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Original Paper

The Prognostic Value of p53 for Long-term and Recurrence-free Survival Following Radical Prostatectomy

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In the present study, 76 specimens (T_1–T_4) from 76 randomly selected patients undergoing radical prostatectomy at Hannover University as well as in the Josef Hospital Regensburg (13 patients) between 1980 and 1992 for whom tissue sections for immunohistochemical investigation were available, were investigated for different biological and clinical characteristics as predictors for long-term and recurrence-free survival: age, depth of tumour infiltration, histological grade, lymph node status, as well as overexpression of the p53 protein (monoclonal antibody DO-1). After a median follow-up of 50 months, 6 of 18 patients (33%) with more than 20% of tumour cells stained positively for p53 died from tumour progression compared with 9 of 58 patients (16%) with less than 20% of tumour cells positive for p53. During univariate analysis, p53 overexpression ($P = 0.011$), histological grading ($P = 0.009$) and tumour stage ($P = 0.024$) were significant prognostic factors for survival, among which only p53 overexpression ($P = 0.026$) remained an independent significant predictor in multivariate analysis. Additionally, 18 of 66 patients (27%) with less than 40% positivity for p53 suffered tumour recurrence in contrast to 6 of 10 patients (60%) with more than 40% tumour cells exhibiting a positive staining reaction. In multivariate analysis, p53 overexpression was identified as the only prognostic parameter for recurrence-free survival ($P = 0.005$). Prospective studies are needed to confirm the independent prognostic potential of p53 overexpression in patients with localised prostate cancer. The availability of more refined prognostic factors should assist decision making regarding the value of radical prostatectomy versus a surveillance strategy for prognostically defined subgroups of patients.

Key words: *p53* tumour suppressor gene, prostate cancer, prognostic value
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INTRODUCTION

ADENOCARCINOMA OF the prostate is one of the most frequent malignancies in Western countries, and in the U.S.A. it has even become one of the most common causes of death from cancer within the male population [1]. However, the clinical course of prostate cancer is rather unpredictable. While some patients die within 1–2 years of diagnosis, other patients presenting with so-called 'latent' tumours will not suffer from any symptoms of prostate cancer during their lifetime. These observations indicate the highly variable biological potential of prostate cancer [2–4].

For the determination of the biological aggressiveness of prostate cancer, easily available prognostic factors are needed which allow the clinical prognosis of the patients to be predicted. The predictive value of 'classical' tumour characteristic, such as histological grading, Gleason score (> 7) and tumour stage, tumour volume (> 12 cm^3), is clearly established [5, 6]. Nevertheless, clinical understaging might alter the utility of these pathological parameters as prognostic tools for guiding therapy. Recent investigations have tried to apply the improved understanding of tumour cell biology to determine additional tumour characteristics as possible prognostic

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parameters [7]. Such prognostically important factors might help determine the optimal treatment strategy based on the biological potential of the individual tumour.

Alterations of the *p53* tumour suppressor gene have been reported with varying frequencies ranging from 0 to 70% in prostate cancer (Table 1). Former studies have positively correlated the occurrence of p53 gene alterations with advanced tumour stages, as well as with a high Gleason score [17–23]. Additionally, a recently published investigation has suggested that overexpression of the p53 oncoprotein and the bcl-2 oncogene product are predictors for hormone-refractory disease [24]. However, the effect of p53 alterations on relapse-free [25–27] and especially long-term survival of patients undergoing radical prostatectomy for the treatment of primary, clinically localised prostate cancer has rarely been investigated [28, 29].

The aim of the present study was to correlate an immunohistochemically detected overexpression of the p53 oncoprotein with other biological variables, such as age, tumour stage, histological grading and regional lymph node status and to investigate the prognostic value of these parameters for relapse-free and overall survival in 76 patients undergoing radical prostatectomy for different stages of newly diagnosed prostate cancer.

