



## Original article

# Limited improvement of incorporating primary circulating prostate cells with the CAPRA score to predict biochemical failure-free outcome of radical prostatectomy for prostate cancer

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## Abstract

**Objective:** To establish a prediction model for early biochemical failure based on the Cancer of the Prostate Risk Assessment (CAPRA) score, the presence or absence of primary circulating prostate cells (CPC) and the number of primary CPC (nCPC)/8 ml blood sample is detected before surgery.

**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 score. Before 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 prostate-specific antigen and P504S, and the presence or absence of CPCs and the number of cells detected/8 ml blood sample was registered. Patients were followed up for up to 5 years; biochemical failure was defined as a prostate-specific antigen >0.2 ng/ml. The validity of the CAPRA score was calibrated using partial validation, and the fractional polynomial Cox proportional hazard regression was used to build 3 models, which underwent a decision analysis curve to determine the predictive value of the 3 models with respect to biochemical failure.

**Results:** A total of 267 men participated, mean age 65.80 years, and after 5 years of follow-up the biochemical-free survival was 67.42%. The model using CAPRA score showed a hazards ratio (HR) of 5.76 between low and high-risk groups, that of CPC with a HR of 26.84 between positive and negative groups, and the combined model showed a HR of 4.16 for CAPRA score and 19.93 for CPC. Using the continuous variable nCPC, there was no improvement in the predictive value of the model compared with the model using a positive-negative result of CPC detection. The combined CAPRA-nCPC model showed an improvement of the predictive performance for biochemical failure using the Harrell's *C* concordance test and a net benefit on DCA in comparison with either model used separately. The use of primary CPC as a predictive factor based on their presence or absence did not predict aggressive disease or biochemical failure.

**Conclusion:** Although the use of a combined CAPRA-nCPC model improves the prediction of biochemical failure in patients undergoing radical prostatectomy for prostate cancer, this is minimal. The use of the presence or absence of primary CPCs alone did not predict aggressive disease or biochemical failure. © 2016 Elsevier Inc. All rights reserved.

**Keywords:** CAPRA score; Prostate cancer; Circulating prostate cells; Biochemical failure

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## 1. Introduction

Prostate cancer is the second commonest cause of cancer death in the Chilean male population [1]; with the

demographic changes and an aging population, the number of men with prostate cancer is increasing worldwide. It is considered that 90% of cancers detected using prostate-specific antigen (PSA) screening is localized to the prostate at the time of diagnosis [2]. However, 20% to 30% of these patients would experience biochemical failure, usually in the first 2 years after surgery [3]. The issue is further complicated by the fact that not all prostate cancers need to be treated. The high prevalence of prostate cancer detected at autopsy [4] and the contrast between the incidence and mortality of prostate cancer implies that more men would die from their cancer [5]. Identifying those men who need active treatment from those who could be observed is of clinical and practical importance. The further identification of those men with a high risk of progression after primary treatment would be of additional importance, especially if this could be determined before deciding the type of primary therapy.

Criteria, such as those reported by Epstein et al. [6] or the more recent PRIAS criterion [7], have been used to determine which patients may be treated by active observation, especially those with estimated low-risk low-volume tumors. Further predictive tools such as the Cancer of the Prostate Risk Assessment (CAPRA) scale [8] have been used to identify men with a higher risk of disease progression after primary treatment using pathological variables obtained from the prostate biopsy.

Early in prostate cancer, there is at least 1 subpopulation of cancer that disseminates first to the neurovascular structures and then into the circulation [9]. Most of these cells are eliminated by host defenses or destroyed by shear forces as they circulate in the blood and lymph systems [10]. These cells, defined as primary circulating prostate cells (CPCs), are not found in small-volume low-grade cancers [11] and have been used as a sequential test to detect men with prostate cancer [12,13]. The presence of primary CPCs may therefore imply a higher risk of aggressive cancer and could be used preoperatively to identify these patients.

In consideration of this hypothesis, we evaluated the use of a model that includes the CAPRA score, the presence or absence of CPCs, and the number of primary CPCs (nCPCs)/sample to predict biochemical failure before radical prostatectomy in men with a diagnosis of prostate cancer.

## 2. Patients and methods

A prospective single-center study of 269 men who underwent radical prostatectomy as monotherapy for prostate cancer between 2005 and 2014. 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: (a) date of radical prostatectomy, (b) age, (c) serum PSA (ng/ml) at diagnosis using the Siemens Advia CentaurXR assay, (d) the pathological study

of the prostate biopsy was performed by dedicated genitourinary pathologists according to the Gleason system, (e) clinical staging by the Urologist (T1c–T3a), and (f) percentage of biopsy cores positive for cancer.

