Journal of Pathology
J Pathol 2002; **197**: 148–154.
Published online 21 March 2002 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1084

Original Paper

Value of tissue markers $p27^{kip1}$, MIB-1, and CD44s for the pre-operative prediction of tumour features in screen-detected prostate cancer

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Received: 23 August 2001
Accepted: 8 January 2002

Abstract

The pre-operative prediction of prognostic tumour features in the radical prostatectomy specimen using routine clinicopathological variables remains limited. The present study evaluated the predictive value of the cell-cycle protein $p27^{kip1}$, the proliferation marker MIB-1, and the cell-adhesion protein CD44s, determined on the diagnostic needle biopsy of asymptomatic men screened for prostate cancer. Of 81 screen-detected prostate cancers, representative biopsy cores and matched radical prostatectomy specimens were immunohistochemically stained for these tissue markers. Conventional pre-operative and post-operative clinicopathological variables were assessed and cancers were divided according to a validated tumour classification model (potentially harmless, clinically significant). Low ($<50\%$) $p27^{kip1}$ expression, high ($\geq 10\%$) MIB-1 expression, and low ($<25\%$) CD44s expression were considered adverse prognostic signs. Binary logistic regression analysis was performed to assess the most valuable predictors of clinically significant disease. An adverse prognostic immunostaining assessment on the biopsy was found in 10 (12.3%), 17 (21.0%), and 25 (30.9%) cases for $p27^{kip1}$, MIB-1, and CD44s, respectively. The concordance in tissue marker assessment between the biopsy specimen and matched radical prostatectomy specimens was low for all three. The positive predictive value (PPV) of $p27^{kip1}$ was 90.0%, remarkably higher than that of MIB-1 and CD44s (41.2% and 52.0%, respectively), indicating that a low radical prostatectomy $p27^{kip1}$ score is expected if the biopsy $p27^{kip1}$ score is low. Logistic regression analysis revealed that biopsy Gleason score ($p<0.01$) and $p27^{kip1}$ assessment ($p<0.01$) remained the only significant predictors of clinically significant disease. All cases with low $p27^{kip1}$ expression were found to have clinically significant disease after radical prostatectomy. The assessment of $p27^{kip1}$ in the biopsy specimen might thus assist in distinguishing between potentially aggressive and potentially non-aggressive disease in prostate cancer screening. **Copyright © 2002 John Wiley & Sons, Ltd.**

Keywords: prostate; carcinoma; screening; $p27^{kip1}$; MIB-1; CD44s; prognostic tissue marker; biopsy; radical prostatectomy

Introduction

Despite prognostic information gained from serum prostate-specific antigen (PSA), clinical tumour stage, and tumour grade assessed on prostatic needle biopsies, the accuracy of predicting prostate cancer tumour characteristics in the radical prostatectomy specimen and with this, the final outcome of screen-detected prostate cancer, remains limited [1–4]. The vast majority of cancers are diagnosed within the PSA range 3.0–9.9 ng/ml, with biopsy Gleason scores 6 or 7, and with clinical tumour stage T_{1c}–T_2 [5,6], but the biological behaviour of these tumours is highly variable. Some might have been treated unnecessarily as the post-operative prognostic tumour features proved highly favourable, while others might have too advanced disease to be cured. It is therefore appropriate to seek to refine the prognostic information gained from pretreatment variables, and from prostate cancer biopsy specimens in particular.

Recently, several immunohistochemical studies have demonstrated that the cell-cycle protein $p27^{kip1}$, the proliferation marker Ki-67 (MIB-1), and the cell-adhesion protein CD44s have independent prognostic value with respect to disease recurrence and patient survival after radical prostatectomy [7–13]. Potentially, these tissue markers might help in differentiating aggressive from non-aggressive cancers before definitive treatment. In the present study, we assessed whether the immunohistochemical expression of these markers on the diagnostic needle biopsy was representative of that in matched radical prostatectomy specimens. We examined the predictive value of these tissue markers for well-established prognostic factors such as pathological tumour stage, tumour grade, and tumour volume in the radical prostatectomy specimen. It was anticipated that the tumour marker thus identified as most suitable for application on the needle biopsy might give the clinician additional information on

tumour aggressiveness and patient prognosis on a pretreatment basis.