PATIENTS AND METHODS

Patients

76 randomly selected patients, treated in the Urology Department of Hannover Medical School and in the Department of Urology of Josefs Hospital Regensburg (13 patients) between 1980 and 1992 for clinically localised prostate cancer, for whom tissue sections for immunohistochemical investigation were available, were included in the present study. Prior to radical prostatectomy, the presence of distant metastases was excluded by abdominal computerised tomography (CT) scans, X-rays of the lungs and bone scans. The median age of the patients was 63 years (range: 51–78 years). Following pelvic lymph node dissection and radical prostatectomy, tumour specimens were pathologically classified as T_1 (5 patients; 7%), T_2 (37 patients; 49%), T_3 (31 patients; 41%) and T_4 (3 patients; 4%) according to the TNM system. Nine tumours (12%) were histologically graded as G_1, 44 tumours (58%) as G_2 and 23 tumours (30%) as G_3. The final histopathological investigation of the dissected regional lymph nodes, in all patients classified as tumour-free during intra-operatively performed histopathological examination, revealed regional lymph node metastases in 6 cases (N_1, 4 patients; N_3, 2 patients). All patients were followed after prostatectomy by transrectal ultrasound, bone scans and determination of the serum PSA levels (Hybritech assay, Hybritech, Germany) every 6 months. The median follow-up after radical prostatectomy was 50 months (3–151 months). In cases of a suspicious digito-rectal examination (DRE) or rising levels of prostate specific antigen (PSA) or prostate acid phosphatase (PAP) (in patients treated before the availability of PSA assays) during follow-up, patients received a scan of the abdomen, an X-ray of the lungs and a bone scan. In cases of local recurrence (8 cases), patients were treated with external beam radiation (60–70 Gy), whereas a systemic tumour progression (16 patients) was treated with complete androgen ablation (CAB) either by bilateral orchidectomy or by the administration of a luteinising hormone-releasing hormone (LHRH) analogue in combination with flutamide.

Immunohistochemistry for the detection of p53 *alterations*

Formalin-fixed and paraffin-embedded tissue sections were investigated for overexpression of the p53 oncoprotein using an immunohistochemical approach. p53 immunoreactivity was also studied in 10 biopsy specimens obtained from non-tumour carrying patients undergoing transvesical prostatectomy for the treatment of benign prostatic hyperplasia (BPH). Positive controls were represented by 13 prostate cancer and 12 bladder tumour specimens known to contain a mutational inactivation of the *p53* tumour suppressor gene as detected by DNA sequence analysis. As an internal negative control for the staining procedure, each tumour in the study was incubated with non-immune mouse IgG instead of the primary antibody, followed by the identical procedure for the application of the secondary antibody. Following dewaxing, 8 μm paraffin-embedded, as well as 4 μm fresh frozen slides were cut serially and stained for the p53 oncoprotein.

Paraffin sections were picked up on 3-aminopropyltriethoxysilan (APES)-coated slides, dried for 1 day at room temperature and for an additional 3–4 h at 40°C. For antigen retrieval, sections were incubated with 0.1 M citrate buffer (pH 6) for 5–6 h at 70°C. Endogenous peroxidase activity was blocked by incubation for 30 min at room temperature in 3% hydrogen peroxidase diluted in phosphate buffered saline (PBS) (0.5 M, pH 7.4). After rinsing in PBS/0.1% Tween 20, the tumour-bearing slides were incubated with normal human serum at a dilution of 1:100 in PBS for 30 min to prevent non-specific binding of the first antibody. Then, the specific monoclonal primary antibody for the detection of the p53 oncoprotein (DO-1) was added. This mouse monoclonal antibody recognises a denaturation-resistant epitope in both wild-type and mutant p53 proteins and enables the detection of altered p53 proteins within the cell nucleus because of their prolonged half-life caused by conformational changes as a result of the genetic mutation [30]. The DO-1 antibodies were applied at a dilution of 1:50 in PBS at room temperature for 1 h in a moist chamber. After rinsing with PBS/0.1% Tween 20 for 10 min, a standard streptavidin–biotin complex (Vectastain, Burlingame, California, U.S.A.) method was applied according to the instructions of the manufacturer.