## 3. CAPRA score

The patients were classified according to the CAPRA score as low risk (CAPRA score 0–5) or high risk (CAPRA score  $\geq 6$ ). Based on the reports that with a CAPRA score of  $\geq 6$ , the biochemical failure rate at 5 years post surgery is significantly increased [8,14].

### 3.1. Detection of primary CPCs

Before the prostate biopsy, all men had an 8 ml venous blood sample taken and collected in a tube containing EDTA (Beckinson-Vacutainer). Samples were maintained at 4°C and processed within 48 hours. CPC detection was independently evaluated with the evaluators being blinded to the clinical details.

### 3.2. Collection of CPCs

Mononuclear cells were obtained by differential centrifugation using Histopaque 1077 (Sigma-Aldrich), washed, and resuspended in a 100  $\mu$ l aliquot of autologous plasma. Further, 25  $\mu$ l aliquots used to make 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 pH 7.4 for 5 minutes and finally washed 3 times in phosphate buffered saline pH 7.4.

### 3.3. Immunocytochemistry

CPCs were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastro Laboratory, UK), and identified using an alkaline phosphatase-antialkaline phosphatase-based system (LSAB2, DAKO, USA), with new fuchsin as the chromogen. Positive samples underwent a second process with anti-P504S clone 13H4 (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 as a cell expressing both PSA and P504S according to the criteria of International Society of Hemotherapy and Genetic Engineering [15] and the expression of P504S defined according to the Consensus of the American Association of Pathologists [16]. The test was classified as negative when no cells were detected and as positive when at least 1 cell was detected/8 ml blood sample, and the number of CPCs detected/8 ml blood sample was registered (nCPC).

Slides were analyzed 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 1 trained observer. Using this method, in the preliminary workup trials of 50 subjects and 3 observers, there was an agreement in 86% of cases among 3 observers.

### 3.4. Follow-up

PSA values were evaluated at 3 and 6 months after surgery and then every 6 months. Biochemical failure was defined as a single PSA value greater than 0.2 ng/ml on 2 consecutive occasions, at least 3 months after surgery. Follow-up was for 5 years.

### 3.5. Statistical analysis

The analysis was performed using the program Stata (Stata/SE 14.0 for Windows, Stata Corp Lp, 2015), described according to the nature and distribution of the quantitative and ordinate variables with measurements of central tendency (mean and median) and of dispersion using the interquartile range (IQR) and standard deviation. The Shapiro-Wilk test was used to test the null hypothesis with respect to a normal distribution [17]. The nominal dichotomous variables were described as proportions with their respective CI [17].

The variables, which formed the CAPRA score and CPCs both as a dichotomous positive/negative test and as a continuous variable test of the number of CPCs detected/8 ml sample, were compared with the presence of biochemical failure at 5 years.

#### 3.5.1. Validation of the CAPRA score in the study population

To evaluate the validity of the CAPRA score for our study group, we analyzed the calibration of the data using partial validation (the closeness of fit of the prediction of possible survival) [18], where the prognostic index (Neperiano logarithm of the hazards ratio [HR]) obtained from published data was applied to the CAPRA score of the observed study data. The aforesaid published survival rate was compared with our observed survival rate using the same categorization of patients. For this model, the discriminatory power was evaluated using Harrell's *C* concordance test [19–21].

#### 3.5.2. Using the CPC test as a dichotomous positive/negative test

The nominal dichotomous variables were described as proportions with their respective CI [16].

The variables that form the CAPRA score were compared with the presence or absence of primary CPCs and the presence of biochemical failure at 5 years.

To predict biochemical failure during the first 5 years of follow-up, 3 models were built using Cox proportional hazard regression method; first, the use of the CAPRA score

groups; second, the use of primary CPCs; and third, the combination. All 3 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) [21,22]. In addition, for each model the log likelihood (LL), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Harrell's *C* test were used to perform the respective predictions of biochemical-free failure at 3 and 5 years [22].

To evaluate the clinical use of primary CPCs in the prediction of biochemical failure, a decision curve analysis [23] was used for the 3 models (CAPRA score groups, CPC detection, and combined CAPRA score groups/CPC) to evaluate and compare them and to determine the clinical consequences of their predictions, that is to treat or not.

