Expression of urokinase-type plasminogen activator system in non-metastatic prostate cancer

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Acknowledgements: none

Keywords: biochemical recurrence, prostate cancer, radical prostatectomy, urokinase-type plasminogen activator

Table: 3**, Figure:** 4 **Supplementary Figure:** 1

Reference: 29

Abstract

Purpose: To investigate the prognostic role of expression of urokinase-type plasminogen activator system members, such as urokinase-type activator (uPA), uPA-receptor (uPAR) and plasminogen activator inhibitor-1 (PAI-1), in patients treated with radical prostatectomy (RP) for prostate cancer (PCa).

Methods: Immunohistochemical staining for uPA system was performed on a tissue microarray of specimens from 3,121 patients who underwent RP. Cox regression analyses were performed to investigate the association of overexpression of these markers alone or in combination with biochemical recurrence (BCR). Decision curve analysis was used to assess the clinical impact of these markers.

Results: uPA, uPAR and PAI-1 were overexpressed in 1,012 (32.4%), 1,271 (40.7%) and 1,311 (42%) patients, respectively. uPA overexpression was associated with all clinicopathologic characteristics of biologically aggressive PCa. On multivariable analysis, uPA, uPAR and PAI-1 overexpression were all three associated with BCR (HR: 1.75, $p<0.01$, HR: 1.22, $p=0.01$ and HR: 1.20, $p=0.03$, respectively). Moreover, the probability of BCR increased incrementally with increasing cumulative number of overexpressed markers. Decision curve analysis showed that addition of uPA, uPAR and PAI-1 resulted in a net benefit compared to a base model comparing standard clinicopathologic features across the entire threshold probability range. In subgroup analyses, overexpression of all three markers remained associated with BCR in patients with favorable pathologic characteristics.

Conclusion: Overexpression of uPA, uPAR and PAI-1 in PCa tissue were each associated with worse BCR. Additionally, overexpression of all three markers is informative even in patients with favorable

pathologic characteristics potentially helping clinical decision-making regarding adjuvant therapy and/or intensified follow-up.

Introduction

In 2018, it was estimated that 164,690 received the diagnosis of prostate cancer (PCa) and 29,430 succumbed to their disease only in the US [1]. Radical prostatectomy (RP) is a standard treatment for the patients diagnosed with non-metastatic PCa resulting in durable local disease control and long-term survival [2,3]. However, approximately 40% of patients treated with RP experience biochemical recurrence (BCR) with a significant proportion experiencing clinical disease progression within a decade from diagnosis on average [4-6]. Identification of patients at an increased risk of BCR would allow tailoring of evidence-based follow-up intensity and improve counseling as well as decision-making regarding adjuvant or early salvage therapy [7-9].

The urokinase-type plasminogen activator (uPA) system, one of the proteolytic enzyme systems in the degradation of the extracellular matrix and basement membrane, is one of the most potent cascades for cancer cell invasion [10]. The uPA system contains uPA, its receptor (uPAR) and its inhibitors such as plasminogen activator inhibitor types 1 and 2 (PAI-1 and PAI-2). Overexpression of uPA system has been reported as a prognostic marker in several malignancies such as breast, colorectal and urothelial cancers [11-13]. uPA and PAI-1, indeed, are one of the best validated prognostic biomarkers currently available for lymph node-negative breast cancer (i.e., level-1 evidence) [14,12]. In PCa, several studies investigated the association of uPA system with oncologic outcomes. We have previously shown that preoperative elevated plasma uPA and uPAR are associated with adverse pathologic features and worse BCR rates in patients treated with RP for clinically localized PCa[15]. In addition, in a smaller hypothesis-generating study, we have shown that the immunohistochemical expression of both uPA and uPAR was associated with worse BCR rates in patients treated with RP for clinically localized PCa

[16,17]. Based on this cumulative data, we hypothesized that the tissue expression of uPA, uPAR and PAI-1 are associated with worse BCR rates in patients treated with RP for non-metastatic PCa. To test this, in this retrospective study, we evaluated the prognostic value of uPA system using immunohistochemical assessment of surgical specimens in patients treated with RP for non-metastatic PCa. Our primary aim was to validate the data from the smaller previous single-center study in a large, multicenter, international cohort as part of a phased biomarker validation process[18]. Moreover, for a biomarker to change clinical practice, it needs to improve the predictive accuracy of currently known standard clinicopathologic characteristics as well as the net-value of our current prediction tool in the clinically relevant threshold range.

Material and Methods

Patient population

After the acquisition of ethical approval in each center, we identified 3,294 patients from European and North America centers, who underwent RP between 2000 and 2011. We excluded the patients with preoperative PSA >50ng/ml, missing preoperative PSA, surgical margin status, lymphovascular invasion status, lymph node status and RP Gleason score. This left 3,121 patients for this study. None of the patients had received neoadjuvant androgen deprivation, chemo- or radiation therapy. None of the patients had clinically evident metastatic disease at the time of RP.

Pathological evaluation and immunohistochemical staining

All RP specimens were evaluated according to a standard pathological procedure and assigned stage and grade based on the American Joint Committee on Cancer TNM staging system and the International Society of Urological Pathology, respectively. Lymphovascular invasion was defined as

the invasion of vessel walls by tumor cells and/or the presence of tumor emboli within a definite endothelial-lined space, at a distance from tumors, or in the prostatic parenchyma surrounding the tumor[19]. Positive surgical margin was defined as the explicit presence of tumor cells at the inked margin of the RP specimen.

Immunohistochemical staining for uPA, uPAR and PAI-1 on a tissue microarray of tumor cores was performed as previously described [16]. Briefly, staining of the serial section from the same paraffin-embedded microarray tissue blocks were performed on the Dako Autostainer (Carpinteria) using mouse monoclonal antibodies against uPA (dilution 1:100), PAI-1 (dilution 1:50), and uPAR (dilution 1:100) (American Diagnostica, Greenwich, CT). Bright-field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System, ChromaVision Medical Systems Inc., San Juan Capistrano, CA) were used for staining assessment. All biomarkers were assigned to one of two categories: overexpression or normal based on the cut-offs established in our previous study[16]; uPA, uPAR and PAI-1 were considered to be overexpressed when 30%, 30% and 50% of the tumor cells or more exhibited positive staining, respectively. Figure1 shows representative slides of immunohistochemical staining for all three markers.

