p53 is an Independent Prognostic Factor for Survival in Thyroid Cancer

KAI BACHMANN, DENISE PAWLISKA, JUSSUF KAIFI, PAULUS SCHURR, JENNIFER ZÖRB, OLIVER MANN, HANS J. KAHL, JAKOB R. IZBICKI and TIM STRATE

Department of General, Visceral and Thoracic Surgery, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

Abstract. Background: p53 has been reported to be of prognostic importance in different types of cancer. Immunohistochemical measurement of p53 antigen activity could be a prognostic marker for aggressiveness and survival in thyroid cancer. Different types of antibodies have been used to detect p53 in previous studies without direct comparison to each other. Patients and Methods: A series of 54 patients with thyroid cancer who had undergone thyroidectomy between 1993 and 1998 is reported. All samples were chosen retrospectively and classified by routine histopathology, followed by immunohistopathological examination with three different types of antibodies (PAb1801, CM1 and DO-7) with the peroxidase method. Survival data was generated. Results: The mean time of follow-up was 9.0 years. Eighteen patients died. Twenty-three (42.6%) samples were positive for p53 using the antibody PAb1801, 17 (31.5%) with using CM1 and only 4 (7.4%) cases with DO-7. Statistical analysis determined that the size (p=0.02) and classification of the tumor (p<0.001), the age of the patients (p=0.036), the presence of lymph node metastasis (p=0.024) and positive staining for p53 (p<0.001) were prognostic factors for overall survival by Kaplan-Meier method. Multivariate analysis revealed overexpression of p53 to be an independent significant prognostic factor of survival. Conclusion: PAb1801 is the most sensitive antibody for detection of p53 protein in this type of cancer, and p53 is a prognostic factor for survival in thyroid cancer. This may provide further information for prognosis and aggressiveness of thyroid cancer.

Thyroid carcinoma represents 0.5-1% of human cancer cases. According to WHO guidelines it is classified as follicular, papillary, anaplastic or medullary carcinoma and 80-90% are highly differentiated tumors (follicular and papillary) with excellent prognosis and five year survival of 95% (1). The anaplastic tumors are undifferentiated with a poor outcome (2, 3). In the past, different antibodies have been used to detect p53 mutations and CM1 and DO-7 were the most commonly used (4). To date no study has evaluated which antibody provides the most sensitive detection of p53 in thyroid cancer. Mutations in the p53 gene are the most common in human cancer (5-9), and they represent the most frequent genetic changes in malignant transformation. p53 protein plays an important role in the regulation of the cell cycle. Wild type p53 protein is capable of inhibiting cell proliferation and transformation and it has been found to be inactive in tumor cells (10-12).

Depending on the localisation of the tumor, the rate of mutations in the p53 gene varies from 0-60% (6). High rates of mutations have been found in cancers of the lung, breast, colon, prostate, liver, oesophagus and ovary (6, 13, 14). Previous studies have shown an association between p53 expression and the degree of differentiation (15). Godballe and colleagues found p53 expression to be a prognostic indicator for survival in follicular and papillary cancer (16). The aim of this study was to find the most sensitive antibody to detect p53. Additionally, p53 was evaluated concerning its prognostic impact in patients with thyroid carcinoma.

Patients and Methods

Study design and patients. Written informed consent was obtained from all patients. Fifty-four patients with thyroid cancer, who had undergone surgery in the Department for Surgery at the University Medical Center Hamburg-Eppendorf between 1993 and 1998, were chosen retrospectively for this study. Thyroid cancer was confirmed by histopathological evaluation in all cases. The resection margins were tumor-free on histopathological examination of the surgical specimens (R0) and no patient had evidence of distant metastasis (M0). Tumor
stage and grade were classified according to the most recent TNM classification of the International Union Against Cancer (17, 18).

Clinicopathological data. All data, including gender, histology, depth of tumor invasion, lymph node metastasis, tumor type and disease stage, were obtained from the clinical and pathological records. Clinical follow-up data were obtained by reviewing the hospital records, direct communication with the attending physicians and from the Cancer Registry of Hamburg. The overall survival was calculated from the date of surgical resection of the tumor to the date of death or last follow-up. The mean follow-up period was 9.0 years overall.

Variables. Histological type (papillary, follicular, medullary and anaplastic), lymph node metastasis, tumor size (T 1-4), gender, age at time of operation, p53 expression using different antibodies (PAb1801, CM1, DO-7) were evaluated as possible factors.