Materials and methods

Patients

Between 1 January 1998 and 15 September 1999, 99 consecutively admitted men within the screening arm of the European randomized study of screening for prostate cancer (ERSPC) underwent bilateral pelvic lymph-node dissection and radical prostatectomy at the University Hospital Rotterdam. In all screened participants, prostate cancer was diagnosed on ultrasound-guided sextant transrectal biopsy of the prostate, prompted by an elevated ($\geq$3.0 ng/ml) serum-PSA level. Neo-adjuvant (hormonal) treatment was not applied in any of the patients. No patient had pelvic lymph-node metastatic disease, either on intra-operative examination of frozen tissue sections, or after the examination of paraffin slides. Of 81 surgically treated patients, tissue from both the diagnostic needle biopsy and the matched radical prostatectomy specimens was available for (immunohistochemical) analysis. Pre-operative PSA and clinical tumour stage were obtained from the ERSPC database.

Pathological tissue examination

All sextant diagnostic biopsy cores were labelled and processed separately. The biopsy cores were routinely fixed in 10% buffered formalin (pH 7.5), embedded in paraffin wax, freshly cut into 4 μm tissue sections, and mounted on glass slides. Haematoxylin and eosin (H&E) slides of three subsequent levels of the needle biopsy were histologically examined and a Gleason score was assigned by a specialist genito-urinary pathologist (THvdK) [14].

Radical prostatectomy specimens were fixed similarly, schematically cut [15], embedded in paraffin wax, cut into 4 μm tissue sections, and mounted on glass slides. The tumour was staged according to the TNM'97 system and the Gleason score was determined. All tumour areas were traced and outlined on the slides. Detailed prostate maps were developed to illustrate the size, extent, and location of the prostate tumour and its different histopathological grades (Figure 1). Morphometric analysis was performed to assess the tumour volume as described by Hoedemaeker *et al.* [16]. Finally, cancers were categorized according to a previously developed and validated prognostic tumour classification model, including pathological tumour stage, tumour volume, and the proportion of high-grade cancer [17]. According to this classification model, organ-confined cancers with a tumour volume less than 0.5 ml, without Gleason growth patterns 4 and 5, were considered potentially 'harmless', while all other cancers were arbitrarily assessed as 'clinically significant' [17,18].

Selection of the most representative slides

The selection of the most representative biopsy core was made by an experienced pathologist (THvdK). The selection was based on the assumption that the observed tumour features within the slide would be most predictive of patient outcome. The most representative core was the core with the highest Gleason score or when the Gleason score was not different between biopsies, the core with the most extensive tumour involvement. The biopsy slides were stored for a maximum of 2.5 years in a dark environment at room temperature until immunostaining was performed.

The most representative slides within the radical prostatectomy were similarly selected. One to three paraffin blocks with tumour tissue most representative of the whole tumour within the radical prostatectomy specimen were selected for immunohistochemical analysis. Using radical prostatectomy maps, the site, range, and trajectory of the individual biopsy cores, and that of the most representative biopsy core in particular, were reconstructed (Figure 1). In doing so, one may determine whether the representative biopsy needle hit or did not hit the representative tumour parts within the radical prostatectomy specimen.

