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A prediction model for early biochemical failure after radical prostatectomy based on the CAPRA-S score and the presence of secondary circulating prostate cells

Nigel P.Murray [1, 2], Socrates Aedo [2], Eduardo Reyes [3, 4], Nelson Orellana [4], Cynthia Fuentealba [1] , and Omar Jacob [1]
[1]Hospital Carabineros of Chile, Nuñoa, 7770199 Santiago, Chile
[2]Faculty of Medicine, University Finis Terrae, Providencia, 7501015 Santiago, Chile
[3]Faculty of Medicine, Diego Portales University, Manuel Rodrıguez Sur 415, 8370179 Santiago, Chile
[4]Hospital DIPRECA, Las Condes, 7601003,Santiago, Chile

Correspondence should be addressed to Nigel P. Murray; nigelpetermurray@gmail.com

Abstract

Objective: To establish a prediction model for early biochemical failure based on the CAPRA-S score and secondary circulating prostate cells.

Patients and Methods: A prospective single center study of men who underwent radical prostatectomy as monotherapy for prostate cancer. Clinical-pathological findings were used to calculate the CAPRA-S score. 90 days after surgery blood was taken for CPC detection, mononuclear cells were obtained using differential gel centrifugation, and CPCs identified using immunocytochemistry. A CPC was defined as a cell expressing PSA but not CD45. The CPC test was defined as positive or negative. Patients were followed up for up to 5 years, biochemical failure was defined as a PSA >0.2ng/ml. The validity of the CAPRA-S score was calibrated using partial validation, and Cox proportional hazard regression to build three models, CAPRA-S, CPC and combined models.

Results: 321 men participated, mean age 65.5 years, after 5 years of follow up the biochemcial free survival was 98.55%. The model using CAPRA-S showed a HR of 7.66, that of CPC 34.52 and the combined model showed a HR of 2.60 for CAPRA-S and 22.5 for CPC. Using the combined model, 23% of men changed from low risk to high risk or vice versa.

Conclusion. The incorporation of CPC detection significantly increased the discrimination in establishing the probability of biochemcial failure, high risk CAPRA-S patients who are negative for CPCs have a much better prognosis. The addition of CPC detection gives clinically significant information of who may be eligible for adjuvant therapy.

Methods and Patients: A single center prospective observational study of men following radical prostatectomy for prostate cancer. CAPRA-S scores were obtained from the surgical specimen analysis; secondary CPCs were detected using inmunocytochemistry three months post surgery, a positive sample contained ≥1 PSA (+) CD45 (-) staining cell/blood sample and BF was defined as a serum total PSA >0.20ng/ml. Five year BF was determined using Cox regression analysis for models using the CAPRA-S, CPC, and combined data, they were compared using a decision analysis curve (DAC), Harrell´s C concordance test and predicted versus observed survival using Kaplan-Meier curves.

Results: 321 men, mean age 65.5yrs participated, in whom 193 (60%) had secondary CPCs detected. After 5 years of follow up the predicted biochemical free survival was 98.6%. For the DAC, the combined CAPRA-S/CPC model was superior to both single variable models with a Harrell´s C score of 0.86. Using the combined model 23.7% of men changed risk group.
Discussion: The incorporation of CPC detection into the CAPRA-S score improved significantly its prognostic value, it identified a low risk CAPRA-S sub-group with intermediate risk and a high risk CAPRA-S subgroup with low risk. The incorporation of CPC detection into the CAPRA-s score provides clinically important information on possible treatment decisions.

Introduction:

Prostate cancer is the second most common cancer diagnosed in men worldwide (1), with radical prostatectomy or radiotherapy being equivalent treatment options for localized cancer (2). However, approximately 20% of patients with develop biochemical failure within 5 years, defined as two or more consecutive PSA values of >0.20ng/ml (3, 4). A fraction of those patients developing biochemical failure will progress to develop clinical recurrence metastasis and cancer specific death. Identifying which patients are at risk is important to individualize patient care, both men with a high risk where early use of androgen blockade or radiation has a greater benefit (5, 6) and those at a low risk who could be spared the potential side effects of therapy.

The University of California, San Francisco produced the Cancer of the Prostate Risk Assessment Score (CAPRA-S) score to predict the risk of biochemical failure at five years, incorporating the pathological findings found at surgery (7) and which has been externally validated in differing populations (8-10).

The presence of secondary circulating prostate cells (CPCs), detected using the same standard immunocytochemistry method of defining CPCs as cells expressing PSA but not CD45, has been associated with a seven fold increased risk of biochemical failure (11) and using RT-PCR versus PSA to define CPCs are found more frequently in men with a rising PSA (12) and using RT-PCR versus PSA and prostate specific membrane antigen (PMSA) to define CPCs with a shorter PSA doubling time (13) and may complement the CAPRA-S or give an improved risk assessment of patients.

