Abstract. Background: The predictive value of TP53 mutations and prostate-specific antigen (PSA) was assessed in prostate needle biopsies of samples without signs of malignancy for later affliction by prostate cancer (PCa). Comparison of mutation frequency and PSA level in prostate needle biopsies with data of patients with benign prostate hyperplasia (BPH) treated by transurethral resection (TURP), patients with prostatic intraepithelial neoplasia (PIN), and patients with PCa, was made. Materials and Methods: A total of 466 samples were analysed from patients with benign and malignant diseases of the prostate, including 52 samples of needle biopsies of the prostate with repeated benign histopathological diagnosis. Analysis of TP53 state by temperature gradient gel electrophoresis of TP53 exon-specific PCR products of exons 5, 6, 7 and 8 was performed. Clinical follow-up of 100 patients with benign diseases of the prostate and with PIN was available. Results: Needle biopsy samples with repeated benign diagnosis resemble BPH specimens taken by TURP in TP53 mutation frequency (TURP: 16.1%, needle biopsy: 21.2%) and later affliction by PCa (TURP: 3/32 =9.4%, needle biopsy: 8/51 = 15.7%, p=0.409). Patients with TP53 mutations in needle biopsy samples showed a significantly reduced disease-free survival time for affliction by PCa (log rank: p=0.0149). This significance is raised by computing exon 6 mutations only with respect to affection by PCa (p=0.0002). In TURP patients, exon 7-mutations were also significant (p=0.0086). Needle biopsy TP53 mutations (p=0.029) had significant predictive values for later affliction by PCa in multivariate analysis. PSA level and patient age had no predictive value for PCa. Conclusion: TP53 mutations reduce the PCa-free survival time in patients with needle biopsy of the prostate and primary benign diagnosis. Exon 6 mutations enhance the risk of being affected by PCa 32-fold.

TP53 mutations are present in tumour tissues with varying frequencies (1). In prostate cancer (PCa) a moderate TP53 mutation frequency in the range of 30% without evident relationship to tumour grade or stage has been reported (2). This may be an indication of an early stage of mutagenesis during tumorigenesis of the prostate. TP53 plays a role as cell cycle regulator and in the stress response. Its contribution in case of mutation to the development of PCa is questioned (3). However, in PCa tissue, the overexpression of the p53 gene product is a risk factor for tumour progression (4, 5). Contribution of TP53 mutation to progression of PCa has been discussed (6-8). Berner et al. have discussed a TP53 exon 8 mutation in codon 273 as a hot spot for tumour progression of PCa (9). In isolated benign cells located near foci of high-grade adenocarcinoma of the prostate one mutation of exon 7 was detected in 20 specimens (6). Navone et al. (1999) found two exon 7 mutations in PCa tissue and also in lymph nodes (8).

In patients with benign prostate hyperplasia (BPH), TP53 mutations are detectable in approximately 19.0% (10). We have analyzed a high proportion of silent mutations in benign prostate tissue samples (recent data: 19/35=54.3%). It is questioned whether TP53 mutations in benign prostate disease contribute to the incidence of PCa. BPH is not considered as a precancerous disease (11), and BPH is treated by transurethral resection (TURP). In the Charité Hospital the prevalence of PCa is 6.6% (10 out of 151) in the case of TURP treatment of patients suspected for BPH.
A needle biopsy is carried out in patients suspected for PCa. The incidence of PCa determined from needle biopsies of patients without any history of PCa is 28.4% (19 out of 67) at the Charité Hospital. A high preoperative serum PSA level one of the main risk factors for PCa in needle biopsies (12). It is known that the correlation between the outcome of needle rebiopsy and PSA is insignificant, even if the serum PSA level is less than 4 ng/ml (13, 14).

In this study, we screened needle biopsy samples of patients with repeated benign diagnosis. The advantage of using such samples consists of better conditions for outpatient follow-up in comparison with TURP patients. The predictivity of TP53 mutations in benign prostatic tissue for later affliction by PCa was evaluated. The aim of this study was to investigate if TP53 mutations could reduce the PCa-free survival time in patients with needle biopsy of the prostate, and if TP53 mutations could enhance the risk of being affected by PCa.

Materials and Methods

Analytical data and summarized follow-up results were available for 466 patients. The patients were sampled in groups according to disease:

i) BPH group. A total of 218 tissue samples of benign prostate hyperplasia, taken by transurethral resection (TURP), were available, in five cases by adenomectomy. Thirty-two patients of this group were followed up for 34 months, including seven patients with TP53 mutations for 38 months (range 3-64) months, and 25 patients with wild-type TP53 for 32 months (range 2-97 months).

ii) Needle-benign group (NB). Samples were taken from 1994 to 1999 at the Outpatient Clinic Department of Urology of the Charité Hospital by ultrasound-controlled prostate needle biopsy of 52 patients suspected for PCa, but without signs of malignant disease in their histopathological examination of all needle cores. Forty-four of these patients had a repeated benign diagnosis 6-58 months after first examination and TP53 analysis. The remaining seven patients had already been diagnosed using TURP for BPH or with needle biopsy without PCa in the past (1-108 months). Fifty-one patients of this group were followed up for 31 months, including eleven patients with TP53 mutations for 29 months (range 14-62), and 40 patients with wild-type TP53 for 32 months (range 2-82 months).