#### 3.5.3. Using the CPC test as a continuous variable with respect to the nCPCs detected per 8 ml blood sample

To construct the Cox's model, the multivariable fractional polynomial assessment was used to establish a functional form for the nCPC, represented by the formula  $[(nCPC + 1)/10]^{-1}$ . The transformed value of nCPC allows a better adjustment and specification for the Cox proportional hazards regression model. Thus, the regression models incorporating nCPC use the functional form (transformed variable). To predict biochemical failure during the first 5 years of follow-up, 3 models were built using the fractional polynomial of the Cox proportional hazard regression method: firstly, the use of the CAPRA score groups; secondly, the use of nCPC; and thirdly, the combination. During the construction of the Cox model, multivariable fractional polynomials to evaluate the transformed predictor nCPC were used to accommodate the nonlinear regression relationship that the Cox model establishes.

All 3 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) and for the correct specification for the respective model (Linktest) [22,23]. In addition, for each model the LL, AIC, BIC, and Harrell's *C* test were used to predict biochemical-free failure [23].

To evaluate the clinical utility of primary CPCs in the prediction of biochemical failure, a decision curve analysis [24] was used for the 3 models (CAPRA score groups; nCPC; and combined CAPRA score groups/nCPC) to evaluate and compare them and to determine the clinical consequences of their predictions, that is to treat or not.

## 4. Results

A total of 267 men with a mean age of  $65.80 \pm 8.78$  years, a median total serum PSA of 5.70 ng/ml (IQR: 3.60 ng/ml), and a median value of percentage of positive biopsy cores of 20% (IQR: 25%) participated in the study.

The p-values of the Shapiro-Wilk test were  $<0.15$  for all the quantitative variables. The median Gleason score, according to the CAPRA point score, had a value of 0, with an IQR of 1 and a minimum and maximum of 0 and 3, respectively. A clinical staging of T1 or T2 was seen in 208 (77.9%; 95% CI: 72.9–82.9%) patients.

The CAPRA score was asymmetrically distributed with a median score of 2 points (IQR: 3), with minimum and maximum scores of 0 and 10, respectively. Further, 31 men (11.6%; 95% CI: 7.7%–15.5%) had a CAPRA score of at least 6 points. In addition, the number of CPCs detected before surgery was asymmetrically distributed with a median score of 4 cells (IQR: 7), with minimum and maximum of 0 and 40 cells, respectively. In 211 men (79.0%; 95% CI: 74.1%–83.9%), primary CPCs were detected.

After 5 years of follow-up, biochemical failure was observed in 97 (36.3%; 95% CI: 30.6%–42.1%) patients. Comparing the presurgery clinical-pathological findings, biochemical failure was significantly associated with a higher total serum PSA, a higher Gleason score, T3 disease, a higher percentage of positive biopsy cores, a higher CAPRA score, a test positive for the detection of CPCs, and a higher nCPCs value ( $P < 0.05$ ) (Table 1).

After 5 years of follow-up, the Kaplan-Meier biochemical-free survival for the whole group was 67.42% (95% CI: 60.63–73.30). The adjusted predicted Kaplan-Meier survival curves for CAPRA score groups based on the original validation population and our Kaplan-Meier estimates (partial validation) are shown in Fig. 1. There is a Harrell's *C* concordance of 0.82 between the predicted survival and the observed survival in our group of patients, which is considered in the range “good.”

#### 4.1. Using the CPC test as a positive/negative result

The log-log plots for  $-\ln(-\ln)$  survival vs.  $\ln$  (time) by categories of a nominal variable using Kaplan-Meier

Table 1

Characteristics of 267 men with and without biochemical failure treated by radical prostatectomy for prostate cancer

Variable	Men without BF, <i>n</i> = 170	Men with BF, <i>n</i> = 97	<i>P</i> value
Age*, <i>y</i> ; mean $\pm$ SD	65.55 $\pm$ 8.78	66.23 $\pm$ 8.81	0.591 <sup>a</sup>
PSA*, ng/ml; median; IQR	5.5; 2.93	6.6; 5.48	$<0.01^a$
Gleason: $\leq 6$ , <i>n</i> (%)	139 (81.76)	36(37.11)	$<0.01^b$
T-stage: T1/T2, <i>n</i> (%)	156(91.76)	52(53.61)	$<0.01^b$
PPB* median; IQR	15; 20	35; 25	$<0.01^a$
CAPRA score*, median; IQR	2; 1	4; 4	$<0.01^a$
CPC positive, <i>n</i> (%)	118(69.41)	93(95.88)	$<0.01^b$

BF = biochemical failure; SD = standard deviation; PPB = percent positive biopsies.