Immunostaining

Slides from biopsies and radical prostatectomy specimens were immunohistochemically stained according to similar protocols. Tissues from the radical prostatectomy specimens were freshly cut, while those of the biopsy specimens were retrieved from storage. After deparaffinization through xylene and 100% ethanol, endogenous peroxidase activity was blocked by immersing the slides for 20 min in 3% H_2O_2 in methanol. The slides were placed in a 10 mmol/l citrate buffer at pH 6.0. Antigen retrieval was performed in a microwave oven at 700 W for 15 min. After cooling, the slides were pre-incubated with 10% normal goat serum in PBS/BSA 5%. The slides were incubated overnight at 4°C with the primary antibody MIB-1 (Immunotech, France) at an optimal dilution of 1 : 3000, $p27^{kip1}$ (Novocastra, UK) at 1 : 40, or CD44s (Bender MedSystems, Austria) at 1 : 200 in PBS/BSA 5%. Negative controls were included with each batch of slides. For MIB-1 and $p27^{kip1}$, the conventional avidin–biotin complex method was applied. Briefly, a 30 min incubation with biotinylated goat-anti mouse antibody (Biogenex, San Ramon, USA) was followed by a 30 min incubation with streptavidin–peroxidase complex (Biogenex). For CD44s, the catalysed signal amplification (CSA, K1500, DAKO) system was used. After overnight incubation with the primary antibody, a 15 min incubation with a linking antibody was followed by a 15 min incubation with streptavidin–biotin complex, a 15 min incubation with an amplification reagent, and a final 15 min incubation with streptavidin–peroxidase. In all slides, the antibody–antigen binding was visualized with diaminobenzidine hydrochloride (Fluka, Neu-Ulm, Germany) with 0.08%

H_2O_2 for 7 min. The specimens were counterstained with haematoxylin, dehydrated, and covered.

Quantitation

All slides were assessed by two independent observers (ANV, BWvR) without knowledge of matched biopsy or radical prostatectomy tumour features. In the event of discrepancy between observers, the slides were reassessed in a combined session. Agreement between observers occurred in over 80% of cases for all three tissue markers. All selected sections contained benign prostatic glands, which could serve as internal positive controls. For all three tissue markers, the immunostaining quantitation was similar for both the biopsy specimens and the radical prostatectomy specimens.

For $p27^{kip1}$, nuclear staining was assessed by estimating a positive-to-total ratio as previously described [7]. A tumour was considered 'high' for $p27^{kip1}$ expression if 50% or more nuclei showed positive immunostaining, and 'low' if a positive-to-total ratio of less than 50% was recorded [7] (Figure 2). In the case of tumour heterogeneity, those parts within the tumour that showed the lowest positive-to-total ratio were assessed. For MIB-1, nuclear staining was assessed by estimating the percentage of MIB-1-positive cells [7]. Tumours with 10% or more nuclei positive for MIB-1 were considered 'high' for MIB-1 expression, whereas those with less than 10% of MIB-1 positivity were assessed 'low' for MIB-1 expression. If the tumour exhibited heterogeneous MIB-1 expression, the area with the highest density of MIB-1-positive cells was selected. Slides stained with CD44s were assessed according to the percentage of cells showing positive membranous immunostaining (Figure 2). Since less than 25% negative immunostaining has been reported to be most predictive of clinical progression after radical prostatectomy [12], this cut-off point was taken for statistical analysis. Slides were assessed as having 'low' ($<25\%$) or 'high' ($\geq 25\%$) tumour CD44s expression. A tumour CD44s score was obtained by taking the lowest assessed score within the tumour sections.

The concordance in tissue marker assessment between the biopsy and the radical prostatectomy specimen was determined for all three tissue markers. An adverse prognostic assessment (low $p27^{kip1}$, high MIB-1, low CD44s) was considered a positive test outcome. Sensitivity, specificity, and the positive (PPV) and negative predictive values (NPV) of tissue marker assessment were calculated. Similar analyses were performed with respect to biopsy tissue marker assessment and clinical significance of disease.

Statistical analysis

Statistical analysis was performed using SPSS 9.0 (SPSS Inc., Chicago, IL, USA). The association between $p27^{kip1}$, MIB-1, and CD44s expression on the biopsy and conventional clinicopathological parameters was evaluated using the Pearson chi-squared (χ^2) test. The pre-operative PSA level was categorized as 3.0–3.9 ng/ml, 4.0–5.9 ng/ml, 6.0–9.9 ng/ml, or ≥ 10.0 ng/ml; clinical tumour stage as T_{1c} or T_{2a-b}; Gleason score as 2–6, 7, or 8–10; and the proportion of high-grade cancer as 0%, 0–9%, 10–49%, or $\geq 50\%$. Post-operative variables were categorized as listed in Table 1.