Thus the objective of the study was to establish a predictive model for biochemical failure based on the CAPRA-S score and secondary CPCs in men who had undergone radical prostatectomy for prostate cancer.

Methods and Patients:

We present a prospective single centre study of men who underwent radical prostatectomy as the sole treatment for prostate cancer between 2005 and 2015. The study was approved by the local ethics committee and complied with the Declaration of Helsinki.

For each patient, after giving informed written consent, the following were recorded; date of prostatectomy radical, age, the clinical details in order to calculate the CAPRA-S score as originally described (7)

a) serum total PSA (ng/ml) at the time of diagnosis using the Siemens Advia CentaurXR® assay

b) The pathological study of the surgical piece was performed by dedicated genitournary pathologists according to the Gleason system

i) presence or absence of extra-capsular extension (ECE),

ii) presence or absence of positive surgical margins, defined as one with cancer cells in contact with the inked surface of the specimen.

iii) infiltration of the seminal vesicles and lymph nodes

The patients were divided into two CAPRA-S score groups, low risk (0-2) and high risk (≥3), corresponding clinically to observation or no adjuvant treatment and to be considered for adjuvant treatment respectively.

Detection of Secondary circulating prostate cells (CPCs):

90 days after radical prostatectomy, an 8ml venous blood sample was taken and collected in a tube containing EDTA (Beckinson-Vacutainer®. Samples were maintained at 4ºC and processed within 48 hours. CPC detection was independently evaluated with the evaluators being blinded to the clinical details.

Collection of CPCs:

Mononuclear cells were obtained by differential centrifugation using Histopaque 1,077 (Sigma-Aldrich), washed, and resuspended in an 50µL aliquot of autologous plasma. 25 µL aliquots were used to make two slides (silanized, DAKO, USA), were dried in air for 24 hours and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate buffered saline (PBS) pH 7.4 for five mintues and finally washed three times in PBS pH 7.4 .

Immunocytochemistry:

CPCs were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastro Laboratory, UK), and identified using an alkaline phosphatase-anti alkaline phosphatase based system (LSAB2, DAKO, USA), with new fuchsin as the chromogen. Positive samples underwent a second process with anti-CD45 clone 2B11 + PD7/26 (DAKO, USA) and were identified with a peroxidase based system(LSAB2,DAKO, USA) with DAB (3,3 diaminobenzidine tetrahydrochloride) as the chromogen. A CPC was defined according to the criteria of ISHAGE (International Society of Hemotherapy and Genetic Engineering) (14) and the expression of CD45 as membrane bound staining. A secondary CPC was defined as a cell that expressed PSA but not CD45, a leucocyte did not express PSA but expressed CD45 (Figure 1 and 2). A test was considered positive for secondary CPCs when at least 1 cell/8mL of blood was detected and the number of CPCs detected/8ml blood simple was registered.

Slides were analysed manually, stained cells were photographed using a digital camera and from the digital images determined if CPCs were present or absent and the total number of CPCs/sample determined by one trained observer. Using this method, in preliminary work up trials of 50 subjects and three observers, there was agreement in

86% of cases between the three observers, in 14% of cases between two observers. The differences were in the number of CPCs/sample and not if the test was positive or negative. As the test was designed as a positive/negative test it was considered appropriate to proceed, acknowledging that there is an interobserver variation in the absolute CPC count.

Follow-up:

All men were followed up with serial total serum PSA measurements; three monthly for two years, then six monthly to detect the presence or absence of biochemical failure (BF) for up to 5 years. BF was defined as a serum total PSA >0.20ng/ml on two separate occasions, taken at least two weeks apart.

Statistical Analysis: The analysis was performed using the program Stata (Stata/SE 14.0 for Windows, Stata Corp Lp, 2015), describing according to the nature and distribution of the quantitative and ordinate variables with measurements of central tendency (mean and median) and of dispersion (interquartile range (IQR) and standard deviation), the Shapiro-Wilk Test was used to test the null hypothesis with respect to a normal distribution (15). The nominal dichotomous variables were described as proportions with their respective confidence intervals (15).