Follow up

All included patients were followed up according to guidelines at that time. Generally, this considered in serum PSA measurement and digital rectum examination every three months for first two years, semiannually from the third to the fifth year, and annually thereafter. BCR was defined as PSA >0.2ng/ml on two consecutive postoperative evaluation and the date of BCR was assigned to the first evaluation. None of the patients received adjuvant androgen deprivation or radiation therapy.

Statistical analysis

The associations of uPA, uPAR and PAI-1 with categorical and continuous variables were evaluated using Chi-square and Mann-Whitney U tests, respectively. Kaplan-Meier curves estimated the correlation of uPA, uPAR and PAI-1 with BCR-free survival. The log-rank test was used to determine the statistical difference between groups. Univariable and multivariable Cox regression analyses were conducted to determine the association of uPA, uPAR and PAI-1 with BCR after RP, while adjusting for the effects of established clinicopathologic characteristics. Discrimination of the models was measured using Harrell's C-index. We built a nomogram based on the Cox regression hazard coefficients to obtain a graphical visualization of the model. Internal validation was performed using 1000 bootstrap resamples. Calibration plots were generated to assess the performance of the model. A decision curve analysis was used to assess the clinical impact on decision making of uPA system and visualize the net benefit over a realistic range of threshold probabilities compared to base model based on standard clinicopathologic characteristics. Data were analyzed using STATA 14.0 (Stata Corp., College Station, TX) and R 3.4.1 (The R Foundation, Vienna, Austria). All tests were two-sided and $p < 0.05$ was considered as statistically significant.

Results

Patients characteristics

The clinicopathologic characteristics of the 3,121 patients and their association with uPA, uPAR and PAI-1 status are shown in Table1. uPA, uPAR and PAI-1 were overexpressed in 1,012 (32.4%), 1,271 (40.7%) and 1,311 (42%) patients, respectively. uPA overexpression was associated with age ($p=0.03$), higher preoperative serum PSA ($p<0.01$) and all adverse pathologic findings ($p<0.01$). uPAR

overexpression was associated with higher RP Gleason sum (p=0.04) and RP lymphovascular invasion (p=0.04).

Association of uPA, uPAR and PAI-1 with BCR after RP

Within a median follow up of 49 months (IQR: 31-68 months), 619 patients (19.8%) experienced BCR after RP. The median time to BCR was 21 months (IQR: 8-43 months). The 5-year BCR-free survival rates for patients with overexpression of uPA was lower than those of patients with normal expression of uPA (70.6% vs. 84.2%, p<0.01; Figure2A). The 5-year BCR-free survival rates for patients with overexpression of uPAR was lower than those of patients with normal expression of uPAR (77.3% vs 81.7%, p<0.01; Figure2B). The 5-year BCR-free survival rates for patients with overexpression of PAI-1 was lower than those of patients with normal expression of PAI-1 (77.9% vs 81.3%, p=0.01; Figure2C). Figure 2D shows that the BCR-free survival decreases incrementally with increasing cumulative number of overexpressed markers (the number of overexpression: 5-year BCR-free survival rate; all normal: 86.1%, one overexpressed: 82.1%, two overexpressed: 74.8%, and all three overexpressed: 61.1%, p<0.01).

On multivariable analysis that adjusted for the effects of standard clinicopathologic characteristics, uPA, uPAR and PAI-1 overexpression were each associated with worse BCR-free survival (HR: 1.75, p<0.01, HR: 1.22, p=0.01 and HR: 1.20, p=0.03, respectively) (Table2). The inclusion of uPA, uPAR and PAI-1 improved the discrimination of a standard prognostic model for BCR-free survival by a small prognostic margin from 80% to 81%. Similarly, the combination of the three markers was associated with cumulative worse BCR rates (one overexpressed HR: 1.38, p<0.01, two overexpressed; HR: 1.82, p<0.01, all three overexpressed; HR 2.66, p<0.01). The inclusion of the combination of the markers

improved the discrimination of a standard prognostic model for BCR-free survival from 80% to 81%. We integrated uPA, uPAR and PAI-1 in addition to standard clinicopathologic characteristics into a nomogram for prediction of the probability of BCR at 6month, 1year, 3years and 5years after RP. The overall bootstrap corrected accuracy of the nomogram was 80.3% (Figure3). Calibration plots of our model showed a good performance in predicting probabilities at any time point after RP (Supplementary Figure1).

Decision curve analysis

Decision curve analysis showed a better net benefit of our model including uPA, uPAR and PAI-1 than that of the base model using standard clinicopathologic characteristics at any time point after RP across the entire range of threshold probabilities (6months: 5-20%, 1year: 5-35%, 3years: 15-50% and 5years: 5-50%, respectively.) (Figure4).

Subgroup analyses in patients treated with RP

Table 3 shows the association of combination of the three markers with BCR in different subgroups of patients treated with RP on multivariable Cox regression models. Overexpression of all three markers remained associated with BCR even in patients with favorable pathologic characteristics such as RP Gleason sum 6 (HR: 3.22, $p<0.01$), localized disease (HR: 3.38, $p<0.01$), negative surgical margin (HR: 3.47, $p<0.01$), no lymphovascular invasion (HR: 3.10, $p<0.01$) and no lymph node involvement (HR:3.46, $p<0.01$). In patients with positive surgical margin and/or those with locally advanced disease, who are often considered for adjuvant radiation therapy, overexpression of all three markers was associated with a worse BCR rate (HR: 2.96, $p<0.01$) (Table3). The inclusion of the combination of the three markers into a standard prognostic model improved its prognostic accuracy with more

than 2.0% discrimination in subgroups of patients with Gleason 6 and those with positive surgical margin and/or locally advanced disease (Table3).

Discussion

In the present study, we confirmed the value of tissue expression of uPA, uPAR and PAI-1 in patients treated with RP for non-metastatic PCa. uPA, uPAR and PAI-1 were each overexpressed in more than one-third of all patients, and at least one of them was overexpressed in 76.3%. We further found uPA and uPAR overexpression were associated with established features of biologically and clinically aggressive PCa such as higher RP Gleason score, positive surgical margin and lymphovascular invasion; in contrast, PAI-1 overexpression was not associated with any of these variables. These results were consistent with previous studies. Gupta et al. reported the association of uPA overexpression with extraprostatic extention[16]. Similarly, Kumano et al. showed the uPA and uPAR overexpression were associated with higher RP Gleason score and positive surgical margin[17]. While the association with clinicopathologic characteristics is important, prognostification of BCR is more relevant to clinical decision-making regarding adjuvant therapy and follow-up intensity.