Binary logistic regression analysis was performed to assess the statistical significance of pre-operative variables. Clinically significant disease was taken as the dependent variable, while conventional pre-operative clinicopathological variables, and the expression level of tissue markers on the biopsy, were taken as covariates. Variables that were not statistically significant at the univariate level were removed, while controlling for

Table 1. The association of the expression of $p27^{kip1}$, MIB-1, and CD44s with pathological tumour stage, Gleason score, and the tumour classification model. Numbers in parentheses are percentages

	Tissue marker expression on the diagnostic biopsy						
	$p27^{kip1}$		**MIB-1**		**CD44s**		
Radical prostatectomy tumour features	**Low ($<50\%$)**	**High ($\geq 50\%$)**	**Low ($<10\%$)**	**High ($\geq 10\%$)**	**Low ($<25\%$)**	**High ($\geq 25\%$)**	**Total**
Pathological stage*							
pT_2	5 (50.0)	64 (90.1)	56 (87.5)	13 (76.5)	20 (80.0)	49 (87.5)	69
pT_{3a}	4 (40.0)	6 (8.5)	7 (10.9)	3 (17.6)	4 (16.0)	6 (10.7)	10
pT_{3b-4}	1 (10.0)	1 (10.0)	1 (1.6)	1 (5.9)	1 (4.0)	1 (1.8)	2
Gleason score							
2–6	2 (20.0)	51 (71.8)	46 (71.9)	7 (41.1)	12 (48.0)	41 (73.2)	53
7 (3+4)	5 (50.0)	17 (23.9)	13 (20.3)	9 (52.9)	11 (44.0)	11 (19.6)	22
7 (4+3)	3 (30.0)	3 (4.2)	5 (7.8)	1 (5.9)	2 (8.0)	4 (7.1)	6
Tumour classification							
Harmless†	0 (0.0)	23 (32.4)	20 (31.3)	3 (17.6)	4 (16.0)	19 (33.9)	23
Clinically significant‡	10 (100.0)	48 (67.6)	44 (68.8)	14 (82.4)	21 (84.0)	37 (66.1)	58
Total	10	71	64	17	25	56	81

*pTNM 1997.
†Possibly harmless disease: organ-confined cancers with a tumour volume of less than 0.5 ml, without Gleason growth patterns 4 and 5.
‡Clinically significant disease: all others.

other variables (backward elimination method). Forward stepwise elimination was performed to verify that the same parameters remained of prognostic significance in the final models. The assumption that no association existed between the variables evaluated (H0) was rejected (H1) if $p < 0.05$.

Results

Patient characteristics

All 81 patients had clinically localized disease at the time of diagnosis. The median serum PSA level was 5.2 ng/ml (range 3.0–15.1), and 71 (87.7%) had a PSA level between 3.0 and 9.9 ng/ml. A total of 48 (59.3%) and 18 (22.2%) men had a Gleason score of 6 or 7 on the biopsy, respectively, and 11 (13.6%) a Gleason score of 7 (4+3) or 8. Within the radical prostatectomy specimen, 69 (85.2%) cancers were organ-confined, 10 (12.3%) had extraprostatic extension, and 2 (2.5%) showed extensive infiltrating disease (Table 1). The Gleason score was 2–6 in 53 (65.4%) and a dominant Gleason growth pattern of 4 or 5 was seen in 6 (7.4%). According to the tumour classification model, 23 (28.4%) cases were considered 'harmless' and 58 (71.6%) 'clinically significant'. Using prostate maps, in 14 (17.3%) cases the selected representative biopsy needle did not hit the site of the prostate that was thought to contain the representative sections within the tumour (Figure 1).