\*Shapiro-Wilk test  $P < 0.15$ .

<sup>a</sup>Mann-Whitney test, a one-tailed.

<sup>b</sup>Pearson's Chi-squared test, a two-tailed. The ordinate and quantitative variables are represented by median and IQR values.

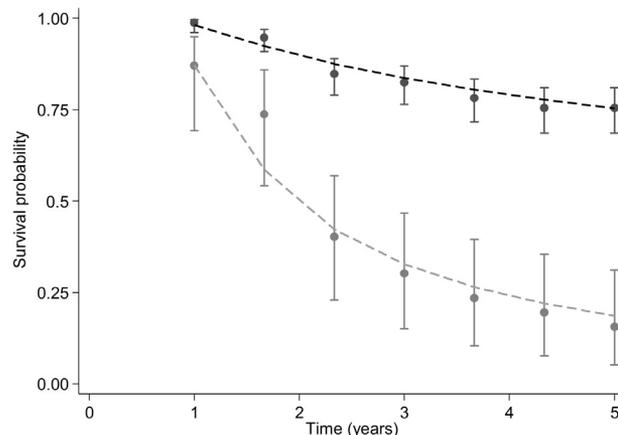


Fig. 1. Partial calibration of a Cox model with the prognostic index estimated from the published regression coefficient and evaluated on the validation dataset with reestimation of the baseline cumulative-hazard function. Smooth dashed lines represent predicted survival probabilities, and vertical capped lines represent Kaplan-Meier estimates with 95% CI. Further, 2 prognosis groups are plotted: the “Good” group (darkest lines, included CAPRA score 0–5) and the “Poor” group (paler lines; included CAPRA score  $\geq 6$ ).

estimates show parallel log curves in the 3 models. The Therneau and Grambsch test and testing for a cohort time relation or the 3 models were not significant ( $P > 0.15$ ).

The model incorporating the CAPRA score groups showed a HR of 5.76 (95% CI: 3.53–12.09;  $P < 0.01$ ) with an LL of  $-358.81$ , an AIC of 719.63, BIC of 723.21, and a Harrell's *C* of 0.63. The CPC model as an independent variable showed HR of 26.84 (95% CI: 3.73–193.33;  $P < 0.01$ ), a LL of  $-359.38$ , AIC of 720.77, BIC of 724.36, and a Harrell's *C* of 0.63. The model using the CPC and CAPRA score groups showed HRs for each predictor of 4.16 (95% CI: 2.55–6.79) for the CAPRA and 19.93 (95% CI: 2.75–144.61) for the CPC, both with  $P < 0.01$ . For this combined model, the values for LL were  $-345.97$ , AIC 695.94, BIC 703.12, and a Harrell's *C* of 0.72. The comparison of either single variable model with the combined variable model showed a likelihood ratio test with  $P < 0.001$ .

Fig. 2 shows the results of the decision curve analysis of the 3 models, for probability threshold values observed between 0% and 82%. The model based on the CAPRA score is superior to that based on primary CPC detection, from a probability threshold of 33.7% of an aggressive cancer that is being present. The combined model did not differ significantly from the model using CAPRA or CPC detection alone for all the thresholds of probability.

In summary, the use of the primary CPCs to predict the occurrence of a future biochemical failure when used as positive/negative proved to be inferior compared with traditional predictive factors. Even when combined with the CAPRA prediction model, it failed to improve the model of predicting biochemical failure. The Harrells' *C* predictive value between 0.63 and 0.71 for the CPC test as a

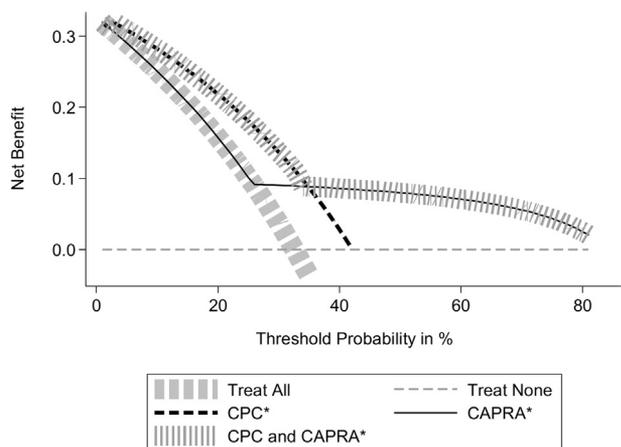


Fig. 2. Decision curve analysis comparing models using CAPRA score groups, presence of PRIMARY circulating prostate cells, and combined in 267 men treated by radical prostatectomy for prostate cancer. \*These models are based on Cox's proportional hazards risk, differing according to the variable used (CAPRA, CPC or both); CPC= indicates the presence of PRIMARY circulating prostate cells; CAPRA = CAPRA score groups according to CAPRA score, low risk (0-5), and high risk ( $\geq 5$ ).

single or combined predictive variable is considered to be poor or at best acceptable.