Classification of immunohistochemistry

The tumours were classified into four groups according to the percentage of nuclei exhibiting a positive immunohistochemical staining reaction for the p53 protein: (1) negative reaction or $< 20\%$ positivity; (2) $\geq 20 - < 40\%$ positivity; (3) $\geq 40 - < 60\%$, (4) $\geq 60\%$ positivity. The immunohistochemical reaction for the p53 protein was considered positive only where there was nuclear staining. Five separate slides per tumour were reviewed and classified by two independent investigators. For the classification of the immunohistochemical staining reaction, the percentage of positively stained tumour cells observed in each of these five tissue slides was estimated in correlation to the total number of tumour cells identified. This kind of semiquantitative analysis has been described previously for the classification of p53 immunohistochemistry in advanced stage bladder cancer [31]. Additionally, in five microscopic fields (magnification 240-fold) per tissue slide, 400–500 tumour cells were counted irrespective of the result of the immunohistochemical staining reaction. Thereafter, the percentage of immunohistochemically positive cells was determined in order to check the result of the previously performed semiquantitative

Table 1. *Summary of literature data regarding the frequency of* p53 *gene alterations in prostate cancer, including the correlation with tumour characteristics such as stage, histological differentiation and responsiveness to hormone ablation as well as the correlation between immunohistochemically detected overexpression of the p53 oncoprotein and detection of* p53 *gene alterations on the RNA and DNA level as detected by a molecular genetic approach*

Reference	Tumours investigated for *p53* gene alterations (*n*)	Investigational approach	Frequency of p53 alterations	Concordance between IHC and molecular genetic investigation	Detection of p53 alterations in correlation with tumour stage	Microdissectional tumour cell preparation
Suzuki and associates [8]	29 (stage B) 22 > stage B, hormone-refractory and metastatic tumours	PCR-directed DNA SSCP and DNA sequence analysis	7 of 51 (14%)	Not investigated	Stage B: 0% > Stage B: 7 of 22 (32%)	None
Latil and associates [9]	20 ($\leq T_2$)	LOH analysis	None	Not investigated	–	None
Moyret and associates [10]	27 (stage A–D)	IHC, DNA SSCP analysis, DNA sequencing	2 of 19 (2%)	100%	No correlation	None
Heidenberg and associates [11]	26 (hormone-refractory, progressive following radiation therapy) 27 (stage A–C) 8 (metastatic tumours)	IHC, cold DNA SSCP, DNA sequencing	26 of 52 (50%)	9 of 11 (82%)	Progressive PCA: 16/17 (94%) Localised PCA: 6/27 (22%) Metastatic PCA 4/8 (50%)	Yes
Mirchandani and associates [12]	65 (stage A–D)	DNA SSCP analysis, DNA sequencing	12%	Not investigated	Localized PCA; 10% Metastatic PCA: 21%	Yes
Kubota and associates [13]	21 (stage A–D)	RNA SSCP, DNA sequencing	4 of 21 (19%)	Not investigated	3/4 PCA revealing p53 alterations: stage C/D	
Konishi and associates [14]	15 (latent PCA) 32 (stage A–D)	IHC, DNA SSCP, DNA sequencing	5 of 32 (16%)	Not given	Stage B: 2/13 (15%) Stage C: 2/7 (29%) Stage D 1/11 (9%) Latent tumours: 0%	None
Hall and associates [15]	37 (stage A–D)	IHC, DNA SSCP, DNA sequencing	1 of 37 (3%) (DNA analysis in case of IHC positivity)	100%	Statement not possible due to low frequency of p53 mutations	Yes
Chi and associates [16]	44 (stage A–D) 4 (metastatic lesions)	RT PCR, Southern blotting, quantitative RNA PCR, RNA SSCP analysis, DNA sequencing	20 of 48 (42%)	Not investigated	Stage B: 11/26 (42%) Stage C: 5/14 (36%) Stage D: 1/4 (25%) Metastases: 3/4 (75%)	Yes
Dinjens and associates [17]	20 primary tumours 15 LN metastases	IHC, DNA SSCP, DNA sequencing	2 of 10 primary tumours (10%), 15% of LN metastases	100%	Frequency of p53 alterations in LN metastases not increased	None
Navone and associates [18]	92 patients (primary tumours stage A–D and bone metastases)	IHC, DNA SSCP, DNA sequencing	20 of 92 (22%) 18 of 40 bone metastases (45%)	100%	Tumours revealing p53 overexpression: stage D, dedifferentiated and hormone-refractory/p53 accumulation identified as typical for hormone-refractory tumours	None
Bookstein and associates [19]	150 patients	IHC, DNA SSCP, DNA sequencing	19 (13%)	Detection of *p53* gene alterations in 9/14 IHC positive and in none of IHC negative tumours	Stage C/D-69 PCA (23%) < Stage C: 4%	None

