between mutations in the p53 gene and antibodies against p53 in sera [3].

Serum tumor markers are routinely used in the management of patients with different cancers [4]. In breast cancer patients carbohydrate antigen 15-3 CA15-3 is considered the serum marker of choice[5].

Breast cancer is the leading cause of death among women. It is responsible for approximately 15% of all cancer-related deaths, and the incidence of Breast cancer may increase substantially in the future [6-8].

The aim of this study was to assess the prevalence of p53-Abs in breast cancer patients and to compare this with the widely used tumor marker CA15-3; the specificity of p53-Abs testing in patients with non-malignant breast diseases; the potential value of p53-Abs monitoring for the management of patients with breast cancer.

Materials and Methods

Patients' characteristics

Sixty breast cancer patients were studied. The mean age was (46,2) years (range 25-71 years). All patients were treated at Al-assad university hospital and Al-bairouny oncology education tumor hospital, Damascus, Syria, between April 2008 to February 2011.

The following data of each patient shown in table 1 were analyzed: age, tumor size, stage of tumor, axillary lymph node metastases.

Table 1. Data on 60 patients with breast cancer

Patients with breast cancer	N=60	
	Number	%
Age (years)		
<50	39	65
>50	21	35
Tumour size (cm)		
<2	10	17
>2	50	83
Clinical stage (TNM)		
I/II	32	53
III/IV	28	47
Axillary lymph node		
Negative	49	82
Positive	11	18
Metastatic disease		
Yes	9	15
No	51	85

Serum samples

Serum samples of the 60 patients with breast cancer were collected after diagnosis but before treatment during routine blood sampling. Serum samples of 35 patients with various non-malignant breast diseases were also collected. Whole blood was centrifuged at 3000 rpm for 10 minutes and the supernatant was stored at -80°C until use.

Analysis of p53 antibodies in the serum

We used IN VITRO p53 antibody ELISA kit (Homburg,Germany) to quantitative determination of human p53 antibodies in serum. The special feature of this ELISA is that cell extracts from human tumor cells are used containing p53. On the antigen plate, this immunoreactive and native p53 protein is fixed on a monoclonal antibody. To monitor nonspecific bonds between the patient serum and the antigen-antibody complex or the microtitre plate, we used a control antigen plate coated with a control antigen.

With the present ELISA the first step is to bind the antibodies which are present in the sample (diluted serum) to the immunoreactive p53 antigen on the micro-titre plate. The bound antibodies from the serum are detected after the next step, which is washing with a second antibody conjugated with an anti-human IgG peroxidase. The specific bond of this antibody to human IgG molecules is definite and makes quantitative determination of the amount of IgG bound to p53 possible across a range from 0.2 to 1.5 at 450 nm. This quantitative evidence of the antigen-antibody complex is followed, after another washing step, by addition of a substrate so that a soluble product emerges. After a stopping stage, the concentration of this product can be measured photometrically at 450 nm in an ELISA reader. To avoid faulty interpretations and as a negative control, the diluted samples are incubated simultaneously in the cavities of the control antigen plate, where the p53 is replaced by a control antigen. Using this method of proof this ELISA permits direct quantitative determination of the anti-p53 antibodies in the serum in $\mu\text{g/ml}$. The **cut-off** value of positive p53 antibodies is $> 0.4 \mu\text{g/ml}$.

Analysis of CA 15-3

We used CA 15-3 IEMA WELL kit (Radim, Roma,Italy) for the quantitative determination of CA 15-3. The CA 15-3 IEMA WELL is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, control sera and patient samples are incubated together with biotinylated Anti- CA 15-3 monoclonal antibody and horseradish peroxidase (HRP) labelled Anti- CA 15-3 monoclonal antibody in streptavidin coated microtiter strips. After washing, buffered TMB-substrate reagent (hydrogen peroxidase and tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportional to the amount of CA 15-3 present in the sample. Normal CA 15-3 value is up to 30 U/mL.

Results

Detection of p53-Abs in serum

Among the 60 patients with breast cancer, there were 8 (13.3%) had p53-Abs, and among 35 patients with non-malignant breast disease, there was 1 (2.9%) had had p53-Abs. The Table 2 describes the clinical characteristics, treatment, and follow up of patients with p53-Abs.

Carbohydrate antigen 15-3 (CA 15-3)

Values for CA 15-3 were above normal in 29 out of 60 patients (48.3%) tested before treatment. Five of these patients also had positive p53-Abs in serum, and three patients with normal values of CA 15-3 (table 2). By contrast, all 35 patients with non-malignant breast disease had normal CA 15-3 values.