iii) PIN group. This group comprised samples with histopathological signs of intraepithelial neoplasia of the prostate (21 cases) or with atypical adenomatous hyperplasia (two cases) but without signs of PCa. Eighteen samples were taken by needle biopsy, five by TURP. Seventeen patients of this group were followed for 20 months, including seven patients with TP53 mutations for 22 months (range 4-40), and ten patients with wild-type TP53 for 20 months (range 6-35 months).

iv) Needle-PCa group (NPCa). This group comprised 50 prostate tissue samples taken by needle biopsy of patients suspected for PCa. Histopathological diagnosis confirmed malignancy in at least one core.

v) Surgery-PCa group (SPCa). A total of 123 PCa tissue samples of first surgical treatment made up this group.

<table>
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<tr>
<th>Table I. Statistical comparison of TP53 mutation frequencies.</th>
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<td>BPH 36/218=16.5% vs. NB 11/52=21.2% 0.428</td>
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<tr>
<td>BPH 36/218=16.5% vs. SPCa 38/123=30.9% 0.002</td>
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<tr>
<td>NB 11/52=21.2% vs. NPCa 17/50=34.0% 0.146</td>
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<tr>
<td>NB 11/52=21.2% vs. PIN 9/23=39.1% 0.105</td>
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<tr>
<td>NB 11/52=21.2% vs. SPCa 38/123=30.9%: 0.190</td>
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| 1Pearson’s Chi-square test (asymptotic significance), patients with TP53 mutations / all patients of indicated groups. |

Only two samples without any sign of prostate disease were available for analysis. These samples had wild-type TP53, but PSA analysis results were not available. Therefore comparison with PSA or mutation analysis data of other patients was not possible, and these patients were excluded from further examination.

PSA level data were taken from medical laboratory results (Innulite procedure) of the Charité Hospital.

TP53 mutation analysis was carried out by temperature gradient gel electrophoresis (TGGE) screening of mobility shifts of GC-clamped PCR products of TP53 exons 5, 6, 7, and 8, in single reactions as described elsewhere (10). Sequence analysis was performed from TGGE extracted lanes by reamplification and solid-phase sequencing reaction (2).

Statistical analyses were carried out using SPSS 12.0.1. A type I error level of $p=0.05$ was used for all statistical tests. Cox regression was performed using the “Forward Conditional” method.

Results

TP53 mutation analysis. Samples of needle biopsy in the NB group contained mutations in 21.2% of cases. TP53 mutations were found in exon 5 (4 pts), exon 6 (3 pts), in exon 7 (5 pts), but not in exon 8. One sample (patient 63 years old, PSA 9.71 ng/ml, follow-up 42 months) contained mutations in exon 5 and in exon 7. The mutation frequency of the NB was compared with those of other patient groups. The results of this computation by Chi-square test showed no significant difference against any other patient groups.

This is outlined in Table I. Significant differences in TP53 mutation frequency between BPH (16.5%) and SPCa (30.9%) groups are reported in Table I ($p=0.001$). BPH exon 6 contained more mutations than other exons. But exon-specific differences of mutation frequencies between groups were not significant. All other patient groups have most mutations in exon 7.

Result of the sequence analysis of patient 1909 is given in Figure 1, showing a silent mutation in codon 213 of exon 6 at the time of benign histopathological diagnosis of the needle biopsy.
PSA level. Metric values of PSA are never normally distributed in any patient group. Median values are indicated as followed: NB (7 ng/ml), BPH (3.7 ng/ml), PIN (9.9 ng/ml), NPCa (11.55 ng/ml) and SPCa (8.65 ng/ml). In the NB, BPH and PIN groups, the PSA median levels were lower in patients with wild-type TP53 in their prostate tissue compared to those of the same group found to have mutated TP53. This tendency was reversed in the NPCa and SPCa groups. However, any significance of this different tendency of PSA in comparison with TP53 state was demonstrated only in the NPCa group (p=0.018: Table II).

Clinical follow-up. One patient (63 years old, PSA 9.71 ng/ml) of the NB group showed a TP53 mutation in exon 5 and in exon 7. However, this patient had no sign of malignancy after 42 months follow-up. One patient (89 years old, PSA 146.90 ng/ml) with wild-type TP53 in their needle biopsy tissue was followed up for 2 months only, but without affliction by PCa.