PCA, prostate cancer; LN, lymph node; IHC, immunohistochemistry; PCR, polymerase chain reaction; RT, reverse transcription; SSCP, single strand conformation polymorphism, LOH, loss of heterozygosity.

analysis. For analytical purposes, the highest category obtained in each patient was considered.

Univariate analysis using a log rank test was employed for each possible prognostic factor alone to determine its prognostic significance for survival. For p53 immunohistochemistry and according to our experience with advanced stage bladder cancer, patients were divided into groups revealing $> 20\%$, $\geq 20 - < 40\%$, $\geq 40 - < 60\%$, $\geq 60\%$ positivity and for each of these cut-off levels, the correlation between positive staining and tumour-free as well as overall survival was calculated according to our previously reported investigations in advanced stage bladder cancer [31]. Spearman correlation coefficients were used to calculate the influence of different variables on the immunohistochemical reactivity for the p53

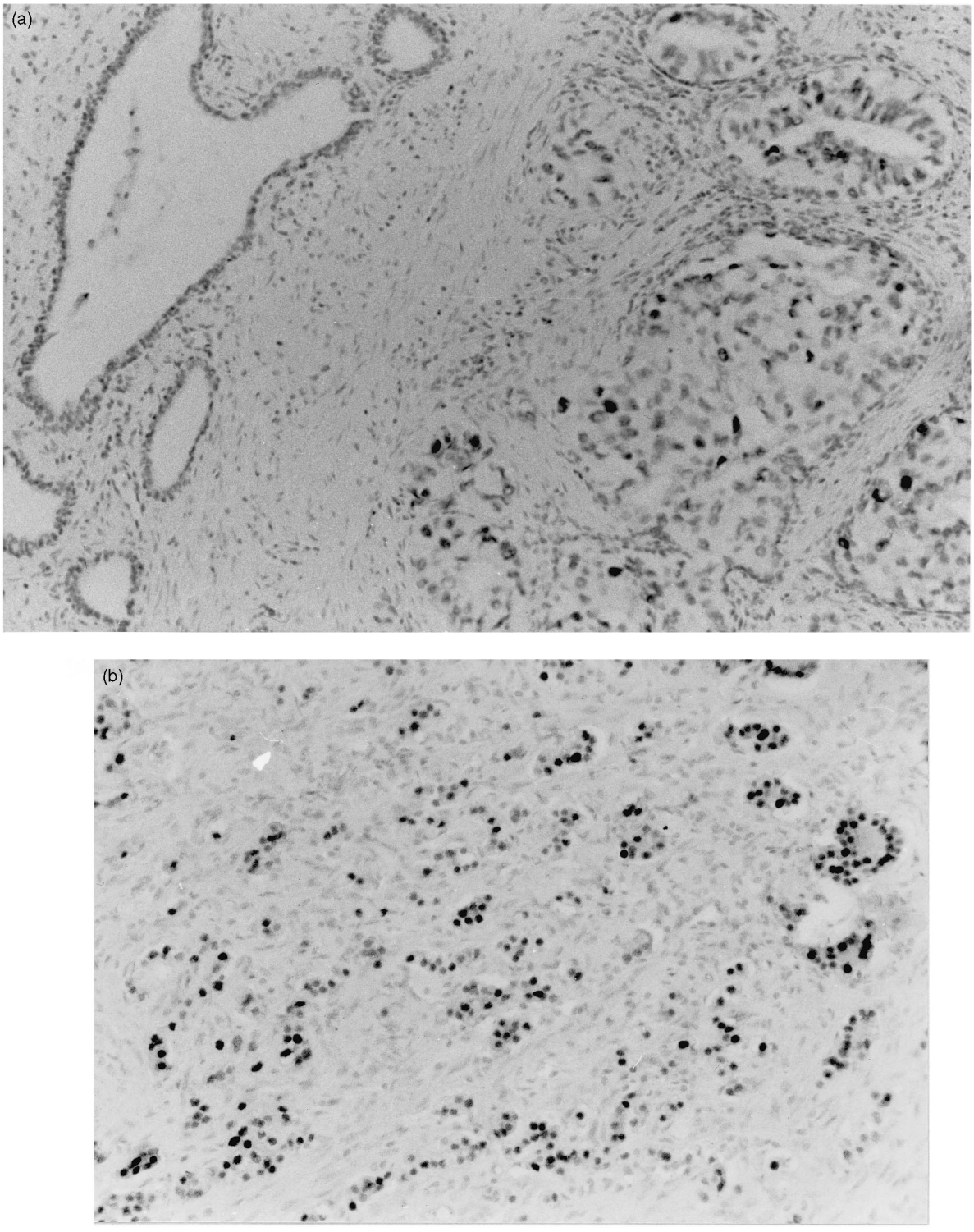

Figure 1. Immunohistochemical staining of p53 oncoprotein in a highly and a poorly differentiated adenocarcinoma of the prostate with a relative amount of (a) 20–40% and (b) > 60% of tumour cells exhibiting a positive staining reaction (magnification 240-fold, ABC staining kit).

protein. Tumour-free survival was calculated from the time of radical prostatectomy to either relapse in the form of rising PSA or PAP levels or the diagnosis of a local recurrence or metastatic lesions. Overall survival was calculated according to the Kaplan–Meier method from the time of radical prostatectomy and either death or date of last follow-up. Finally, multivariate Cox regression analysis was used to determine whether any of the factors tested—age, sex, tumour stage, lymph node status, histological grade or p53 positivity—could be identified as independent prognostic factors.

RESULTS

p53 overexpression and overall survival