We confirmed the findings of Gupta et al[16]. in a large international multi-institutional cohort showing that uPA, uPAR or PAI-1 overexpression were each independent predictors of BCR after RP on multivariable Cox regression analyses that adjusted for the effects of established clinicopathologic features. We further confirmed the clinical value of our model including these markers and found a prognostic improvement of the net benefit for decision making over a base model comprising standard clinicopathologic features across all threshold probabilities.

The uPA system is known to play a significant role in tumor growth, angiogenesis, tumor cell invasion,

migration and metastasis [20]. At the blood level, Shariat et al. assessed the value of uPA system preoperatively in patients who underwent RP for non-metastatic PCa and reported that higher uPA and uPAR levels were both associated with aggressive pathologic features as well as worse BCR-free survival[15]. Similarly, elevated activation and expression of different members of the uPA system have been reported as a predictive biomarker in other malignancies [21-23]. In fact, both uPA and PAI-1 are categorized as one of the best available validated biomarkers for lymph node-negative breast cancer with level 1 evidence [14,12].

Interestingly, in subgroup analyses of the current study, we found that the combination of the three markers predicted worse BCR rates in patients with both favorable and unfavorable pathologic features. Additionally, we showed that the addition of the combination of the three markers improved the prognostic accuracy of a base model comprising established clinicopathologic characteristics by more than 2.0% in patients with Gleason 6 and those with positive surgical margin and/or locally advanced disease.

The accurate identification of the patients with an increased probability of BCR after RP is essential for individualized risk-based PCa management. Several nomograms predicting BCR after RP comprising standard clinicopathologic features have been validated [24,25]. Adjuvant therapy such as radiation therapy is usually considered for patients with adverse pathologic features such as seminal vesicle invasion, extraprostatic extension and/or positive surgical margins at this moment, due to the lack of the evidence of superiority between adjuvant vs early salvage radiation therapy, which are still ongoing trials, such as RADICALS [26] and RAVES trials [27]. Meanwhile, patients with low Gleason score, negative surgical margin and no lymph node involvement are considered as

survivors after RP. Nevertheless, in these patients with favorable pathologic features in RP specimen, 10 to 20% of patients will experience BCR[28,29]. To improve predictive accuracy, we need to capture the biologic and clinical potential of the tumors by integrating validated markers into established predictive models.

Taken together, our results highlight that uPA, uPAR and PAI-1, as well as their combination, could help identify the patients who are most likely to experience BCR after RP with curative intent and, therefore, are likely to benefit from adjuvant therapy and/or intensified follow up. In addition to its promising role as a marker, targeting therapy against members of the uPA system is being assessed in preclinical studies showing an efficacious interruption of tumor growth and invasiveness[20]. Further studies to assess the therapeutic potential of blockade for the uPA system are envisaged with high expectation.

This study has limitations including its retrospective design which may have led to selection bias. Due to the multicentric nature, we could not control for surgeon and pathologist performance and experience. Additionally, immunohistochemistry has inherent limitations such as its reproducibility and reliability. For example, **immunohistochmical stained nomal epithelium and cancerous cells are not separated and identified correctly in this study**To reduce these limitations, we used a microtissue array and staining protocols coupled with automated scoring systems based on bright-field microscopy imaging and advanced color detection software. Another limitation was that the main outcome of our interest in our study was BCR and the follow up was relatively short. Larger studies with longer follow up to evaluate the association of uPA system with BCR as well as more significant endpoints such as metastasis and cancer-specific survival in PCa patients are needed.

Conclusions

Overexpression of uPA, uPAR and PAI-1 were each independently associated with worse BCR-free survival. The inclusion of these markers improved the discrimination of conventional prognostic tools and decision curve analyses across all threshold probabilities. Additionally, overexpression of the combination of the three markers was prognostically informative even in patients with favorable pathologic characteristics potentially improving and individualizing further the clinical decision-making regarding adjuvant therapy and/or follow-up scheduling.

Authors' Contribution

Shoji Kimura: Protocol/project development, Data collection, Data analysis, Manuscript writing/editing,

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Disclosure of potential conflict of interest: The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals: This article does not contain any studies with animals performed by any of the authors.

Informed consent: Informed consent was obtained from all individual participants included in the study

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Figure legends:

Figure1. Immunohistochemical staining of (A) uPA, (B) uPAR and (C) PAI-1 expression in radical prostatectomy specimens (x200 magnification)

PAI-1: plasminogen activator inhibitor-type1, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

Figure2. Biochemical recurrence-free survival estimates in 3,121 patients treated with radical prostatectomy for non-metastatic prostate cancer according to the expression of (A)uPA, (B) uPAR

and (C) PAI-1 and (D) the combination of these three markers.

PAI-1: plasminogen activator inhibitor-type1, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

Figure3. Postoperative nomogram to predict biochemical recurrence at 6months, 1year, 3year and 5years in 3,121 patients treated with radical prostatectomy for non-metastatic prostate cancer.

BCR: biochemical recurrence, LVI: lymphovascular invasion, PAI-1: plasminogen activator inhibitor type1, PSA: prostate specific antigen, uPA: urokinase-type plasminogen activator-type1, uPAR: urokinase-type plasminogen activator receptor

Figure4. Decision curve analyses comparing the added benefit of uPA, uPAR and PAI-1 (red dot line) in addition to standard clinicopathologic features (black dot line) within 6 months, 1year, 3years and 5years in 3,121 patients treated with radical prostatectomy for non-metastatic prostate cancer. Treat all is represented by the gray line and treat none by the black line.

PAI-1: plasminogen activator inhibitor-type1, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

Supplementary Figure1. Calibration plots with 1000 bootstrap resample for the nomogram including uPA, uPAR and PAI-1 predicting biochemical recurrence-free survival at 6months, 1,3 and 5years in 3121 patients treated with radical prostatectomy for non-metastatic prostate cancer.