#### 4.2. Using the CPC test as a continuous variable

The 3 models the CAPRA score group, functional form of nCPC, and the combined model, constructed using the Cox proportional hazards model show parallel log curves for the log-log plots for  $-\ln(-\ln)$  survival vs.  $\ln(\text{time})$  by categories of a nominal variable using Kaplan-Meier estimates show parallel log curves in the 3 models. The Therneau and Grambsch test and testing for a cohort time relation or the 3 models were not significant ( $P > 0.15$ ). Likewise, the Linktest showed a proper specification of all the 3 models aforementioned.

The model incorporating the CAPRA score groups showed a HR of 5.76 (95% CI: 3.53–12.09;  $P < 0.01$ ) with an LL of  $-358.81$ , an AIC of 719.63, BIC of 723.21, and a Harrell's  $C$  of 0.63.

The model for the functional form of nCPC as an independent variable showed HR of 0.66 (95% CI: 0.57–0.78;  $P < 0.01$ ), a LL of  $-350.22$ , AIC of 702.45, BIC of 706.0336, and a Harrell's  $C$  of 0.74.

The combined model showed HRs for each predictor of 2.93 (95% CI: 1.74–4.92) for the CAPRA and 0.72 (95% CI: 0.62–0.84) for the nCPC, both with  $P < 0.01$ . For this combined model, the values for LL were  $-342.81$ , AIC 689.61, BIC 696.78, and a Harrell's  $C$  of 0.77.

The comparison of either single variable model with the combined variable model showed a likelihood ratio test with a  $P < 0.001$ .

Fig. 3 shows the Kaplan-Meier survival curves, whereby the observed survival curves agreed with the predicted survival of the 3 models.

Fig. 4 shows the results of the decision curve analysis for the 3 models for the range of probability threshold values observed between 0% and 80%. For a probability threshold of 45% for an aggressive prostate cancer being present, the model based on the CAPRA score is inferior to the model based on nCPC alone and the combined model. The combined model did not differ significantly from the nCPC alone model in predicting biochemical failure under a probability threshold of 47%. When the threshold of probability of biochemical failure is more than 47%, the models of CAPRA alone and combined are superior to the nCPC alone model. In this range of prediction, there was no significant difference between the CAPRA alone and CAPRA combined models.

The 3 models of patient survival complied 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 ( $P > 0.15$ ). The increase in the LL 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 nCPC model, CAPRA score group model, and combined model. This observation was ratified using the likelihood ratio test ( $P < 0.01$ ) when comparing the combined model with the 2 models containing a single variable.

Notwithstanding, the LL of the models suggests a closer proximity between the models for the CAPRA score and CPC detection, showing that both have a similar predictive capacity, with an approximate Harrell's  $C$  of 0.63, which is interpreted as poor in its predictive capacity in our study population. The combined model of CAPRA score plus CPC detection produced an increase in the Harrell  $C$  value to 0.71, considered to be borderline between acceptable and regular values [18,19]. The predictions of all 3 models are shown in Table 2 (comparing predicted vs. observed survival); the combined use of CAPRA and CPC detection did not show a significant increase in the capacity of predicting biochemical failure for intermediate groups.

When used as a continuous variable in patients classified as high risk ( $> 47\%$  probability), the use of nCPCs did not improve the prediction of biochemical failure. In patients with lower risk, the use of nCPCs improved the predictive model; however, the Harrell's  $C$  between 0.63 and 0.71 is considered to be poor or at best acceptable.

## 5. Discussion

Predicting the long-term results after radical prostatectomy is an essential part of pretreatment counseling of men with prostate cancer. The use of nomograms to predict biochemical failure permits the evaluation of treatment options in individuals as well as to compare clinical outcomes. The CAPRA score improves the pretreatment assessment and has been externally validated in North American and European populations [8,25].

