Clinical Significance of Serum p53 Antibodies as a Tumor Screening Marker

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Background p53 is the gene for a transcription factor that is activated in response to deoxyribonucleic acid (DNA) damage, and research has revealed that abnormalities of this gene show a high frequency in gastrointestinal cancers.

Methods We investigated the positivity rate of p53 antibodies in serum from 890 subjects, and found seropositivity in 74 subjects vs. seronegativity in 816 subjects.

Results There was little difference between males and females, but there was a trend for the seropositive rate to increase with advancing age from the 30s onwards, peaking among subjects in their 60s. The 5 subjects with positive antibody titers and cancer were all men, but there was no correlation between the antibody titer and the presence of cancer.

Conclusion Based on the results of the present investigation, detection of p53 serum antibodies is not an effective cancer screening tool compared with existing tumor markers. (Ningen Dock 2007; 21: 15—18)

Key Words: p53 serum antibody titer, tumor marker, cancer screening

Since the 1980s, the three major causes of death for people aged 40 years or older have been malignancy, cardiac disease, and cerebrovascular disease. For all these diseases, the prognosis is influenced by early detection and early treatment. Numerous genes are involved in the development of cancer, and it has been ascertained that abnormal expression of tumor promoter genes and tumor suppressor genes is involved at several stages in the process of carcinogenesis.¹ It is well known that mutations of the p53 tumor suppressor gene are common in various malignancies. The p53 gene plays a key role in cell death by inducing apoptosis in response to deoxyribonucleic acid (DNA) damage, and its expression is not detected in normal cells.²,³ When mutations of this gene occur and cause the mutant protein to be overexpressed, abnormal cells are allowed to proliferate. Recently, it has become possible to measure an antibody to overexpressed p53 protein, and it has also been suggested that this antibody may serve as a useful marker for cancer screening.⁴⁻⁷ Here we report our investigation into the value of serum p53 antibodies for cancer screening.

Methods

Measurements of p53 autoantibodies were only carried out after review and approval by the ethics committee established at the center. Before measurement of p53 autoantibodies, we handed subjects an explanatory pamphlet noting that participation in the measurement of p53 autoantibodies was voluntary, and that the p53 autoantibody assay was a method for testing blood samples for the presence of antibodies for p53 protein in which mutations had occurred. The doctors and nurses on staff were to provide additional explanations if there were any questions, and patients who gave their consent to participate completed a p53 autoantibody test screening form. Blood samples were then collected, and the results were later returned to individual patients.

Using an enzyme-linked immunosorbent assay (ELISA), we measured p53 autoantibodies levels in serum samples collected from 890 subjects (572 males and 318 females with a mean age of 58.1 years) who volunteered for this study at the Institute of Geriatrics of Tokyo Women’s Medical University between October 2003 and October 2005.

On the measurement method for p53 autoantibodies, and the cut-off value for antibody measurements, we used an ELISA assay kit, the MESACUP anti-p53 Test Kit (Medical and Biological Laboratories Co., LTD., Nagoya, Japan), developed by Shimada et al.⁸ The method uses a microplate containing the marker protein, normal human p53 protein, produced by a baculovirus. Serum samples diluted 100-fold are allowed to react at room temperature, producing secondary antibodies, and these secondary antibodies are visualized by peroxidase-labeling, and the absorbance is measured at a wavelength of 450 nm. Serum obtained beforehand from lung cancer patients was used as a positive control, and selecting a 3-fold dilution as 15 U/ml, the antibody titer was calculated from a linearly-plotted absorbance curve. It was also confirmed that
neither bilirubin nor Hb levels had any effect on the measured values. The cut-off value of 1.3 U/ml was selected from the 95% distribution interval of antibody titers for 205 healthy individuals measured using this technique, and results above these antibodies levels were regarded as positive.

Results

Among the 890 subjects, p53 serum autoantibodies were detected in a total of 74 subjects (8.3%), comprising 47 (8.2%) out of 572 males and 27 (9.2%) out of 318 females (Fig. 1, 2 and Table 1). Among both males and females, the positivity rate of p53 serum autoantibodies increased with advancing age from the 20s onwards, peaking in the 60s, and then decreasing again (Fig. 3). Gastric fluoroscopy or endoscopy, abdominal ultrasound, routine blood tests, urinalysis, and a fecal occult blood test were performed in all subjects who tested positive for p53 autoantibodies.