PAI-1: plasminogen activator inhibitor-type1, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

Table1. Association of uPA, uPAR and PAI-1 expression with clinicopathological characteristics in 3,121 patients treated with radical prostatectomy for non-metastatic prostate cancer										
Variables	Total	uPA			uPAR			PAI-1		
		Normal	Overexpression	p-value	Normal	Overexpression	p-value	Normal	Overexpression	p-value
Number of patients	3,121	2,109 (67.6)	1,012 (32.4)		1,850 (59.3)	1,271 (40.7)		1,810 (58.0)	1,311 (42.0)	
Median (IQR)										
Age, years	62 (58-67)	62 (58-67)	63 (58-67)	0.03	62 (58-67)	62 (58-67)	0.44	62 (58-67)	62 (58-67)	0.36
Preoperative PSA (ng/ml)	7 (6-10)	7 (5-10)	8 (6-11)	<0.01	8 (6-10)	7 (5-10)	0.36	7 (6-10)	7 (6-10)	0.75
Gleason sum at biopsy (n, %)										
6	2,036 (65.2)	1,421 (69.8)	615 (30.2)	<0.01	1,235 (60.7)	801 (39.3)	0.11	1,189 (58.4)	847 (41.6)	0.90
3+4	572 (18.4)	386 (67.5)	186 (32.5)		315 (55.1)	257 (44.9)		328 (57.3)	244 (42.7)	
4+3	362 (11.6)	222 (61.3)	140 (38.7)		210 (58.0)	152 (42.0)		205 (56.6)	157 (43.4)	
8-10	151 (4.8)	80 (53.0)	71 (47.0)		90 (59.6)	61 (40.4)		88 (58.3)	63 (41.7)	
Gleason sum at RP (n, %)										
6	1,093(35.0)	760 (69.5)	333 (30.5)	<0.01	684 (62.6)	409 (37.4)	0.04	634 (58.0)	459 (42.0)	0.97
3+4	773 (24.8)	537 (69.5)	236 (30.5)		452 (58.5)	321 (41.5)		443 (57.3)	330 (42.7)	
4+3	1049 (33.6)	706 (67.3)	343 (32.7)		596 (56.8)	453 (43.2)		612 (58.3)	437 (41.7)	
8-10	206 (6.6)	118 (57.3)	88 (42.7)		118 (57.3)	88 (42.7)		121 (58.7)	85 (41.3)	
Extracapsular extension (n, %)	828 (26.5)	505 (61.0)	323 (39.0)	<0.01	480 (2580)	348 (42.0)	0.37	473 (57.1)	355 (42.9)	0.56
Seminal vesicle invasion (n, %)	231 (7.4)	129 (55.8)	102 (44.2)	<0.01	132 (57.1)	99 (42.9)	0.49	122 (52.8)	109 (47.2)	0.10
Positive surgical margin (n, %)	522 (16.7)	311 (59.6)	211 (40.4)	<0.01	297 (56.9)	225 (43.2)	0.23	315 (60.3)	207 (39.7)	0.23
Lymphovascular invasion (n, %)	324 (10.4)	196 (60.5)	128 (39.5)	<0.01	175 (54.0)	149 (46.0)	0.04	182 (56.2)	142 (43.8)	0.48
Lymph node status (n, %)	74 (2.4)	30 (40.5)	44 (59.5)	<0.01	36 (48.6)	38 (51.4)	0.06	37 (50.0)	37 (50.0)	0.16
IQR: interquartile range, PAI-1: plasminogen activator inhibitor-type 1, PSA: prostate-specific antigen, RP: radical prostatectomy, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor										

Table2.Univariable and multivariable Cox regression analyses for the prediction of biochemical recurrence in 3,121 patients treated with radical prostatectomy for non-metastatic prostate cancer.

Variable	Univariable			Multivariable		
	HR	95%CI	p	HR	95%CI	p
Age	1.01	0.99-1.02	0.24	-	-	-
Preoperative PSA	1.06	1.05-1.07	<0.01	1.04	1.03-1.05	<0.01
Gleason sum at RP						
6	ref			ref		
3+4	2.02	1.57-2.59	<0.01	1.46	1.19-1.80	<0.01
4+3	3.10	2.46-3.89	<0.01	1.62	1.29-2.04	<0.01
8-10	10.2	7.84-13.2	<0.01	2.62	1.99-3.43	<0.01
Extracapsular extension	4.64	3.96-5.45	<0.01	2.43	2.00-2.95	<0.01
Seminal vesicle invasion	6.58	5.47-7.91	<0.01	1.65	1.32-2.07	<0.01
Positive surgical margin	3.28	2.78-3.88	<0.01	1.93	1.62-2.30	<0.01
Lymphovascular invasion	2.23	1.83-2.73	<0.01	1.53	1.25-1.88	<0.01
Lymph node status	12.48	9.66-16.11	<0.01	3.03	2.28-4.03	<0.01
Harrell's C-index	79.95%					
uPA	2.27	1.94-2.66	<0.01	1.75	1.49-2.06	<0.01
uPAR	1.27	1.88-1.49	<0.01	1.22	1.04-1.44	0.01
PAI-1	1.23	1.05-1.44	0.01	1.20	1.02-1.40	0.03
Harrell's C-index	80.83%					
No. Overexpression						
0	ref			ref		
1	1.56	1.23-1.99	<0.01	1.38	1.08-1.76	<0.01
2	2.24	1.76-2.86	<0.01	1.82	1.42-2.33	<0.01
3	3.74	2.72-5.16	<0.01	2.66	1.91-3.69	<0.01
Harrell's C-index	80.67%					

CI = Confidence Interval, HR = Hazard Ratio, PAI-1: plasminogen activator inhibitor-type 1, PSA: prostate-specific antigen, RP: radical prostatectomy, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

Table3. Multivariable Cox regression analyses for prediction of biochemical recurrence according to the number of overexpression of uPA, uPAR and PAI-1 in subgroups of patients treated with radical prostatectomy for non-metastatic prostate cancer.