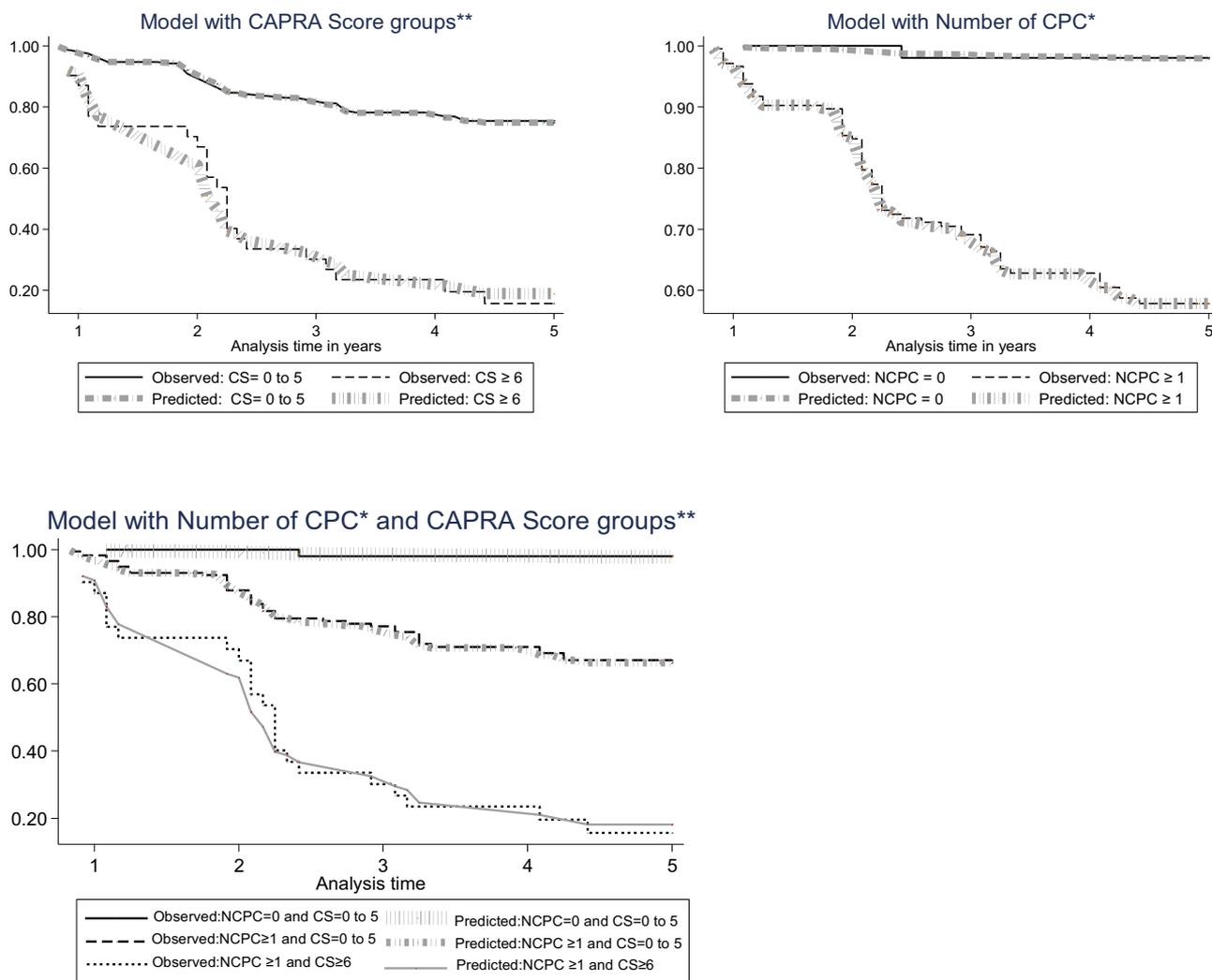


Fig. 3. Biochemical failure-free progression at 5 years. Comparing “Predicted” (according to the models of Cox<sup>a</sup>) vs. “Observed” survival (model Kaplan-Meier) for CAPRA score, nCPC, and combined models, in 267 men treated by radical prostatectomy. nCPC= number of circulating prostate cells prior to surgery; CS=the CAPRA Score. A These models are based on Cox’s proportional hazards risks according to the different dependent variables (nCPC, CS Groups or both).\* A functional form of variable “nCPC” represented by “[nCPC+1]/10<sup>-1</sup>·[1.229281768]”, were used for the models with this dependent variable. \*\*CS groups according to the prognostic index (CAPRA score), low risk (0-5), and high risk (≥6), were used for the models with this dependent variable. (Color version of figure is available online.)