Evaluating the validity of the CAPRA-S score for our study group, we analysed the calibration of the data using partial validation (where the prognostic index and prognostic groups are derived from published information and the baseline distribution function is estimated in the validation dataset) (16); where the prognostic index (Neperiano logarithm of the hazard ratio) obtained from published data is applied to the CAPRA-S score of the observed study data. The aforesaid published survival is compared with our observed survival using the same categorization of patients. For this model the discriminatory power is evaluated using Harrell´s C concordance test (17-19). In order to predict biochemical failure during the first five years of follow up, three models were built using Cox proportional hazard regression method; firstly the use of the CAPRA-S score groups, second the use of secondary CPCs and thirdly the combination. All three models were tested for compliance with the Cox proportional

hazards model (log-log plots, Therneau and Grambsch test and testing for a cohort time interaction) (20-21). In addition, for each model the log likelihood (LL), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) and Harrell´s C to perform the respective predictions of biochemical free failure at three and five years (21).

To evaluate the clinical utility of secondary CPCs in the prediction of biochemical failure, a decision curve analysis (22) was used for the three models (CAPRA-S score groups; CPC detection; combined CAPRA-S score groups/CPC) to evaluate and compare them and to determine the clinical consequences of their predictions, that is to treat or not.

Results: 321 men participated with a mean age of 65.5 ± 8.3 years, and median serum total PSA at the time of diagnosis of 5.48 ng/ml (IQR= 3.26ng/ml), the median CAPRA-S score for the Gleason Index was 0 (IQR=1), ECE was present in 128/321 (40.0% ,95% CI 35.9-44.1); positive surgical margins in 55/321 (17.1% ,95% CI 13.4-21.7), seminal vesicle invasion in 8/321 (2.5%, 95% CI 1.3-4.9) and lymph node infiltration in 4/321 (1.3% 95% CI 0.5-3.3).

The CAPRA-S score was asymmetrically distributed, with a median score of 1 (IQR of 3) and a minimum and maximum score of 0 and 9 respectively. 69.5% (95% CI 64.2-74.3) of men had a CAPRA-S score of ≤ 2. Secondary CPCs were detected in 193 men (60.1%, 95% CI 54.6-65.4%).

After 5 years of follow up, the Kaplan-Meier biochemical free survival for the whole group was 98.55% (95% CI 94.31-99.64). The adjusted predicted Kaplan-Meier survival curves for CAPRA-S score groups based on the original validation population and our Kaplan Meier estimates (partial validation) are shown in Figure 3. There is a Harrell´s C concordance of 0.82 between the predicted survival and the observed survival in our group of patients.

The log-log plots for -ln(-ln)survival versus ln(time) by categories of a nominal variable using Kaplan-Meier estimates, show in the three models parallel log curves. The Therneau and Grambsch test and testing for a cohort time relation or the three models

were not significant (p>0.05).

The model incorporating the CAPRA-s scores groups showed a hazard ratio of 7.66 (95% CI 4.86-12.09 p<0.01) with an LL of -432, an AIC of 867, BIC of 871 and a Harrell´s C of 0.73. The CPC model as an independent variable showed hazards ratio of 34.52 (95% CI 13.97-852.9 p<0.01) a LL of -399, AIC of 801, BIC of 804 and a Harrell´s C of 0.81. The model using the CPC and CAPRA-S score groups showed hazard ratios for each predictor of 2.60 (95% CI 1.62-4.15) for the CAPRA-S and 22.5 (95% CI 8.85-57.3) for the CPC, both with values of p<0.01. For this combined model, the values for LL were -391, AIC 785, BIC 793 and a Harrell´s C of 0.86. The comparison of either single variable model with the combined variable model showed a likelihood ratio test with a p value of <0.001.
Figure 4 shows the results of the Decision Curve Analysis of the three models, for probability threshold values observed between 0 and 67%, it can be seen that the combined model is superior to the CPC and CAPRA-S score groups when used separately.

Risk Groups:
Of the 223 men with a CAPRA-S score group of ≤ 2 (low risk), 53 (23.8%) were CPC positive, thus according to the combined risk group changed from low to intermediate risk. Conversely of the 98 men with a CAPRA-S score group of ≥3 (high risk), 23 (23.5%) were CPC negative, thus changing from high risk to low risk according to the combined model. In total, 76 (23.75%) of patients changed risk groups when using the combined CAPRA-S score group/ CPC model of risk assessment.

Discussion:

The CAPRA-s scoring system is one of the many published predictive tools addressing clinical outcomes in men with prostate cancer (23); it is based on pathological data from the radical prostatectomy specimen and has a relatively high concordance value (7-9).

The frequency of biochemical failure observed in men with CAPRA-S scores ≤2 is similar to those previously published (Table 1). Similarly the biochemical failure free survival at 3 and 5 years in the study group of men with a CAPRA-S score of ≤ 2 are similar to those previously published (Table II).