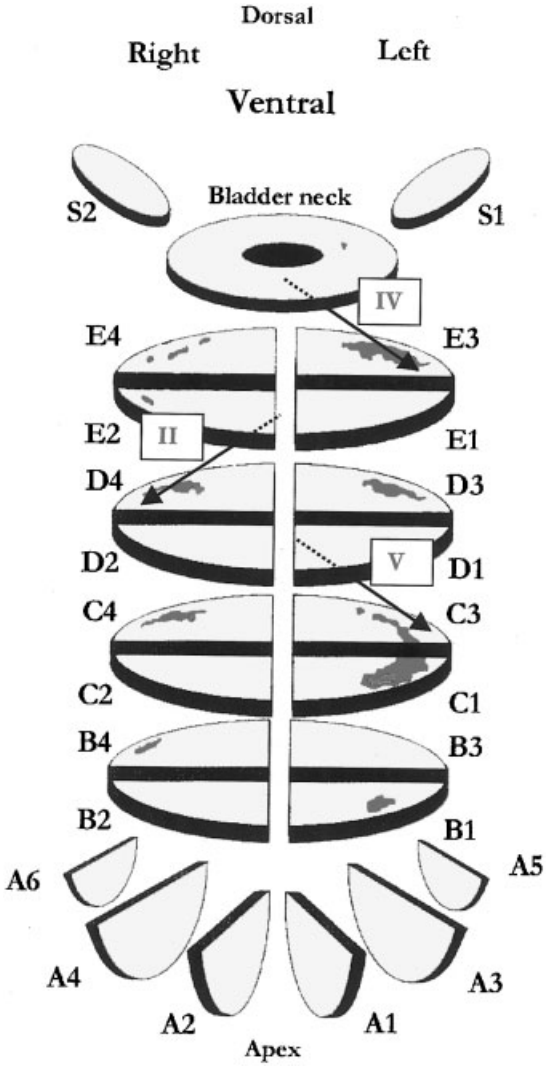

Figure 1. A prostate map showing the site, range, and trajectory of the most representative biopsy core (IV) and two other biopsy cores (II and V), as well as the location of the tumour in the prostate and its corresponding grade of differentiation according to Gleason [14]. The tumour is shown in black (Gleason growth pattern 3) and grey (Gleason growth pattern 4). Prostate sections C1 and D4 were assessed as the most representative of the tumour in the prostate, whereas biopsy core IV contained the highest amount of cancer (approximately 60%). Biopsy V missed the tumour completely, while biopsy II was only marginally involved with cancer (less than 10%)

Immunostaining assessment

Of 81 patients, 10 (12.3%) and 35 (43.2%) had low tumour $p27^{kip1}$ expression on the diagnostic needle biopsy and radical prostatectomy specimen, respectively. These figures were 17 (21.0%) and 26 (32.1%) for high MIB-1 expression, and 25 (30.9%) and 26 (32.1%) for low CD44s expression. Table 2 shows the sensitivity, specificity, PPV, and NPV of tissue marker assessment. The sensitivity of tissue marker assessment was low for all three tissue markers, implying that a substantial proportion of cases were incorrectly designated a favourable prognostic outcome. Nine of 10 (PPV = 90.0%) cases with low biopsy $p27^{kip1}$ expression had low $p27^{kip1}$ expression in the prostate, whereas 26 of 71 cases designated as having high tumour $p27^{kip1}$ expression pre-operatively changed category after radical prostatectomy (NPV = 63.4%). The PPV of

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the expression of $p27^{kip1}$, MIB-1, and CD44s as determined on the biopsy specimen. Low (less than 50%) tumour $p27^{kip1}$, high (10% and more) tumour MIB-1, and low (less than 25%) tumour CD44s expression on the biopsy were considered positive test outcomes (i.e. adverse prognostic indicators)

Biopsy tissue marker	Sensitivity*	Specificity†	PPV‡	NPV¶
$p27^{kip1}$ expression	25.7%	97.8%	90.0%	63.4%
MIB-1 expression	26.9%	81.8%	41.2%	70.3%
CD44s expression	50.0%	78.2%	52.0%	76.8%

*Sensitivity = number of adverse prognostic assessments determined on the biopsy divided by the number of adverse prognostic outcomes in the radical prostatectomy specimen.

†Specificity = number of favourable prognostic assessments on the biopsy divided by the number of favourable prognostic outcomes in the radical prostatectomy specimen.

‡PPV = the proportion of men with an adverse prognostic assessment on the biopsy who also had an adverse prognostic outcome in the radical prostatectomy.

¶NPV = proportion of men with a favourable assessment on the biopsy who also had a favourable outcome in the radical prostatectomy specimen.