Patient subgroup	No. Overexpression of uPA, uPAR and PAI-1 (ref: 0)									Accuracy improvement (Harrell's C-index)
	1			2			3			
	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p	
RP Gleason sum 6[a]	1.82	1.01-3.27	0.04	2.58	1.41-4.74	<0.01	3.22	1.43-7.24	<0.01	+2.2%
RP Gleason 3+4[b]	2.19	1.22-3.94	<0.01	2.64	1.47-4.73	<0.01	4.79	2.22-10.3	<0.01	+1.3%
RP Gleason 4+3[b]	1.20	0.83-1.74	0.34	1.61	1.10-2.35	0.01	2.57	1.55-4.26	<0.01	+0.9%
RP Gleason sum 8-10[c]	1.19	0.69-2.04	0.53	1.43	0.85-2.40	0.18	2.42	1.15-5.08	0.02	+1.3%
Localized disease[d]	1.73	1.21-2.48	<0.01	2.09	1.44-3.03	<0.01	3.38	2.07-5.51	<0.01	+1.0%
Locally advanced disease (pT3a/b) [d]	1.38	1.00-1.91	0.05	1.77	1.27-2.46	<0.01	2.42	1.54-3.80	<0.01	+1.7%
Negative surgical margin [e]	1.54	1.15-2.07	<0.01	1.84	1.36-2.49	<0.01	3.47	2.34-5.15	<0.01	+1.0%
Positive surgical margin [f]	1.36	0.88-2.09	0.16	1.80	1.17-2.76	<0.01	1.96	1.07-3.58	0.03	+1.1%
No lymphovascular invasion [g]	1.44	1.11-1.88	<0.01	1.82	1.39-2.38	<0.01	3.10	2.16-4.45	<0.01	+1.0%
Lymphovascular invasion[g]	1.55	0.83-2.87	0.17	1.97	1.07-3.62	0.03	1.68	0.71-3.99	0.24	+0.5%
No lymph node involvement[h]	1.45	1.13-1.88	<0.01	1.92	1.48-2.48	<0.01	3.46	2.45-4.90	<0.01	+0.8%
Lymph node involvement[*]	-	-	-	-	-	-	-	-	-	-
Positive surgical margin and/or Locally advanced disease (pT3a/b)[i]	1.48	1.10-1.98	<0.01	1.89	1.41-2.55	<0.01	2.96	1.98-4.43	<0.01	+2.0%

CI = Confidence Interval, HR = Hazard Ratio, PAI-1: plasminogen activator inhibitor-type 1, PSA: prostate-specific antigen, RP: radical prostatectomy, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor

[a] Multivariable Cox regression adjusted for PSA, , positive surgical margin, Extracapsular status and lymphovascular invasion

[b] Multivariable Cox regression adjusted for PSA, , positive surgical margin, Extracapsular status, seminal vesicle invasion, lymphovascular invasion and lymph node involvement

[c] Multivariable Cox regression adjusted for PSA, positive surgical margin, Extracapsular status, seminal vesicle invasion, lymphovascular invasion and lymph node involvement

[d] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, positive surgical margin, lymphovascular invasion and lymph node involvement

[e] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, Extracapsular status, seminal vesicle invasion, lymphovascular invasion and lymph node involvement

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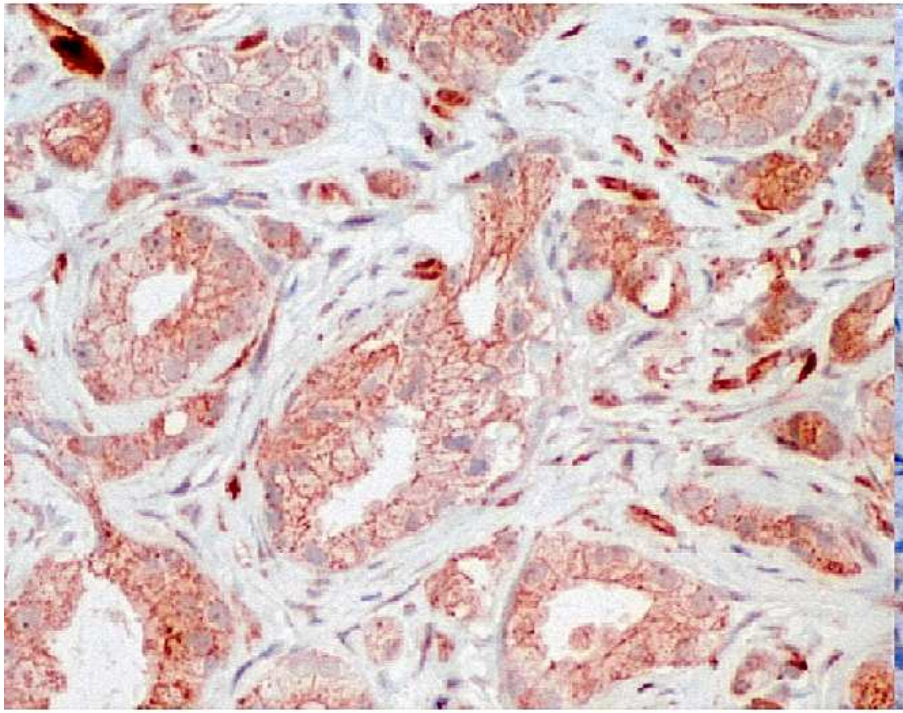

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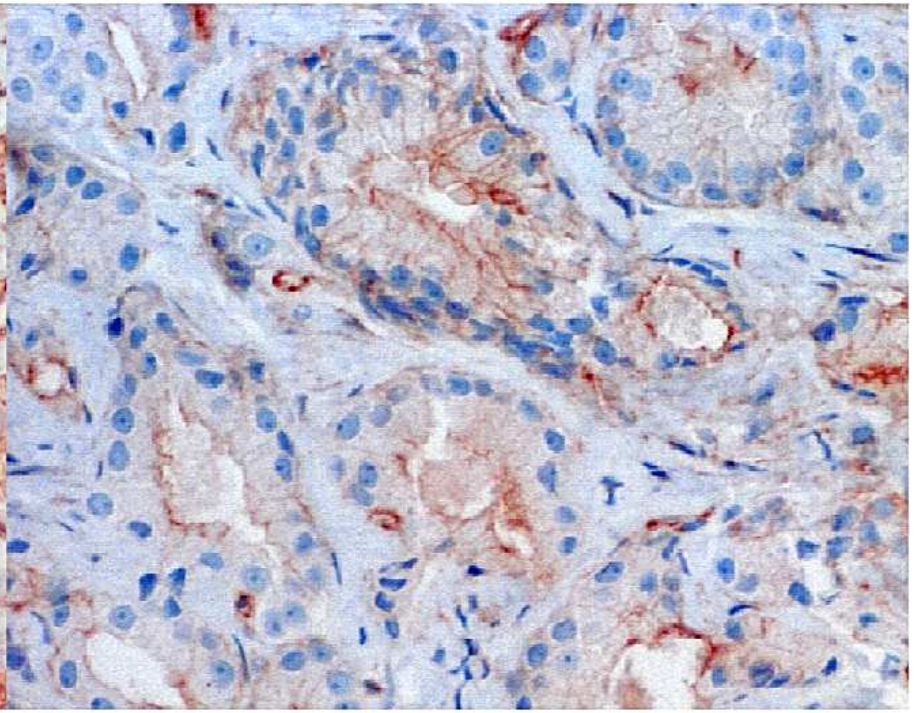