The decision to group all men with a CAPRA-S score ≤ 2 was based on the clinical observation that at this cut off point of the CAPRA-S score the treatment recommended is that of observation. In men with higher CAPRA-S scores the management is variable. As such the introduction of CPC testing in this context permits a simpler algorithm to modify risk categories in these men.

There are two fundamental aspects in the evaluation and as a result the validation of the prognostic model. The discrimination is a measure of how risk estimates of different models characterize or classify treatment failure (16), while the calibration is the accuracy in the prediction of treatment failure (16). Applying the hazard ratio obtained from previously published work (7) and using it in our study group, we obtained a performance in the of classification of patients and as such a discrimination with the Harrell´s C test of 0.82 concordance, which is considered good to excellent, being equivalent to an area under the curve of 0.8-0.9 (18-19). In the same model we can observe that using the hazard ratio weighting that the predicted survival for CAPRA-S score groups based on the original validation data are equivalent to the values observed in the Kaplan-Meier. This confirms the validation partial (16) of the CAPRA-S scoring system for our data, as is suggested as a substitute for the lack of the original data which validated the CAPRA-S score.

The three models of patient survival comply with the assumptions of the Cox proportional hazards model, using graphical techniques, the Therneau and Grambsch non-proportionality test and testing for a cohort time interaction (p values >0.05). The increase in the log likelihood values, and reduction in AIC and BIC indicate the best-fit model. Thus based on these values the best-fit model from worse to best is; CAPRA-S score group model, CPC model and combined model. This observation was ratified using the likelihood ratio test ($p<0.01$) when comparing the combined model with the two models containing a single variable.

The model using CAPRA-S score group only give a Harrell´s C value of 0.73, which is interpreted as a predictive capacity considered acceptable and is in agreement with the results published by other workers. The incorporation of CPC as a sole variable and then in combination with the CAPRA-S score groups produces an increase in the Harrell C value to 0.81-0.86, considered to be excellent in its capacity to classify the patients (18, 19). The predictions of all three models are shown in Table 2, the combined use of CPC and CAPRA-S score groups shows a significantly increased discrimination in establishing the probability of biochemical failure.

The decision curve analysis (Figure 2) confirms that the combined group of CAPRA-S ≥ 2 cut off point and CPC detection results in a net benefit through all the range of levels of threshold probability of a disease in comparison with those achieved using either of the two parameters alone.

From the clinical viewpoint, after radical prostatectomy for prostate cancer, the question arises of which patients are likely to suffer treatment failure. The CAPRA-S score incorporates the known risk factors for treatment failure and classifies the patient into low, intermediate and high-risk groups. Low risk patients are observed; intermediate and especially high-risk patients may be offered treatment to decrease the risk of biochemical failure, although adjuvant therapy post-surgery remains controversial. The CAPRA-s uses simple clinical-pathological parameters, producing clinically important information about treatment failure. However, the clinical parameters are fixed, after “additional treatment” the CAPRA-S is no longer valid. Neither does it predict whether the failure will be local or systemic in origin and as such does not help in deciding between radiotherapy to the prostate bed or systemic hormonal manipulation.

Secondary CPC detection likewise gives important clinical information on the risk of treatment failure; equally the presence of secondary CPCs does not indicate whether the failure will be local or systemic in nature. However, differing from the CAPRA-S score, secondary CPCs can be used to monitor the effect of treatment, simply on a positive/negative score or as cells/blood sample detected. In

addition phenotypic analysis of CPCs may help to determine which type of systemic therapy may have a greater possibility of success (ref).

The combined model using both CAPRA-S and secondary CPC changes risk groups in a significant of patients. There is a subgroup of low risk CAPRA-S patients who are CPC (+), who have a significantly worse prognosis. While a group of high risk CAPRA-S patients who are CPC (-) have a much better prognosis, almost as good as low risk CAPRA-S CPC (-). Due to this, we believe that the addition of CPC detection gives clinically significant information of who may be eligible for adjuvant therapy, and inversely who may not need adjuvant therapy.

Circulating tumour cell detection is dependent on the method used, producing discordant results. It has been reported that using the EpCAM based CellSearch® system, approved by the FDA for use in metastatic prostate cancer, that CPCs have been detected in between 37-80% of studies (24-26). The failure to include tumour cells that have reduced or absent EpCAM expression may limit detection methods. There is down regulation of EpCAM with disease progression and metastasis and during the epithelial to mesenchyme transition (27). Similarly cytokeratins are heterogeneously expressed in tumour cells and also may be down-regulated during disease progression or in poorly differentiated tumours (28). Thus the use of an anti-PSA based detection system avoids this problem, however it must be mentioned that high grade or poorly differentiated tumours may have reduced or absent PSA expression. The problems with the use of different methods to detect circulating tumour cells has been extensively reviewed (29). This may explain why the EpCAM based systems have not been able to differentiate between controls and patients with localized cancer (25, 26).