MIB-1 and CD44s expression was lower than $p27^{kip1}$, while the NPVs were only slightly higher (Table 2). The expression of $p27^{kip1}$ and CD44s was almost absent (i.e. low) in intraluminar growing strands of cribriform and intraductal prostate cancer (Figure 2).

Association of tissue marker expression with pre- and post-operative variables

The association of the expression of the tissue markers on the biopsy with pre-operative and post-operative clinicopathological parameters is given in Table 3. While $p27^{kip1}$ expression was highly associated with most of the pre-operative and post-operative variables, as well as with MIB-1 and CD44s expression, the latter two tissue markers were not, or were only weakly, correlated with these same parameters (Table 3). Low expression of $p27^{kip1}$ had a high predictive value for the presence of clinically significant disease (Table 1). In fact, all cases with low pre-operative $p27^{kip1}$ turned out to have clinically significant disease after radical prostatectomy (PPV = 100.0%). For biopsy Gleason scores of 7 or higher, all cases but one (22 of 23) were found to have clinically significant disease after radical prostatectomy (PPV = 95.7%). Logistic regression analysis revealed that biopsy Gleason score ($p < 0.01$) and low expression of $p27^{kip1}$ ($p < 0.01$) on the biopsy were most valuable as predictors of clinically significant disease, though with wide confidence intervals (Table 4). CD44s and MIB-1 expression on the biopsy did not remain in the final models as independent predictors of clinically significant disease.

Discussion

In prostate cancer screening, no reliable method exists today that may identify the patients with non-aggressive disease and those with fatal disease beyond cure. Such tools are required, for it is considered that a