PET scans were also obtained in the 12 subjects who gave consent to this test. Among the 74 subjects who were positive for p53 autoantibodies, only 5 (6%) had cancer. All of them were elderly men in their 70s or older who had previously been diagnosed as having prostate cancer and were undergoing treatment. Thus, no cancers were newly detected by the present assay.

Discussion

When the p53 tumor suppressor gene shows abnormal overexpression, the mechanism of apoptosis is disrupted and abnormal cells are able to proliferate. Recently, it has become possible to measure the serum level of autoantibodies to mutant p53 genes, and several researchers have claimed that this test is effective as a marker for detecting early cancer or that it is useful for post-operative monitoring of colon cancer and other tumors. Trivers et al. reported that serum p53 autoantibodies are already present in the precancerous state of esophageal cancer and lung cancer, suggesting that detection of such antibodies may not only be useful in the diagnosis of cancer, but also for identifying the precancerous state and thus the population with a high risk of developing cancer.9,10

In the present investigation, the mean seropositive rate for p53 autoantibodies was 8%, which was higher than that obtained by other researchers, but we did not find any correlation between p53 autoantibody seropositivity and the incidence of cancer.4

Presently, efforts for the early detection of cancer include screening via regular medical check-ups or focusing on the at-risk groups for specific malignant can-

<table>
<thead>
<tr>
<th>Result/Sex</th>
<th>Male</th>
<th>Female</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Negative</td>
<td>525</td>
<td>291</td>
<td>816</td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
<td>27</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>572</td>
<td>318</td>
<td>890</td>
</tr>
</tbody>
</table>

Table 1. Sexual differences of positive rate of p53 autoantibodies

Fig. 1. Positive rate of p53 autoantibodies in all subjects

Fig. 2. Age and sex distribution of subjects
cancers (e.g., the chronic hepatitis C/liver cirrhosis group, smokers, persons with a family history of cancer, etc.) and for people in those groups, it is most often the case that their clinical course is monitored through regular outpatient contact. In this context, tests for tumor markers are especially significant as non-invasive and highly accurate cancer screening techniques. Already, there are well-known tumor markers such as CEA for pre- and post-operative testing in gastric and colon cancer, CA125 for cancers in the gynecological area, and CA19-9 for biliary and pancreatic tumors.

However, the p53 autoantibody values obtained in this study suggest that it may become a screening tool for prostate cancer in middle-aged and older men, given that positive results were returned for the 5 patients who had prostate cancer. According to Shimada et al., who are involved in the development of methods for measuring p53 autoantibodies, p53 is positive in various tumors, similar to CEA. In fact, comparison of the positive rates for p53 and CEA in subjects who tested positive yielded almost equivalent results, 23.2% and 25.5% respectively. Furthermore, analysis of the data by types of tumor tissue showed that the p53 autoantibody titer tended to be higher in squamous cell carcinoma, while the positivity rate for CEA, in contrast, tended to be higher in adenocarcinoma.

Other possible causes of this high positive rate may be that: 1) our investigation was a prospective study and it is possible that the assessment of tumor markers was inadequate because we have not yet conducted a periodical follow-up, or 2) the subjects undergoing screening were largely considered to be healthy and had a higher mean age than usual for outpatients, so the possibility of a link between cellular aging and p53 autoantibody expression cannot be ruled out. However, increasing age is also known to be a risk factor for the occurrence of cancer, but we did not discover any new cancers and only detected previously known prostate cancer. In addition, there was no correlation between the extent of seropositivity and the cancer detection rate or cancer morbidity rate. Thus, measurement of p53 autoantibodies still seems to have limitations compared with established tumor markers. In our current patients, those with positive results all had prostate cancer of the adenocarcinoma type, but as Shimada and colleagues note, p53 already tests positive in individuals with early cancer, suggesting that it has the potential to be a new tumor marker or monitoring marker of post-operative recurrence or treatment efficacy.

Further studies should be performed in a larger number of subjects to better determine the significance of measuring p53 autoantibodies.

**Conclusion**

We investigated whether measurement of p53 autoantibodies was useful for cancer screening, but were unable to find evidence that this test was effective. It may be necessary to conduct further investigations in a larger number of subjects.

**References**