There are 2 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 [17], whereas the calibration is on the accuracy in the prediction of treatment failure [17]. Applying the HR obtained from previously published work [8] and using it in our study group, we obtained a performance in the 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 to 0.9 [18,19]. In the same model, we could observe that using the HR weighting, the predicted survival for CAPRA score groups based on the original validation data is equivalent to the values observed in the study of

Kaplan-Meier curves. This confirms the partial validation [17] of the CAPRA scoring system for our data, as is suggested as a substitute for the lack of the original data, which validated the CAPRA score.

The transfer of nomograms to populations different than the original dataset may reduce the predictive accuracy [25]. Although our population is small, 267 patients, the CAPRA score performed equally well in a Chilean population as with the original study groups.

The decision to categorize the CAPRA score into 2 groups, high and low risk, was related to management options; given that men with high-risk scores, ≥6, may need a more aggressive treatment, whereas those with low-risk CAPRA scores may benefit from active observation as has been suggested [25]. The division of the CAPRA score

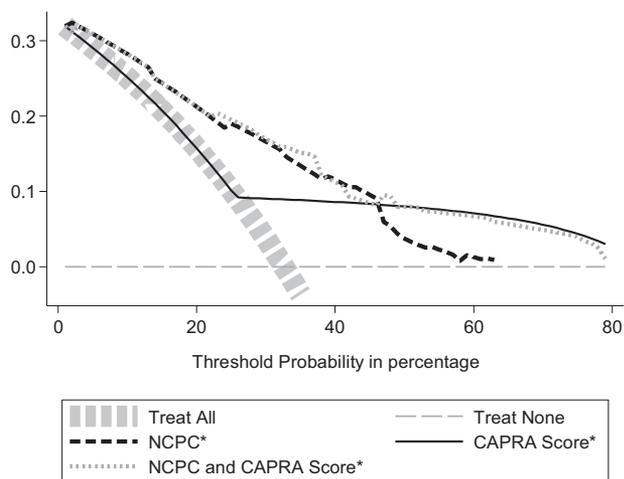


Fig. 4. Decision curve analysis comparing models using CAPRA score groups, number of prostate cells before surgery, and combined in 267 men treated by radical prostatectomy for prostate cancer. nCPC= indicates the number of circulating prostate cells prior to surgery; CAPRA Score = CAPRA score groups according to CAPRA score, low risk (0-5), and high risk ( $\geq 6$ ). \*These models are based on Cox's proportional hazards risk, differing according to the variable used (CAPRA Score, nCPC or both). A functional form of variable "nCPC" represented by " $[(nCPC+1)/10]^{-1} - 1$  [1.229281768]" , were used for the models with this dependent variable.

into 2 groups simplified its interpretation within a clinical context and in view of the small cohort studied.

The addition of the detection of primary CPCs, both as a positive/negative test and as a continuous variable of the number of cells detected per sample, did not improve the predictive capacity of the CAPRA score to identify patients

with a higher risk of biochemical failure as seen in the decision curve analysis.

Recently published by Meyer et al. [26], using the EpCAM-based CellSearch CTC detection system in 152 patients and a median follow-up of 4 years, the authors reached the conclusion that the primary CPC detection did not correlate with the risk of biochemical failure. They only found that 11% of men were positive for CPCs, corresponding to those with a higher-grade cancer and T3 disease. Using anti-PSA/anti-P504S, we found primary CPCs to be present in 76% of patients. The EpCAM-based system has previously been reported to detect CPCs in few patients with localized disease [27,28], differing from that reported by Fizazi et al. [29] using a telomerase-based system. This highlights the importance of which system of detection of CPCs is being used; primary CPCs may not express EpCAM in most patients due to the epithelial-mesenchyme transition [30] and thus may not be the most adequate system to be used. The study of Meyer et al. used a novel cutoff value of CTCs to determine a positive value of  $\geq 1$  cell/7.5 ml sample, and although having fewer patients in the study, they reported similar results in which CPC detection was not associated with predicting biochemical failure.

The reason for this discrepancy and the difference in comparison with the prognostic significance of secondary CPCs lies in the clinical significance of these cells. Primary CPCs disseminate early in the tumor process, as described by Moreno et al. [9]; however, most of these cells would be eliminated by host defense mechanisms [10]. Thus, complete removal of the primary tumor prevents further

Table 2

Biochemical failure-free progression at 3 and 5 years. Comparing predicted (according to the models of Cox) vs. observed survival (model Kaplan-Meier) in 267 men treated by radical prostatectomy.