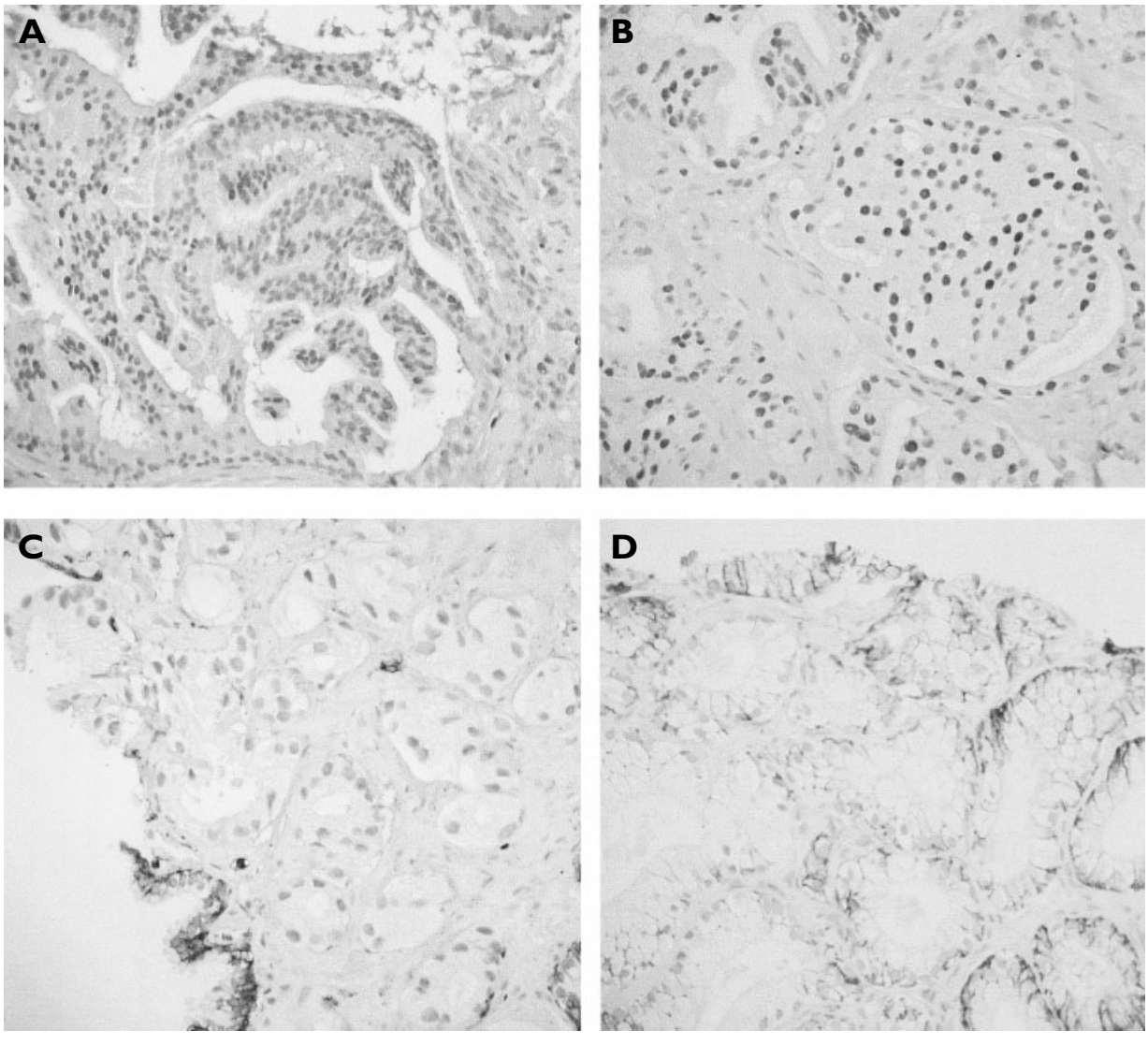

Figure 2. Immunostaining images of tissue marker expression on the diagnostic needle biopsy (×400). (A) Low (<50%) tumour $p27^{kip1}$ expression; (B) high (≥50%) tumour $p27^{kip1}$ expression; (C) low (<25%) tumour CD44s expression; (D) high (≥25%) tumour CD44s expression

Table 3. Correlation of $p27^{kip1}$, MIB-1, and CD44s with pre-operative clinicopathological parameters and tumour characteristics determined on the radical prostatectomy specimen. The figures presented are *p* values

	Tissue marker expression on the diagnostic biopsy		
Variable	**$p27^{kip1}$***	**MIB-1†**	**CD44s‡**
PSA level	<0.01	0.06	ns
Clinical tumour stage	0.01	ns	ns
Biopsy Gleason score	<0.01	<0.01	ns
Biopsy high-grade cancer	<0.01	0.01	0.02
Biopsy $p27^{kip1}$	–	0.05	<0.01
Biopsy MIB-1	0.05	–	0.01
Biopsy CD44s	<0.01	0.01	–
Pathological tumour stage	<0.01	ns	ns
Prostatic Gleason score	<0.01	ns	ns
Tumour classification model	<0.01	ns	ns

*Dichotomized as low $p27^{kip1}$ (less than 50%) and high $p27^{kip1}$ (50% and more) expression.
†Dichotomized as high MIB-1 (10% and more) and low MIB-1 (less than 10%) expression.
‡Dichotomized as low CD44s (less than 25%) and high CD44s (25% and more) expression.
ns = not significant.

substantial proportion of screen-detected prostate cancers may have been overdiagnosed (and subsequently overtreated), while others may not have been detected (and treated) early enough. Unfortunately, the predictive value of conventional clinicopathological parameters for powerful prognosticators such as pathological tumour stage and lymph-node metastatic disease remains limited, and with this, the identification of aggressive but curable cancers.

Recent studies demonstrated that the expression of the cell-cycle protein $p27^{kip1}$, the proliferation marker MIB-1, and the cell-adhesion protein CD44s within tumours was of prognostic importance in men treated with radical prostatectomy, in addition to grading and staging [7–13]. Valuable tissue markers assessed on the diagnostic needle biopsy may aid in the selection of patients to undergo (or to have withheld) radical surgery for clinically localized prostate cancer. In the current study we report a relatively poor concordance for the expression level of $p27^{kip1}$, MIB-1, and CD44s on the diagnostic needle biopsy and representative sections of the corresponding radical prostatectomy specimen (Table 2). The sensitivity was low for all three tissue markers, indicating that prognostically adverse tumour areas within the prostate were missed in a substantial number of cases. These results are in line with those of previously published and similarly performed studies [19–21]. Furthermore, the PPV was high only for low $p27^{kip1}$ expression (PPV = 90%), while comparably low for MIB-1 and CD44s (41.2% and 52.0%, respectively).