We recognise that the study is limited in that it is a single centre, that the method of CPC detection is manual, though could be semi-automated. That the test is deemed to be positive or negative decreases the inter-observer variation according to our pilot study. With adequate training and capacitation this variation could be kept to a minimum. Our definition of a CPC is different than that using EpCAM based systems, however it would seem logical to use PSA as a marker for CPCs.

The study should be confirmed using a larger population and multiple centres, this would define inter-centre variability and determine the test´s use in the routine immunocytochemical laboratory of a general hospital.

Conflicts of Interest: None of the contributing authors have any conflicting interests, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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Table I. Characteristics of 321 men with and without biochemical failure treated by radical prostatectomy for prostate cancer.

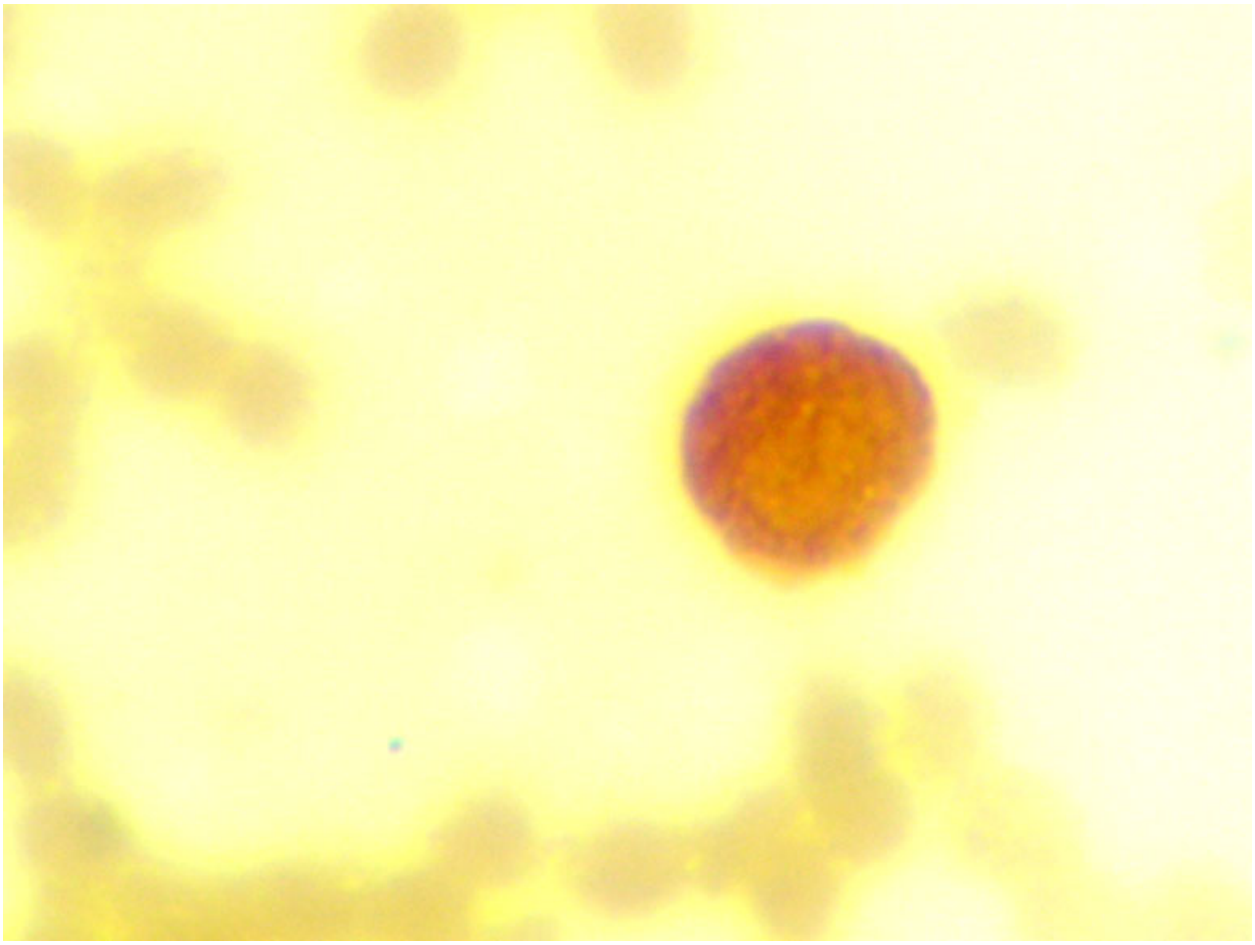
variable	Men without biochemical failure n=190	Men with biochemical failure n= 131	p- value two tails
Age, years mean ± de	64.71±8.07	66.52 ± 8.64	0.04 [a]
PSA, ng/ml median (IQR)	5.21 (1.92)	6.03 (3.84)	< 0.00 [a]
Gleason score Median (IQR)	0 (0)	1 (2)	< 0.00 [a]
Surgical margin positive n (%)	14 (7.37)	41 (31.30)	< 0.00 [b]
Infiltration of seminal vesicles n (%)	0 (0.00)	8 (6.11)	< 0.00 [c]
Extracapsular extension positive n (%)	42 (22.11)	88 (67.18)	< 0.00 [b]
Lymph node invasion positive n (%)	0 (0.00)	4 (3.05)	< 0.00 [c]
CAPRA-S score median (IQR)	0 (2)	3 (4)	< 0.00 [a]
CAPRA-S score ≥3 n (%)	30 (15.79)	68 (51.91)	< 0.00 [b]
CPC positive n (%)	18 (9.47)	110 (83.97)	< 0.00 [b]