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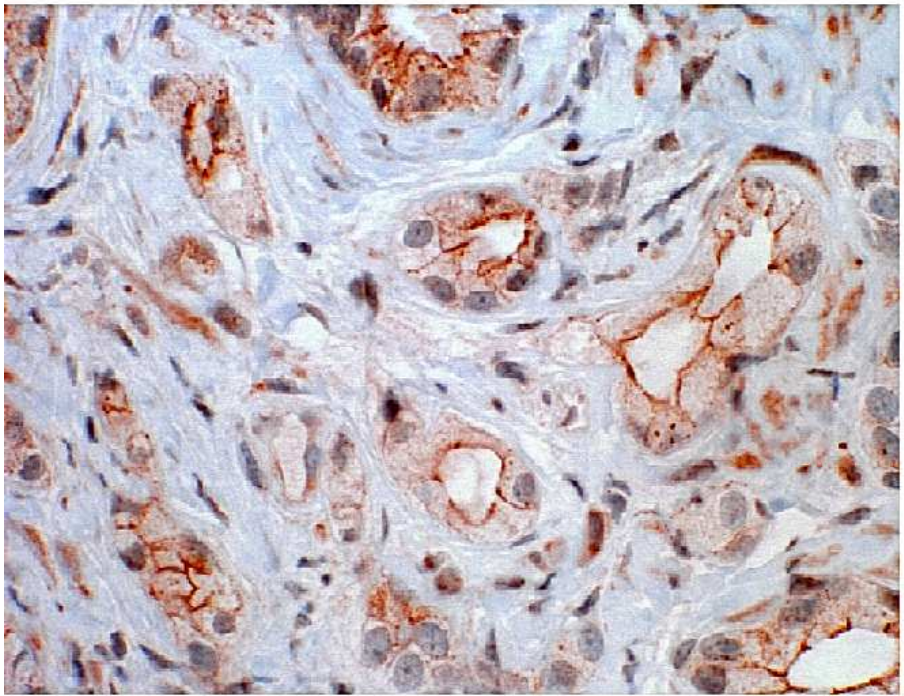

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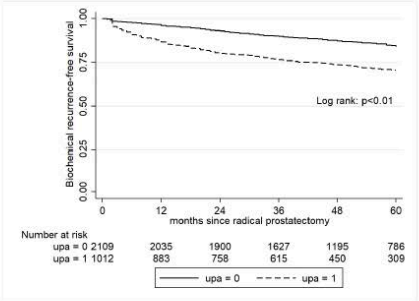
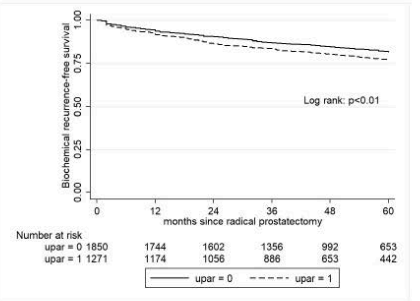

Biochemical recurrence-free survival
1.00
0.75
0.50
0.25
0.00
Log rank: p<0.01
0
12
24
36
48
60
months since radical prostatectomy
Number at risk
upa = 0 2109 2035 1900 1627 1195 786
upa = 1 1012 883 758 615 450 309
upa = 0
upa = 1

B.


Biochemical recurrence-free survival
1.00
0.75
0.50
0.25
0.00
Log rank: p<0.01
0
12
24
36
48
60
months since radical prostatectomy
Number at risk
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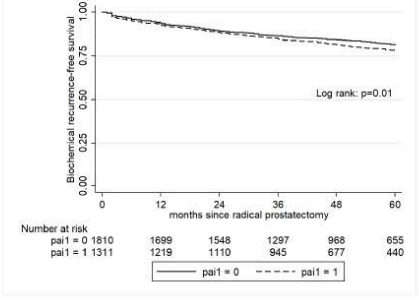
upar = 1 1271 1174 1056 886 653 442
upar = 0
upar = 1

C.