After a median follow-up of 50 months (3–151 months) 15 of 76 patients (20%) had died from tumour progression and 24 patients (32%) had a tumour recurrence. The radical prostatectomy specimens investigated for overexpression of the p53 oncoprotein using the monoclonal antibody DO-1 were classified as follows: negative reaction, 51 patients (67%); 1–20% positivity, 7 patients (9%); 20–40% positivity, 8 patients (11%); 40–60% positivity, 9 patients (12%); 60–80% positivity, 1 patient (1%) (Figure 1). All 10 cases of benign prostatic hyperplasia, as well as the stromal cells within the prostate cancer specimens, were completely negative for p53 immunostaining. A positive staining reaction as a predictor of overall survival achieved statistical significance at a cut-off value of $\geq 20\%$ of cells stained positively for the p53 oncoprotein (log rank test). Based on this result, 58 patients (76%) were classified into group A ($< 20\%$ positivity for the p53 oncoprotein) and 18 patients (24%) into group B ($\geq 20\%$ positivity) (Table 2).

6 of 18 (33%) patients from group B died from tumour progression, in contrast to 9 of 58 (16%) patients of group A. The calculated mean survival times were 71 months (31–151 months) for patients from group A, median follow-up for group A patients: 60 months, (9–151 months and 47 months (4–106 months) for group B patients median follow-up for group B: 41 months (3–106 months). This difference was statistically significant ($P = 0.01$) (Table 3). Median survival is shown in Figure 2. 8 of 9 group A patients dying from tumour progression exhibited a completely negative staining reaction for the p53 oncoprotein, while 1 had $< 20\%$ positivity.

With 12 of 18 (67%) T3, and 8 of 18 (44%) G3 patients in group B, there was a slightly higher tendency towards more advanced tumour stages and histological dedifferentiation in comparison with group A (Table 2). With respect to other clinical characteristics, patients from groups A and B were comparable.