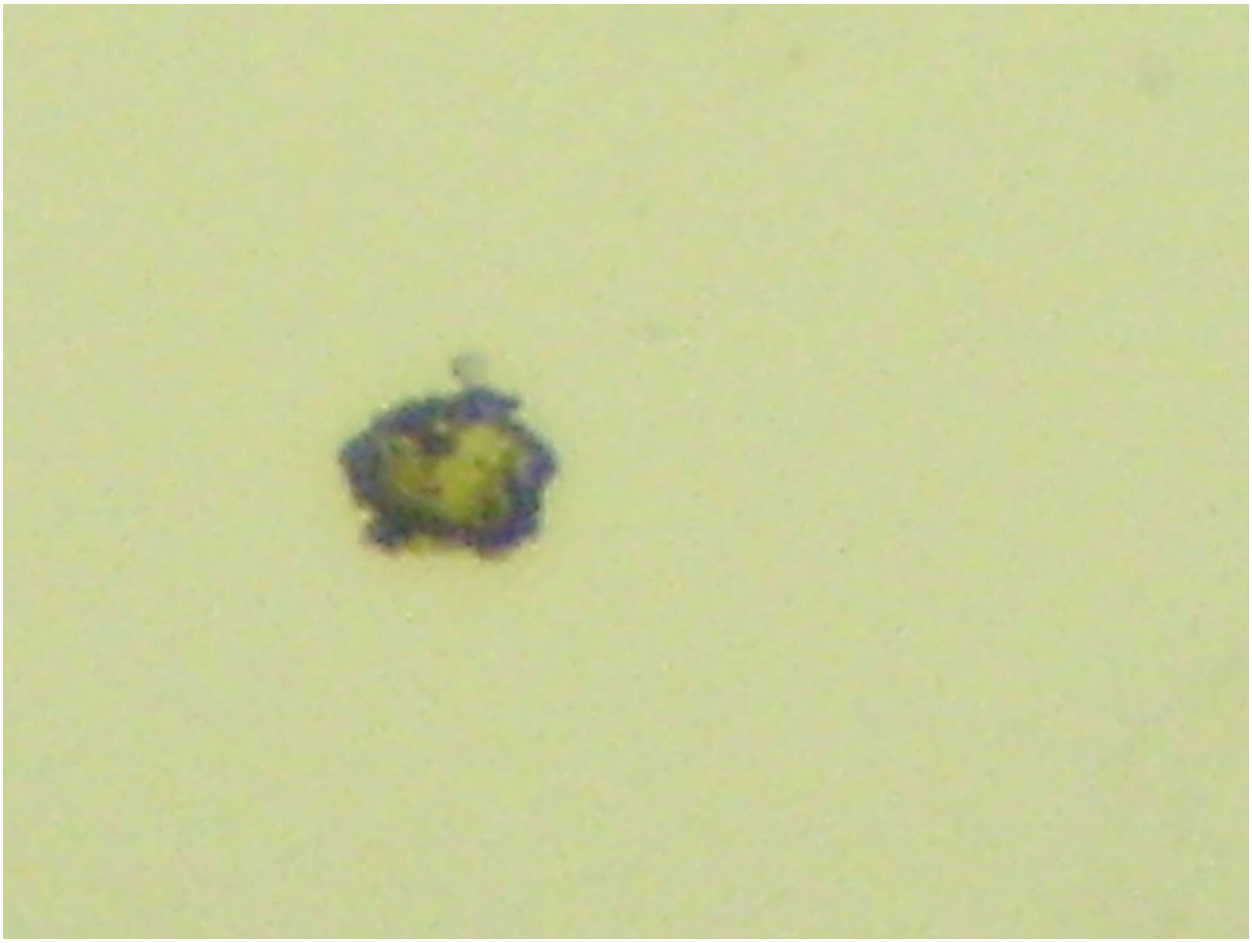
PSA= serum total PSA at diagnosis; Gleason Score = CAPRA-S score classification of Gleason score; CPC= Presence of circulating prostate cells 90-120 days post surgery. [a]Mann- Whitney Test; [b] Pearson´s Chi squared test; [c] Fisher´s Exact Test