Biochemical recurrence-free survival
1.00
0.75
0.50
0.25
0.00
Log rank: p=0.01
0
12
24
36
48
60
months since radical prostatectomy
Number at risk
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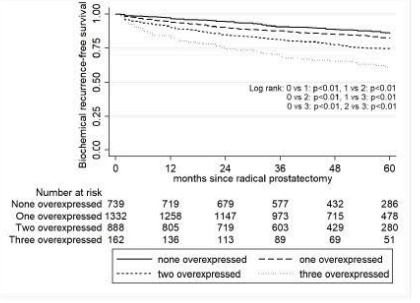
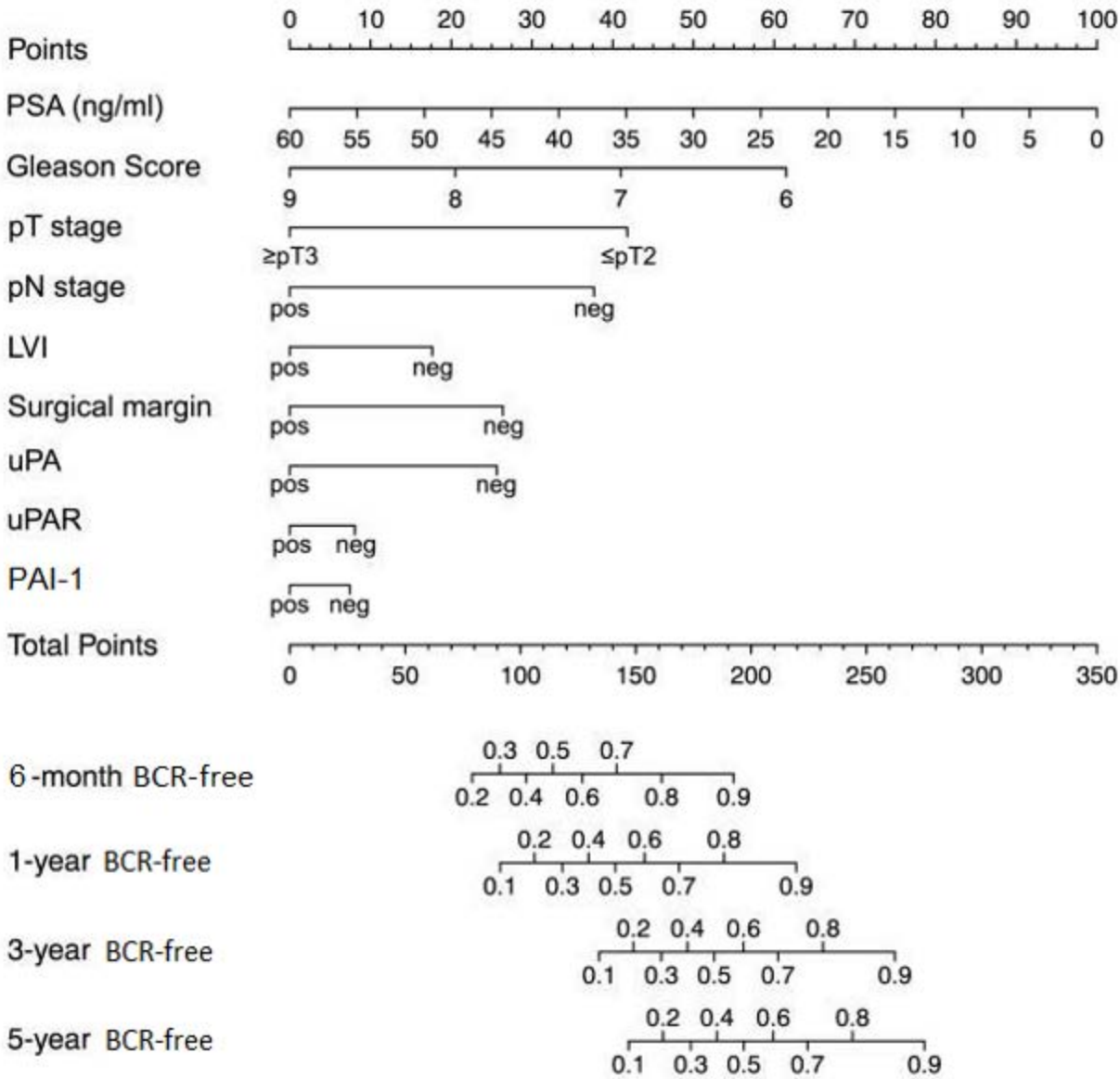
pai1 = 1 1311 1219 1110 945 677 440
pai1 = 0
pai1 = 1

D.


Biochemical recurrence-free survival
1.00
0.75
0.50
0.25
0.00
Log rank: 0 vs 1: p<0.01, 1 vs 2: p<0.01
0 vs 2: p<0.01, 1 vs 3: p<0.01
0 vs 3: p<0.01, 2 vs 3: p<0.01
0
12
24
36
48
60
months since radical prostatectomy
Number at risk
None overexpressed 739 719 679 577 432 286
One overexpressed 1332 1258 1147 973 715 478
Two overexpressed 888 805 719 603 429 280
Three overexpressed 162 136 113 89 69 51
none overexpressed
one overexpressed
two overexpressed
three overexpressed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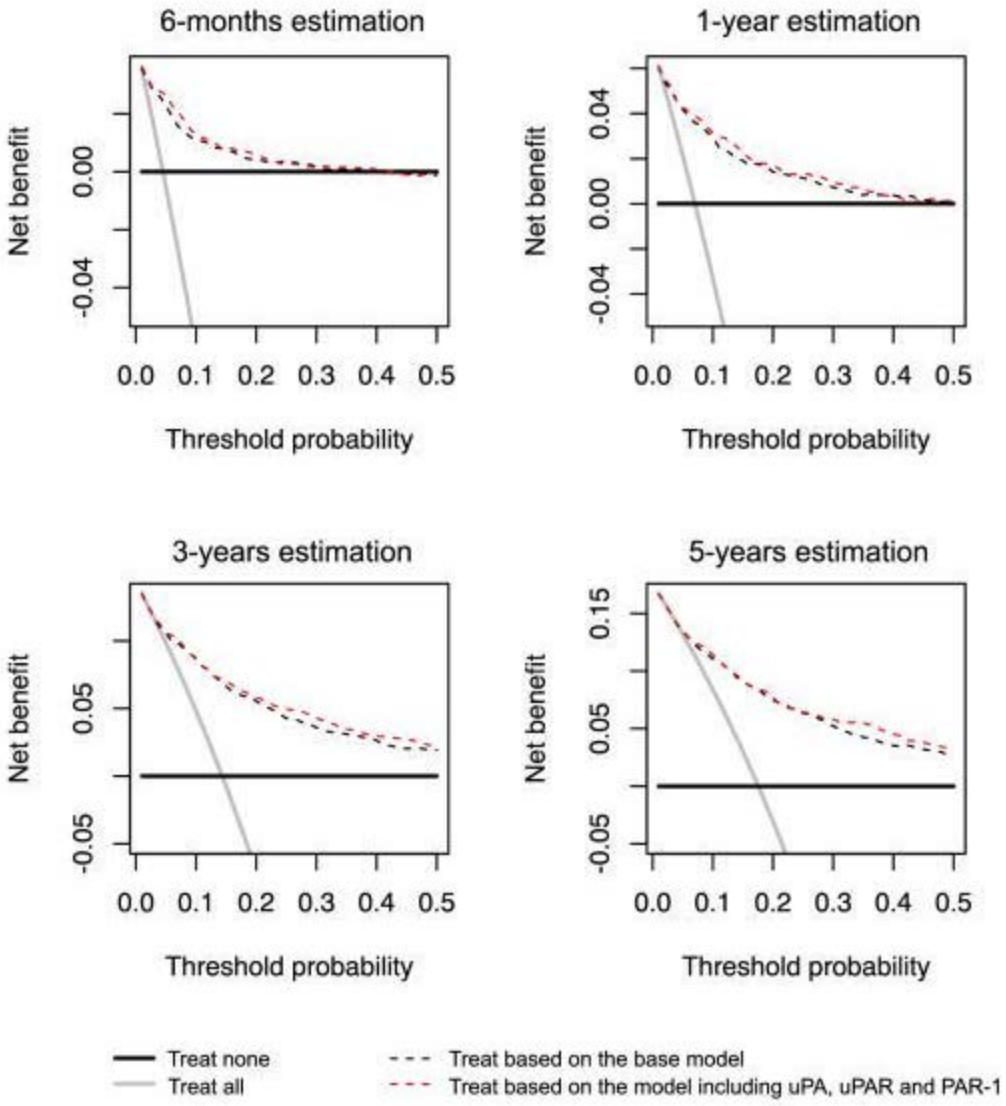
PSA (ng/ml)
60 55 50 45 40 35 30 25 20 15 10 5 0
Gleason Score
9 8 7 6
pT stage
≥pT3 ≤pT2
pN stage
pos neg
LVI
pos neg
Surgical margin
pos neg
uPA
pos neg
uPAR
pos neg
PAI-1
pos neg
Total Points
0 50 100 150 200 250 300 350
6-month BCR-free
0.3 0.5 0.7
0.2 0.4 0.6 0.8 0.9
1-year BCR-free
0.2 0.4 0.6 0.8
0.1 0.3 0.5 0.7 0.9
3-year BCR-free
0.2 0.4 0.6 0.8
0.1 0.3 0.5 0.7 0.9
5-year BCR-free
0.2 0.4 0.6 0.8
0.1 0.3 0.5 0.7 0.9