Statistical analysis of further prognostic parameters

Univariate statistical analysis demonstrated that the time of survival following radical prostatectomy was independent of age ($P = 0.83$), the diagnosis of regional lymph node metastases ($P = 0.476$), the histological growth pattern ($P = 0.109$) and the serum PSA and PAP levels ($P = 0.385/P = 0.923$). However, for overall survival, a significant correlation was found with histological grading ($P = 0.009$) and tumour stage ($P = 0.024$). With a cut-off value of $\geq 20\%$ for overall survival, p53 positivity was significantly correlated with the clinical prognosis of the patients (Figure 2; $P = 0.011$). Spearman correlation coefficients were determined to compare p53 overexpression with patient age ($P = 0.27$), PSA level ($P = 0.96$), PAP level ($P = 0.54$) and the presence of regional lymph node metastases ($P = 0.21$). These variables were not significantly correlated with p53 protein overexpression. However, p53 positivity was significantly correlated with the histological grading of the tumours ($P = 0.01$) and with tumour stage ($P = 0.001$). In a multivariate analysis, reactivity for the p53 oncoprotein ($\geq 20\%$) proved to be the only

Table 2. Prostate cancers investigated for the correlation between p53 overexpression and long-term survival of patients. Detailed characterisation of patients from group A ($< 20\%$ relative amount of tumour cells stained positively for the p53 oncoprotein) and group B ($\geq 20\%$ positivity). Except for the higher number of advanced stage tumours classified into group B, patients from groups A and B were comparable regarding other characteristics of possible prognostic value

Patient and tumour characteristics	Patients from group A ($< 20\%$ of tumour cells stained positively for the p53 protein)	Patients from group B ($\geq 20\%$ of tumour cells stained positively for the p53 protein)
Patients (*n*)	58	18
Age (years)	62 (52–76)	65 (54–75)
Average follow-up (months)	60 (9–151)	41 (3–106)
T stage (%)		
T1	5 (9)	–
T2	32 (55)	5 (28)
T3	19 (33)	12 (67)
T4	2 (3)	1 (6)
Histological grading (%)		
G1	9 (16)	–
G2	34 (59)	10 (56)
G3	15 (26)	8 (44)
Presence of regional lymph node metastases at the time of radical prostatectomy		
N1	3	1
N2	–	–
N3	1	1
Median long-term survival (months)	71 (31–151)	47 (4–106)

Table 3. *Prognostic factors for long-term survival in* 76 *patients following radical prostatectomy*

Factor investigated	Univariate prognostic value (*P*)	Multivariate prognostic value (*P*)
Age	No (0.83)	No (0.081)
Histological grading	Yes (0.009)	No (0.064)
Tumour stage (T)	Yes (0.024)	No (0.705)
Lymph node metastases	No (0.476)	No (0.784)
p53 positivity ($\geq$ 20% positive)	Yes (0.011)	Yes (0.026)
Serum PSA level	No (0.385)	No (0.531)
Serum PAP level	No (0.923)	No (0.761)

PSA, prostate specific antigen; PAP, prostate acid phosphatase.

statistically relevant prognostic factor for overall survival of patients ($P = 0.026$) (Table 3).

p53 overexpression and tumour recurrences

Local recurrences (8 patients) and the development of distant metastases (16 patients) were observed in 24 of 76 (32%) patients. Univariate analysis using the log rank test was employed for each biological variable alone to determine its prognostic significance for relapse-free survival. For p53 immunohistochemistry, patients were divided into groups revealing > 20, > 40 and > 60% positivity and, according to our previously reported experience regarding the evaluation of the prognostic importance of p53 protein overexpression for advanced stage bladder cancer [31], for each of these three cut-off levels, the correlation between positive staining and time to tumour recurrence was calculated. A positive staining reaction as a predictor of recurrence-free survival achieved statistical significance at a cut-off level of $\geq$ 40% of cells stained positively for the p53 oncoprotein (log rank test). Based on this calculation 66 patients (87%) were classified as < 40% positivity and 10 patients (13%) as $\geq$ 40% positivity. 18 of 66 patients (27%) with < 40% positivity (local recurrence, 5 patients; systemic progression, 13 patients) and 6 of 10 (60%) patients with $\geq$ 40% positivity (local recurrence, 3 patients; systemic progression, 3 patients) developed recurrent disease. The mean relapse-free survival time following radical prostatectomy was 40 months (7–133 months) for patients with < 40% positivity (median follow-up was 58 months, range 3–151 months) and 30 months (5–72 months) for patients with $\geq$ 40% positivity (median follow-up was 40 months, range 4–106 months) ($P = 0.003$). There were no imbalances for other investigated variables between the two groups.