Table II: Biochemical failure free progression at 3 and 5 years, according to the model of survival prediction in 321 men treated by radical prostatectomy.

Model	Individual Groups	Progression- free probability (95% CI)	
		3 years	5 years
Kaplan-Meier	CSG (-)	91.30 (86.54 to 94.43)	85.94 (80.26 to 90.09)
	CSGG(+)	49.30 (38.38 to 59.32)	31.59 (21.67 to 41.96)
	CPC (-)	99.39 (95.75 to 99.91)	96.71 (92.27 to 98.62)
	CPC (+)	50.29 (41.20 to 58.69)	34.49 (26.20 to 42.91)
	CSG(-) CPC(-)	1[a]	98.55 (94.31 to 99.64)
	CSG (+) CPC (-)	93.33 (61.26 to 99.03)	74.86 (39.11 to 91.46)
	CSG(-) CPC (+)	66.04 (51.64 to 77.06)	50.65 (36.51 to 63.18)
	CSG(+) CPC(+)	38.64 (27.36 to 49.77)	22.29 (13.34 to 32.68)
CSG[b]	CSG (-)	91.16 (87.07 to 94.00)	85.95 (80.36 to 90.04)
	CSG(+)	49.19 (39.00 to 58.60)	31.34 (21.87 to 41.24)
CPC[b]	CPC(-)	98.10 (95.47 to 99.21)	98.10 (95.47 to 99.21)
	CPC(+)	51.52 (42.56 to 59.76)	33.87 (27.75 to 42.15)
CSG and CPC[b]	CSG(-) CPC(-)	98.37 (96.09 to 99.33)	97.26 (93.60 to 98.84)
	CSG(+) CPC(-)	95.82 (89.56 to 98.36)	93.04 (83.14 to 97.22)
	CSG(-) CPC(+)	69.07 (56.99 to 78.39)	53.50 (39.68 to 65.49)
	CSG(+) CPC(+)	38.27 (27.88 to 48.56)	19.72 11.89 to 28.99)

[a]no biochemical failures registered at time of censoring; [b] These models are based on Cox´s proportional hazards risks according to the different parameters (CSG, CPC y/o ambos); CPC= indicates the presence of secondary circulating prostate cells; CSG= CAPRA-S score group (-)= low risk (0-2), and (+)=high risk (≥3).