In all groups and subgroups with benign disease some patients were diagnosed with PCa later. In the BPH group two out of 17 patients followed (2 out of 4 with TP53 mutation, 0 out of 13 with wild-type TP53) were later affected by PCa. In the NB group 7 out of 51 (5 out of 11 with TP53 mutation, 2 out of 40 with wild-type TP53) were diagnosed with PCa during the follow-up. In the PIN group 4 out of 16 (2 out of 7 with TP53 mutation, 2 out of 9 with wild-type TP53) were affected by PCa at a later time. Data of all patients later affected by PCa are given in Table III. The effect of PCa during follow-up was computed by the Kaplan-Meier method. Results of computation are shown in Figure 2. Results of given statistical tests for the Kaplan-Meier method show significantly reduced tumour-free survival of patients with TP53 mutation in the NB group, but not of all in the BPH or PIN groups (Figure 2 a-c). In the case of available computation of the mutation status of single TP53 exons by the Kaplan-Meier method, significance was found for exon 6 and exon 7. Exon 7 results in better statistical significance in comparison with the summarized TP53 status of exons 5-8. These statistical results are shown in Figure 2 d-f, indicating significance for exon 7 in the BPH group, too. Cox regression was carried out to analyze the impact of the factors TP53-mutation, PSA-level and patient age on later affliction by PCa in the NB group. The output of the beginning block 0 (univariate analysis) by the “Forward Conditional” method shows a significance for TP53 (p=0.002), but no significance for PSA (p=0.371) or for age (p=0.658). The same tendency results from computation of the effect of exon 6, and exon 7 (Table III). In block 1 (multivariate analysis) of same computations of Cox regression, significance is given with p=0.009 for TP53 only, p=0.012 for exon 6, and p=0.001 for exon 7, respectively. This is outlined in Table IV. In the case of a one-step categorical factor, as mutation and significance for this factor, the head Exp(B) in Table IV refers to the increased probability of developing PCa. Thus Exp(B) is 15.141 for exon 7 mutations, and 8.956 for any TP53-mutation.

The difference between affliction by PCa in patients with primary benign disease (BPH+NB groups: 9/68=13.2%) versus PIN (4/16=25.0%) is not significant (Chi-square test: p=0.242) with our data.

### Table II. Statistical comparison of median PSA levels (ng/ml) between subgroups of patients with prostatic TP53WT against TP53Mutated in indicated patient groups.

|                | TP53WT | TP53Mut | p-value
|----------------|---------|---------|----------
| BPH            | 3.68    | 5.80    | 0.486    |
| NB             | 6.90    | 8.80    | 0.179    |
| PIN            | 9.65    | 9.90    | 0.777    |
| NPCa           | 15.60   | 8.03    | 0.018    |
| SPCa           | 9.49    | 6.80    | 0.358    |
| NPCa + SPCa    | 10.18   | 7.35    | 0.051    |

1Mann-Whitney U-Test (asymptotic significance), median PSA level is given in parentheses.

Figure 1. BPH patient No.1909: Sequence analysis of TP53 exon 6, region of codons 202-222. Mutation A =>G (arrow) in map position 13399 in third position of codon 213 Arg-silent.
Discussion

Assessment of p53 and PSA in combination for the outcome in prostate diseases, potentially offering improved prediction, has not yet been performed. In this study of 52 patients, using multivariate analysis to evaluate the predictive value of TP53 mutations and PSA level, TP53 was strongly predictive of PCa in patients who had a needle biopsy and a diagnosis of primary benign disease. PSA was not a significant factor for PCa affection in univariate analysis either.

In breast cancer patients a combination analysis of p53 expression and PSA in serum was done by Yu et al. in 1999 (15). It was shown that PSA(-)/p53(+) patients have a significantly higher risk for tumour relapse and death than PSA(+)/p53(-) patients. The main result of the analysis was the demonstration of the advantage of combined PSA and p53 expression status.

Poor prognosis is a well known phenomenon in tumour patients with TP53 mutations in their malignant cells (16-23). This is confirmed by our results for patients with benign prostate disease. The reason for the high significance of exon 7 mutations for this predictive value cannot be answered with our data. Exon 7 contains the region for the L3-loop of p53 (codons 236-251), partly responsible for DNA-binding by p53.
Kucera et al. did not find a relationship between prognosis and TP53 mutations in L2/L3-loop regions in breast cancer patients (22). Barnabas et al. (2001) describe exon 7 mutations conferring six out of seven patients with chronic lymphocytic leukemia to a clinically aggressive disease (25).

PSA is the best tumor marker currently available and used for screening of PCa (14, 26-28). As in breast cancer the KLK3 gene encoding PSA is downregulated in PCa cells. This gene is suspected to be a tumor suppressor. Serum levels of PSA, as reported in this study, are never indicators of gene regulation. Therefore, PSA as a prognosis factor, should be questioned for the prostate.

TP53 mutations may be factors of cell proliferation and angiogenesis in prostate tissue (29). It would be interesting to examine histological differences between wild-type and mutated TP53 prostate tissues including PSA expression.

**Conclusion**

TP53 mutations reduce the PCa-free survival time in patients with needle biopsy of the prostate and diagnosis of primary benign disease. TP53 mutations, especially the exon 7 mutations, enhance the risk of being affected by PCa 15-fold. Therefore, a TP53 mutation analysis should be carried out on needle biopsy tissues of the prostate of urological outpatients with a benign histopathological result.

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References