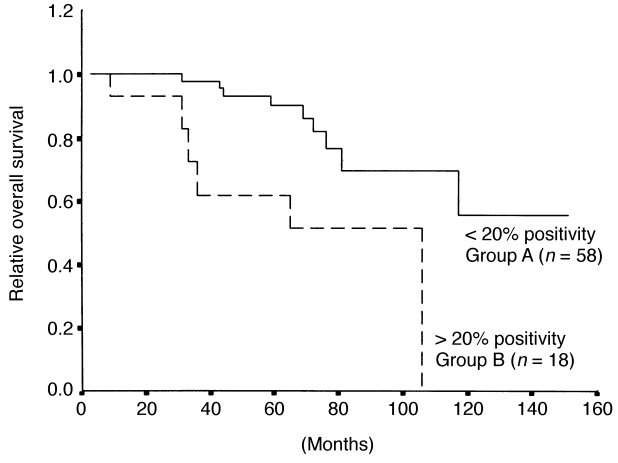

Figure 2. Long-term survival following radical prostatectomy, calculated according to the Kaplan–Meier method. Classification into Group A (< 20% positively stained tumour cells) and Group B ($\geq$20% positivity). Group B patients had a significantly decreased long-term survival following radical prostatectomy ($P = 0.01$, log rank test).

Statistical analysis of further prognostic parameters

Univariate analysis demonstrated that tumour-free survival following radical prostatectomy was independent of age ($P = 0.714$), histological grading ($P = 0.06$), the presence of regional lymph node metastases ($P = 0.33$), as well as serum PSA and PAP levels ($P = 0.814$/P = 0.401). Tumour stage ($P = 0.04$) and, with a cut-off value of $\geq$ 40%, p53 positivity were significantly correlated with the recurrence-free survival of the patients ($P = 0.0025$) (Table 4). In multivariate analysis, reactivity for the p53 oncoprotein ($P = 0.005$) proved to be the only statistically relevant prognostic factor for recurrence-free survival of patients.

DISCUSSION

In the present study, p53 accumulation was demonstrated in only 5 patients with tumours $\leq$ stage T_2. Similar to the outcome of previously reported investigations, this finding might indicate that, in contrast to bladder cancer, inactivation of the *p53* tumour suppressor gene seems not to participitate in the very early stages of tumour development. Overexpression of the p53 oncoprotein was clearly identified as an independent prognostic factor for survival in patients with clinically localised prostate cancer. Patients with $\geq$ 20% of cells positive for p53 had a mean survival of 47 months compared with 71 months for patients with < 20% of cells with p53 positivity (multivariate $P = 0.026$). With a cut-off value of 40%, p53 positivity was also an independent predictor of the likelihood for tumour recurrence (univariate $P = 0.0025$; multivariate $P = 0.005$).

Visakorpi and colleagues [29] were the first to correlate p53 alterations with a decreased recurrence-free and long-term survival of patients with primary prostate cancer. Thomas and associates [32] have also correlated an immunohistochemically detected overexpression of the p53 oncoprotein with decreased long-term survival and a tendency towards tumour progression in 68 patients undergoing radical prostatectomy for the treatment of clinically localised prostate cancer. The recurrence-free survival time for patients with and without p53 alterations was 12 and 24 months, respectively, with

Table 4. *Prognostic factors for relapse-free survival in* 76 *patients following radical prostatectomy for the treatment of clinically localised prostate cancer*