Monitoring of p53-Abs

Eight patients were positive p53-Abs, in the first stage of the study (before tumor resection) that extended 10 months. Later, they were tested in two stages to identify the variation of p53-Abs within the follow up period. In the second stage (after tumor resection and during chemotherapy or radiotherapy) that extended 14 months, five of the eight patients (1,3,5,6,8), who were tested, three of the five patients (1,5,8) remain positive p53-Abs, two of the five patients (3, 6) were negative p53-Abs. In the last 10 months; third stage, (after tumor resection, chemotherapy, and radiotherapy) we could follow up four breast cancer patients, three of the four patients (1,3, 5) were positive p53-Abs, and the patient (6) was negative p53-Abs (table 2).

p53-Abs were present in the five patients(3,4,5,7,8) with high CA 15-3 in the first stage, and in the two patients (1, 8) in the second stage, and in thetwopateints (1, 3) in the third stage (table 2).

Table.2 Clinical characteristics, treatment, and follow up of the 8 patients with breast cancer and p53 antibodies

Evaluation after tumor resection	(The cut-off value of p53 Abs is 0.4 µg/mL) (Normal CA 15-3 value is up to 30 U/mL)						Classification		Age (Years)	No
	Study stage 3		Study stage 2		Study stage 1		Stage	TNM		
	CA 15-3	p53 Abs	CA 15-3	p53 Abs	CA 15-3	p53 Abs				
Bad condition	High	4.9	High	3.21	Normal	6.89	III	T ₂ N ₃ M ₊	45	1
Lost to follow up	-	-	-	-	Normal	1.603	III	T ₂ N ₀ M ₀	50	2
Recurrence	High	0.76	Normal	0.1	High	1.30	IV	T ₄ N _x M ₊	52	3
Died	-	-	-	-	High	2.744	III	T ₂ N ₂ M ₀	46	4
14 months/good condition	Normal	0.92	Normal	1.6	High	6.090	II	T ₂ N ₀ M ₀	44	5
Good condition	Normal	0.09	Normal	0.2	Normal	0.840	II	T ₂ N ₁ M ₀	66	6
Lost to follow up	-	-	-	-	High	0.728	III	T ₄ N ₂ M ₀	43	7
Died	-	-	High	0.721	High	1.342	III	T ₃ N ₊ M ₀	48	8

Discussion

Serum p53-Abs were first discovered by Crawford et al (1982)[9], who reported 9% p53-positive sera in 155 breast cancer patients. This discovery did not attract much attention until recently. Later, several studies were performed to investigate p53-Abs in breast cancer patients[10-12]. In the present study, we found that (13.3 %) of breast cancer patients and (2.9%) of non-malignant breast disease patients had p53-Abs.

In the course of monitoring developments in patients with progressive tumor disorders, a seroconversion was noted in 40% of the patients over a period of 1-2 years. In some patients, postoperatively a reduction of the p53 antibodies was observed so that p53 may be used for monitoring developments or monitoring therapies [13].

Variations of p53-Abs during treatment of patients with cancer have been poorly studied. Angelopoulou et al[14] reported p53-Abs monitoring in five patients with ovarian cancer and one with breast cancer.

In our study, we currently reported the results of 8 patients with p53-Abs. In two of them (2,7), the follow up was lost to draw any conclusions. Among the eight patients followed up for 14 months, thetwopateints (3, 6) showed a negative p53-Abs, but only three of them (1, 5, 8) remained positive p53-Abs. In the three patients, the persistent positive p53-Abs may be due to persistent immunisation against p53 protein.

After a longer follow up period (the third stage of study), we could follow up the four patients (1, 3, 5, 6).

In patient 1, p53-Abs were positive, and there was an increase in CA 15-3 concentration. Perhaps p53 mutation could be viewed as an event that speeds up the disease development, and thus be regarded as a marker for a more aggressive behaviour of the tumor [15].

In patient 3, p53-Abs were negative in the second stage, but became positive in the third stage. Increased serum levels of CA15-3 may therefore mirror the presence of cells bearing a particularly aggressive phenotype. Moreover, it has already been suggested that a possible explanation for the association between CA15.3 and worse prognosis may be that the marker is elevated in patients with micrometastatic disease, which is not detected by standard radiological imaging.

In patient 5, p53-Abs were positive in the second and third stage, and CA15-3 concentration was normal in the second and third stage. Transient tumor marker increases during treatment may be explained by tumor lysis and marker release from disintegrating cells and may provide false signals of tumor progression. An early change of treatment would withhold these patients from possibly effective therapy.

In patient 6, A postoperative significant drop in p53-Abs may be the result of complete tumor resection and a successful djuvant therapy.

Patient who has benign mass has ductal hyperplasia, this type may be progressed to a malignance. But we can not depend on the detection of p53- Abs to evaluate the alterations in p53 gene or abnormalities of p53 protein itself, if it is wild or mutated protein.

We conclude that p53- Abs may be indicative for a poor prognosis and a higher risk of tumor relapse, and play a useful potential biomarker in patient monitoring during therapeutical follow-up. CA15.3 provides additional information to the common prognostic factors.

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