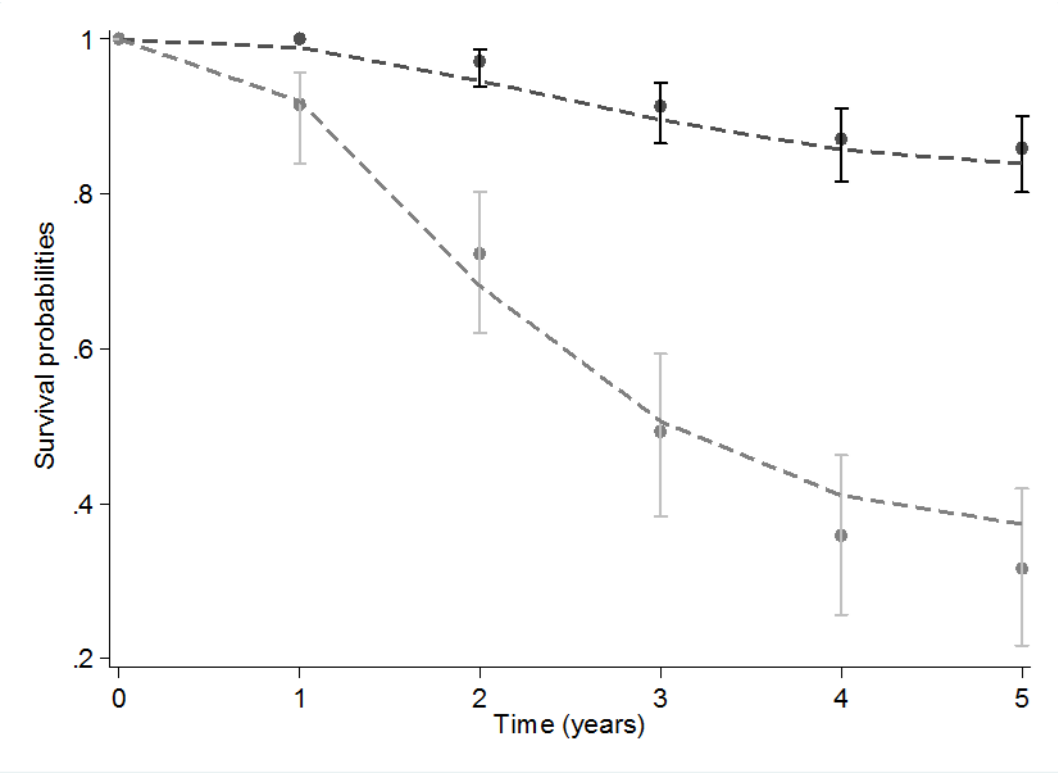
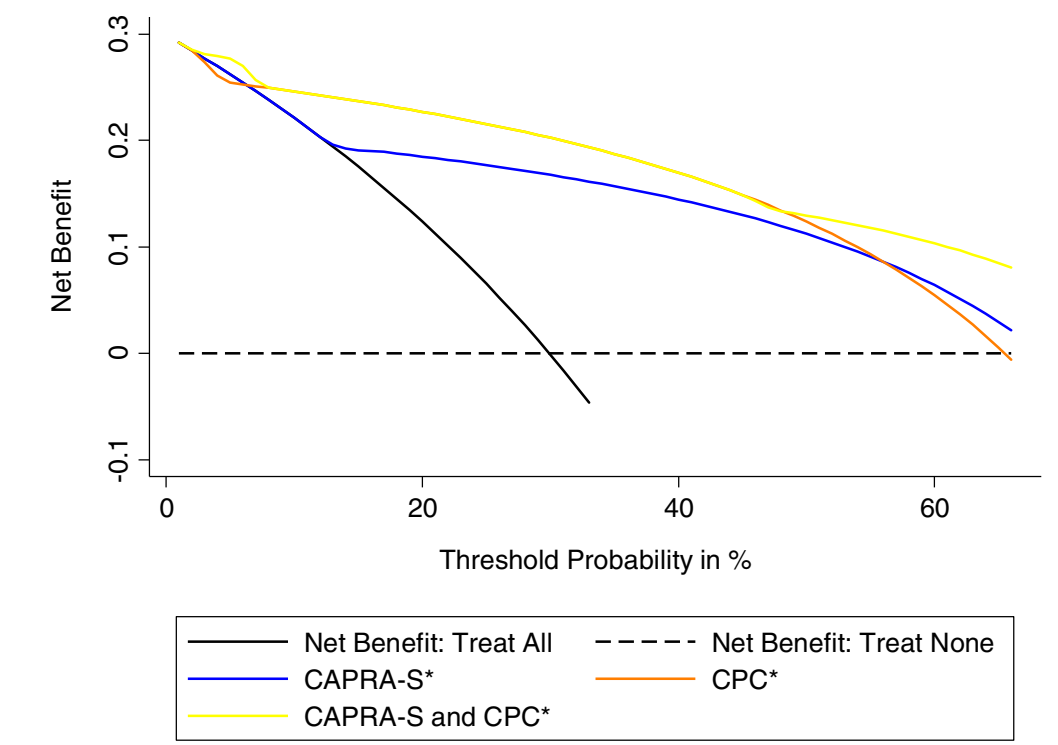


Figure 1 Partial calibration of a Cox model with the prognostic index estimated from the published regression coefficient and evaluated on the validation dataset with reestimation of the baseline cumulative-hazard function. Smooth dashed lines represent predicted survival probabilities, and vertical capped lines represent Kaplan–Meier estimates with 95% confidence intervals. Two prognosis groups are plotted: the "Good" group (darkest lines, included CAPRAS-S score 0 to 2), and the "Poor" group (paler lines; included CAPRAS-S score ≥3).

Figure 2: Decision curve analysis comparing models using CAPRA-S score groups, presence of secondary circulating prostate cells and combined in 321 men treated by radical prostatectomy for prostate cancer.



*These models are based on Cox´s proportional hazards risk, differing according to the variable used (CAPRA-S, CPC or both); CPC= indicates the presence of secondary circulating prostate cells; CAPRAS-S= CAPRAS-S score groups according to CAPRA-S score, low risk (0-2), and high risk (≥3).