Model	Grupos individual groups	Progression-free probability (95% CI)	
		3 years	5 years
Kaplan-Meier	CAPRA-LR	82.41 (76.41–87.02)	75.47 (68.58 –81.07)
	CAPRA-HR	30.15 (15.11–46.73)	15.63 (5.24–31.11)
	CPC (–)	98.00 (86.64–99.72)	98.00 (86.64–99.72)
	CPC (+)	69.15 (61.74–75.41)	57.88 (49.71–65.20)
	CAPRA-LR CPC (–)	98.00 (86.64–99.72)	98.00 (86.64–99.72)
	CAPRA-HR CPC (–) <sup>b</sup>		
	CAPRA-LR CPC (+)	77.10 (69.47–83.05)	67.05 (58.22–74.42)
	CAPRA-HR CPC (+)	30.15 (15.11–46.73)	15.63 (5.24–31.11)
CAPRA <sup>a</sup>	CAPRA-LR	80.89 (74.96–85.55)	74.87 (68.06–80.44)
	CAPRA-HR	29.50 (6.29–43.99)	18.91 (8.30–32.82)
CPC <sup>a</sup>	CPC (–)	98.51 (90.16–99.78)	97.98 (86.92–99.70)
	CPC (+)	66.85 ( 59.42–73.22)	57.85 (49.83–65.05)
CAPRA and CPC <sup>a</sup>	CAPRA-LR CPC (–)	98.54 (90.48–99.78)	97.96 (86.97–99.70)
	CAPRA-HR CPC (–)	94.05 (64.45–99.15)	91.78 (54.02 to 98.81)
	CAPRA-LR CPC (+)	74.53 (67.00–80.59)	66.27 (57.67–73.53)
	CAPRA-HR CPC (+)	29.47 (16.25–43.98)	18.09 (7.76–31.86)

<sup>a</sup>These models are based on Cox's proportional hazards risks according to the different parameters (CAPRA, CPC y/o ambos); CPC = the presence of secondary circulating prostate cells; CAPRA-LR = low risk (CAPRA score 0–5), and CAPRA-HR = high risk (CAPRA score  $\geq 6$ ).

<sup>b</sup>No CAPRA-HR CPC (–) cases were observed.

dissemination from this source. If none of the primary CPCs, for their phenotypic characteristics, have been able to implant and survive, then the presence of these cells detected before surgery would have no prognostic effect. Men, in whom primary CPCs were eliminated by surgery, have a better prognosis than those who remain positive for CPCs after radical prostatectomy [31]. The detection of secondary CPCs, which are detected after primary curative therapy, is associated with an increased risk of biochemical failure [32] and represents minimal residual disease. That is, not all the disease would be eliminated by the primary curative therapy and thus may in the future cause treatment failure. When compared with the CAPRA-S score to predict treatment failure, the use of CPC detection alone was superior; combined with the CAPRA-S score, 23% of men changed from a low-risk to a high-risk group in terms of the probability of biochemical failure [33].

The study has its limitations, firstly, the cohort of 267 men may not be sufficient to detect a difference in the predictive value, although the study of Meyer et al. showed similar results, and the follow-up may not be sufficient to have detected all men who would ultimately suffer biochemical failure, especially those failing after 5 years. However, the time period studied was sufficient to detect most men who would undergo failure for aggressive disease that usually occurs in the first 2 to 3 years. As a manual test, the interobserver variability for the number of CPCs detected/sample is higher than an automatized detection system, which is the reason why the test was designed as a positive/negative result to decrease this variability. Notwithstanding, we consider that the biological significance of CPCs is that owing to their phenotypic characteristics and host defenses, the fact that not all CPCs would implant or survive is the reason why they do not predict treatment failure. However, that they are present in more than 70% of men with prostate cancer suggest that they could be useful in cancer detection as has been suggested by some [12].

This may explain in part why primary CPCs do not improve the CAPRA predictive model.

## 6. Conclusions

The use of primary CPC detection as part of a predictive prognostic model does not improve the identification of men with a high risk of aggressive disease. As such, their detection cannot be recommended as a prognostic factor; it may be that phenotypic or genotypic characterization of these cells may identify those cells able to survive and implant or inversely those which would not.

## Conflict of interests

The authors report no conflicts of interest.

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