Immunohistochemistry for p53 protein. Paraffin-embedded tissue (archival material) was used for immunohistochemical examination. The paraffin was removed from the 4-μm sections and the samples were rehydrated in descending alcohol concentrations. Endogenous peroxidase activity was blocked by H2O2 (Fluka, Neu-Ulm, Germany). The antigens were retrieved by heating in a microwave oven and then cooled down to 20°C. The samples were washed with phosphate-buffered saline (PBS).

For saturation of unspecific binding the sections were incubated with 100 μl PBS bovine serum albumin (BSA). Then the primary antibody was added.

p53 was detected by using three different types of commercial antibodies. PAb1801 (Oncogene Research Products, Cambridge, UK), a monoclonal mouse antibody, CM1, a polyclonal rabbit antibody (Novocastra, Newcastle, UK) and DO-7 (Dako, Glostrup, Denmark) a monoclonal mouse antibody, which detects stable epitopes of human p53 after denaturation (15, 19, 20).

After an incubation time of 30 minutes the antibodies were washed out with PBS. The slides were incubated with 100 μl labelled streptavidin biotin horseradish peroxidase for 30 minutes. Diaminobenzidine (DAB) (ImmunoPure Metal Enhanced DAB Substrate KIT, Pierce, Rockford, IL, USA) was used as chromogen (21-23). Counterstaining with haematoxylin A was performed. All immunohistochemical examinations were performed twice to improve the reliability. The slides were counted by means of a light microscope using a grid and at least 500 tumor cell nuclei were counted in five randomly selected areas. Positive and negative controls were included. The rate of p53 positive cells per total number of counted cells was calculated.

Statistical analysis. SPSS® for Windows® (Version 11.5.1) (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The survival curves of the patients were plotted using the Kaplan-Meier method and analyzed using the log-rank test. Cox regression analysis was used for multivariate analysis to assess the independent influence of p53 simultaneously with other covariates. Significance statements refer to p-values of two-tailed tests that were less than 0.05. For all variables cross tables were generated, followed by calculation of p-value using Chi-square test/Fisher’s exact test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>PAb1801</th>
<th>CM1</th>
<th>D-O7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>40</td>
<td>17</td>
<td>42.50%</td>
<td>14</td>
</tr>
<tr>
<td>male</td>
<td>14</td>
<td>6</td>
<td>42.90%</td>
<td>3</td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>20</td>
<td>6</td>
<td>30.00%</td>
<td>4</td>
</tr>
<tr>
<td>≥45</td>
<td>34</td>
<td>17</td>
<td>50.00%</td>
<td>13</td>
</tr>
<tr>
<td><strong>Tumor size:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>13</td>
<td>4</td>
<td>30.80%</td>
<td>2</td>
</tr>
<tr>
<td>T2</td>
<td>24</td>
<td>6</td>
<td>25.00%</td>
<td>8</td>
</tr>
<tr>
<td>T3</td>
<td>7</td>
<td>7</td>
<td>100.00%</td>
<td>4</td>
</tr>
<tr>
<td>T4</td>
<td>10</td>
<td>6</td>
<td>60.00%</td>
<td>3</td>
</tr>
<tr>
<td><strong>Histological classification:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>29</td>
<td>10</td>
<td>34.50%</td>
<td>6</td>
</tr>
<tr>
<td>Follicular</td>
<td>13</td>
<td>5</td>
<td>38.50%</td>
<td>5</td>
</tr>
<tr>
<td>Medullary</td>
<td>9</td>
<td>5</td>
<td>55.60%</td>
<td>5</td>
</tr>
<tr>
<td>Anaplastic</td>
<td>3</td>
<td>3</td>
<td>100.00%</td>
<td>1</td>
</tr>
<tr>
<td><strong>Lymph node metastasis:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>31</td>
<td>9</td>
<td>29.00%</td>
<td>7</td>
</tr>
<tr>
<td>N1</td>
<td>23</td>
<td>14</td>
<td>60.90%</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>23</td>
<td>42.59%</td>
<td>17</td>
</tr>
</tbody>
</table>

Data are presented in cross tables. P-values were determined by using two-sided Chi-square / Fisher’s exact tests.
Results

Characteristics of the patients. Patient characteristics are listed in Table I. The mean age at surgery was 51 years with a range from 15 to 92 years and a median age of 53 years. The male-female ratio was 1:2.86 with 14 (25.9%) men and 40 (74.1%) women.