Table 4. Logistic regression analysis for the prediction of 'clinically significant' disease using conventional clinicopathological variables and the expression of $p27^{kip1}$, MIB-1, and CD44s on the diagnostic needle biopsy

	Logistic regression analysis		
	e^{β}	**95% CI**	***p* value**
PSA level	–	–	ns
Clinical tumour stage	–	–	ns
Biopsy Gleason score	13.01	1.78–96.03	<0.01
Proportion high-grade	–	–	ns
Biopsy $p27^{kip1}$*	8.97	1.03–76.92	<0.01
Biopsy MIB-1†	–	–	ns
Biopsy CD44s‡	–	–	ns

95% CI = 95% confidence interval; e^{β} = odds ratio; ns = not significant.
*Low $p27^{kip1}$ (less than 50%) expression.
†High MIB-1 (10% and more) expression.
‡Low CD44s (less than 25%) expression.

Our analysis by logistic regression showed that biopsy $p27^{kip1}$ expression and biopsy Gleason score were significant predictors of clinically significant disease (Table 4). Despite wide confidence intervals due to small patient series, the observation of low $p27^{kip1}$ expression on the diagnostic biopsy might thus be indicative of biologically aggressive disease. In our study, all men with a low (<50%) tumour $p27^{kip1}$ score on the biopsy were found to have clinically significant disease after radical prostatectomy, using the definitions of the tumour classification model. On the other hand, a high (≥50%) biopsy $p27^{kip1}$ score poorly predicted the presence of a prostate cancer with prognostically favourable tumour features. Therefore, the assessment of $p27^{kip1}$ expression on the biopsy is not helpful in identifying patients who are most likely to benefit from conservative treatment and surveillance. Moreover, our study did not provide data on whether 'aggressive' cancers identified by a low biopsy tumour $p27^{kip1}$ may be cured by the currently available treatment options, or on the other hand, may already be beyond the reach of cure.

The interpretation of our results may be limited by various factors. Multifocality and tumour heterogeneity may have contributed to the sampling error of the diagnostic needle biopsy, and to the poor concordance of tissue marker assessment between the biopsy and the radical prostatectomy specimen. As only one or two biopsy cores per patient were stained immunohistochemically, i.e. those that were assumed to be most representative within the biopsy sextant, tissue marker assessment may not have reflected the entire primary tumour within the prostate. On the other hand, it is not likely that an adverse prognostic immunostaining assessment would have been found in one of the 'non-representative' biopsy cores, especially when taking into account that these were mostly of lower grade and of low tumour volume. The frequency of adverse prognostic immunostaining assessments was low in our screened population (e.g. 12.3% for low $p27^{kip1}$ expression) and as a consequence, definite conclusions on the predictive value of tissue markers may only be given using larger patient series. It is likely that the proportion of adverse prognostic indicators may have

been higher in other patient groups that lacked the favourable prognostic features observed in our screening group. Finally, the long-term prognostic significance of our tumour classification model remains to be established. It might well be that some men classified as having clinically significant disease in our study population would never have experienced signs or symptoms of prostate cancer; and conversely, that some men designated as having potentially harmless disease might still have had clinically manifest disease if not treated.

At present, the routinely performed diagnostic technique of systematic sextant prostate biopsy has a limited ability to predict the tumour characteristics in the prostate gland, and with this, the expected biological course of disease. The present study has provided some further evidence that tissue marker assessment on the biopsy, $p27^{kip1}$ in particular, might help in discriminating between potentially aggressive and potentially non-aggressive cancers in prostate cancer screening. Before its clinical application is considered, our promising results on the value of $p27^{kip1}$ protein expression on prostatic needle biopsies in men with screen-detected prostate cancer will have to be confirmed, preferably in prospective, multi-institutional studies with larger numbers of patients.

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