Factor investigated	Univariate prognostic value (*P*)	Multivariate prognostic value (*P*)
Age	No (0.714)	No (0.124)
Histological grading	No (0.06)	No (0.353)
Tumour stage (T)	Yes (0.04)	No (0.06)
Lymph node metastases	No (0.33)	No (0.312)
p53 positivity ($\geq$ 40% positive)	Yes (0.0025)	Yes (0.005)
Serum PSA level	No (0.814)	No (0.635)
Serum PAP level	No (0.401)	No (0.872)

PSA, prostate specific antigen; PAP, prostate acid phosphatase.

a long-term survival of 40 versus 76 months for patients with and without overexpression of the p53 oncoprotein. Shurbaji and colleagues [27] investigated p53 overexpression as a predictor of tumour-free survival in 109 patients treated with radical prostatectomy with a median follow-up of 3.8 years (1.3–9.3 years). For tumours with a Gleason score between 2 and 7, p53 overexpression was identified as the only biological variable of independent prognostic value during multivariate analysis ($P < 0.007$).

Bauer and associates [28] investigated the prognostic importance of immunohistochemically detected p53 and bcl-2 overexpression for the relapse-free survival of 175 patients undergoing radical prostatectomy for the treatment of primary prostate cancer. With an overall 5-year recurrence rate of 67%, 114 of 175 tumours (65%) exhibited a positive staining reaction for the p53 oncoprotein. Recurrence rates for patients with and without p53 overexpression were 51% versus 22% [28]. Overall, 24% of tumours exhibited a positive staining reaction for the p53 protein, confirming the relatively low frequency of p53 alterations in primary and clinically localised prostate cancer reported. In the study reported by Bauer and associates [28] the enormously high frequency of positively stained tumours (65%) might either be due to a low specificity of the antibody used or a relatively high amount of advanced stage tumours included in this study, possibly explaining the high recurrence rate of > 60%.

Yang and colleagues [26] suggested that only a small population of cells within the primary tumour exhibiting p53 positivity might be responsible for metastatic potential and biological aggressiveness. Consequently, p53 positivity was defined as at least 15 positively stained tumour cells within a $300 \times 400\ \mu m^2$ field, a feature termed 'clustered staining reaction'. Whereas clustered immunopositivity was demonstrated in 10 of 16 patients (63%) with tumour recurrence following radical prostatectomy, only 7 of 33 patients (21%) presenting with a negative staining reaction suffered from recurrent disease ($P < 0.01$).

In contrast to the results reported by Thomas and associates [32], only 8 of 58 (14%) of our patients exhibiting a completely negative staining reaction for p53 oncoprotein died from cancer progression during the observation period and only 18 of 66 (27%) patients with < 40% of positively stained tumour cells had a tumour recurrence.

The slightly higher cut-off value of 40% positivity identified as prognostically important for recurrence-free survival might be explained by the hypothesis that a higher number of cells with *p53* gene alteration will lead to a faster recurrence. However, death also occurred in p53 negative patients, indicating that either our methods are not thorough enough or that other factors may also be involved. Another factor of interest may be the bcl-2 oncogene [24, 28].

In our study, the presence of regional lymph node metastases was not identified as a prognostic parameter for long-term and recurrence-free survival. This might be explained by the small number of patients with lymph node metastases included in the present investigation. For accepted prognostic factors, including tumour stage and histological grading, an independent prognostic value was not demonstrated by multivariate analysis, possibly resulting from the limited number of patients investigated. Although all patients were randomly selected from the whole group of men undergoing radical prostatectomy at Hannover Medical School between 1980 and 1992, according to our experience, the limited availability of tissue sections, as well as the difficult evaluation of long-term follow-up in these elderly patients, hamper retrospective studies for determining prognostically important biological characteristics. Consequently, these difficulties for the recruitment of patients might result in an unintentional selection bias and a possible impact on the statistical analysis cannot be excluded. Although current data are promising, prospective trials will have to confirm the prognostic role of p53 for long-term and recurrence-free survival of patients with primary prostate cancer, either in addition to or independent of established prognostic parameters, before molecular prognostic parameters should affect our decisions about treatment options for prostate cancer, such as radical prostatectomy or a surveillance strategy.

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