Clinicopathological data. The tumor classification was associated with a significantly better prognosis for survival for papillary and follicular tumors and poorer prognosis for anaplastic tumors with \( p < 0.001 \) using the log-rank test. The median survival of patients with positive p53 antigen was 6.55 years (95% CI 4.47-8.63) and 12.43 years for p53 negative (95% CI 11.44-13.42).

Positive staining for p53 and survival. The examination with the CM1 antibody determined 17 out of 54 (31.48%) samples to be p53 positive. Twenty-three out of 54 (42.6%) samples of thyroid carcinoma were p53 positive using the PAb1801 antibody. Due to the low sensitivity of DO-7 in our series, with p53-positive staining in only 4 out of 54 cases (7.41%) this antibody was excluded from further statistical analysis.

Follow-up data was obtained for all the cases. Statistical analysis using the log-rank test revealed a significantly poorer prognosis for survival for patients with p53 positive staining of the samples \( p < 0.001 \) (PAb1801) and \( p = 0.036 \) (CM1). Survival curves plotted using the Kaplan-Meier method for p53 expression using the antibodies PAb1801 and CM1 for overall survival are shown in Figures 1 and 2.

Using PAb1801 the mean survival for p53 positive patients was 6.55 years (95% CI 4.47-8.63) and 12.43 (95% CI 11.44-13.42) years for p53 negative patients. The mean survival using CM1 was 8.33 years (95% CI 5.77-10.89) for p53 positive patients and 11.08 (95% CI 9.58-12.57) years for p53 negative patients. Multivariate
analysis with Cox regression found PAb1801 to be an independent prognostic factor for survival ($p=0.011$), while CM1 was not an independent prognostic factor ($p=0.312$) (Table II).

**Discussion**

The clinical relevance of thyroid carcinomas is caused by the high prevalence in the European population. The most common type is the papillary carcinoma (24-26).

Using the UICC cut-off point of 45 years the age was found to be an important prognostic factor in accordance to previous trials (27, 28). As expected, gender was not a prognostic factor for survival. The established prognostic factors of age, tumor-size, prevalence of lymph node metastasis and histological classification of the carcinoma were confirmed to be of prognostic significance for survival in long term follow-up. Therefore our cohort of patients seemed to be quite representative of patients with thyroid cancer, despite the fact that this was a relative small series of 54 patients (16, 29, 30).

The role of p53 in the cell cycle is well-known and several previous studies have shown the clinical relevance of the mutation or rather the expression of p53 (6, 15, 16). Overexpression of p53 protein and p53 gene mutation can be detected in thyroid cancer and in other organs, such as lung, liver, colon and ovary (6, 13, 14).

All antibodies are directed against the N terminal part of the protein and react with the mutant as well as the wild type of p53 (19, 20, 31). This type has a short half-life of 5-6 minutes, while the transformation by point mutation has a half-life of a few hours (12, 32, 33). We were not able to show whether the observed expression was the wild type or the mutant. Nevertheless, previous studies have shown a high correlation of occurrence between p53 gene mutations and the p53 protein (15, 31, 34) and we were able to demonstrate a significantly poorer prognosis in patients with p53 positive samples. Additionally, the coexpression of p53 and c-myc in early stages of oral oncogenesis was found to be a possible indicator for premalignant lesions (35).

The antibody DO-7 was positive in 4 out of 54 samples only, where 3 patients died. Therefore this data were excluded from further statistical analysis. In univariate analysis CM1 was found to be a significant prognostic indicator for survival, but it failed to reach statistical significance in multivariate analysis ($p=0.312$). The antibody PAb1801 was found to be the most sensitive marker (23 out of 54 samples with positive staining) and was, even in a limited number of patients, an independent prognostic indicator for survival (Table II).

Previous studies have shown p53 to be a prognostic marker for papillary and follicular carcinoma (16, 30). No direct comparison of the antibodies for detection of p53 is available. PAb1801 has been identified to be a highly sensitive antibody with a staining rate in thyroid carcinoma of up to 75%; no follow up was performed in this trial (31). Nishida et al. used the antibody CM1 and found p53 to be an independent prognostic marker (30). Godballe and colleagues confirmed this result using the antibody DO-7 (16). In our series DO-7 and CM1 were significant prognostic markers in univariate analysis, but DO-7 had to be excluded due to unsatisfactory sensitivity, while CM1 failed to demonstrate significance in multivariate analysis. Possible explanations for this difference are modifications in grade of dilution of the antibodies and different durations of incubation.

**Conclusion**

The expression of p53 antigen is an independent prognostic factor for survival in patients with thyroid carcinoma. In direct comparison PAb1801 is the most sensitive antibody for detection of the p53 antigen.

**References**