6-months estimation
1-year estimation
3-years estimation
5-years estimation
Net benefit
Threshold probability
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-0.04 0.00
-0.04 0.00 0.04
-0.05 0.05
-0.05 0.05 0.15
Treat none
Treat all
Treat based on the base model
Treat based on the model including uPA, uPAR and PAR-1

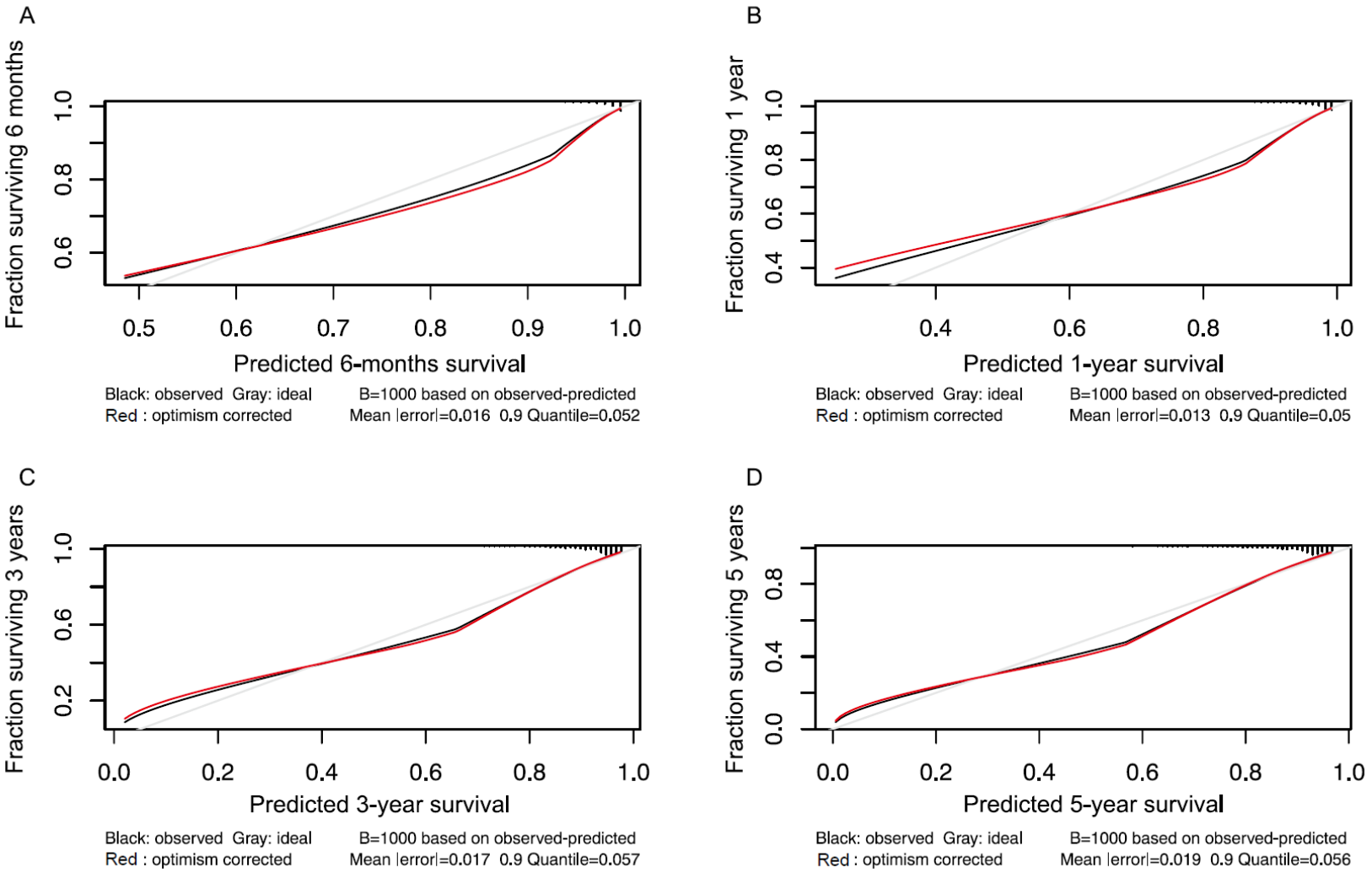
[f] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, Extracapsular status, seminal vesicle invasion and lymph node involvement

[g] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, positive margin, Extracapsular status, seminal vesicle invasion and lymph node involvement

[h] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, positive margin, Extracapsular status, seminal vesicle invasion and lymphovascular invasion

[i] Multivariable Cox regression adjusted for PSA, , Gleason sum at RP, lymphovascular invasion and lymph node involvement

* In patients with lymph node involvement, multivariable analysis was not performed because no association was already found between the number of uPA, uPAR and PAI-1 and BCR on univariable analysis.



Supplementary Figure1. Calibration plots with 1000 bootstrap resample for the nomogram including uPA, uPAR and PAI-1 predicting biochemical recurrence-free survival at 6months, 1,3 and 5years in 3121 patients treated with radical prostatectomy for non-metastatic prostate cancer.
PAI-1: plasminogen activator inhibitor-type1, uPA: urokinase-type plasminogen activator, uPAR: urokinase-type plasminogen activator receptor