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ORIGINAL ARTICLE

Serum Chromogranin A as a Complementary Marker for the Prediction of Prostate Cancer-Specific Survival

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Abstract Better prognostication of clinically localized prostate cancer (PCA) is urgently needed. Former studies using different study end-points provided controversial results regarding the prognostic value of serum chromogranin A (CGA) in clinically localized PCA. However, serum CGA was not tested for correlation with the most significant study end-point of long-term disease-specific survival (DSS). CGA and matrix metalloproteinase-7 (MMP7) levels were measured by the BRAHMS KRYPTOR in two independent patient groups with 127 serum and 110 plasma samples. CGA and MMP7 concentrations were correlated with clinicopathological and survival data. In addition, we tested the combinations of CGA with PSA and with a currently identified prognostic factor, MMP7, for their prognostic value. CGA concentrations were significantly elevated in advanced compared to clinically localized cases both in serum and plasma samples (45 vs. 23 ng/ml, $p < 0.001$ and; 41 vs. 22 ng/ml; $p = 0.002$ respectively). In accordance, high CGA levels were correlated with poor DSS. In clinically localized cases, CGA levels alone were not prognostic, but its dichotomized combinations with PSA or MMP7 were independently associated with DSS (HR: 4.88, 95% CI: 1.35–17.71, $p = 0.016$, HR: 7.46, 1.65–33.63, $p = 0.009$, respectively). Elevated serum CGA levels in progressed PCA and its prognostic value suggest a potential for CGA in disease monitoring. Our results revealed no independent prognostic value for CGA as a single serum marker in clinically localized cases. However, when combining with PSA or MMP7, CGA may improve both marker's performance in distinguishing between clinically significant and indolent PCAs.

Keywords Chromogranin A · CGA · Prostate cancer · Prognosis · KRYPTOR

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Introduction

Prostate cancer (PCA) with a worldwide annual mortality of over 200.000 is one of the leading cause of cancer-related deaths in men [1]. First symptoms are mostly experienced in progressed stages of PCA when a curative treatment cannot be performed anymore. Therefore, early detection of PCA in asymptomatic men is essential to decrease mortality. PSA-based screening detects a large number of clinically insignificant PCAs. These malignancies, because of their low progression would most probably never cause any symptoms for patients and therefore would remain undetected in patients' life. Many of these asymptomatic patients will be unnecessarily treated and will suffer under the side-effects of the treatment [2]. Therefore, population-based PSA screening for PCA is not recommended by the current guidelines [2]. A possible solution of this problem would be the improvement of prognostication in order to distinguish between indolent and high-risk PCAs. Therefore, preoperatively available biomarkers are urgently needed for the better prognostication of PCAs.

Chromogranin A (CGA) is a glycoprotein commonly expressed by neuroendocrine cells. In addition of neuroendocrine tumors, neuroendocrine activity can also be detected in other tumors, such as breast and prostate cancer [3, 4]. CGA is physiologically released by exocytosis and can be detected in blood [5]. When a tumor develops in a neuroendocrine tissue, it becomes the main source of circulating CGA [6]. Neuroendocrine differentiation in PCA has been received considerable attention in the last few years because of its potential implication as a prognostic factor [7]. It is known, that neuroendocrine differentiated PCA cells do not secrete PSA. As a consequence, this cell population remains undetected by serum PSA analysis. Based on these, it can be hypothesized that the combination of CGA may be complementary to PSA in the prediction of the clinical behaviour of PCA. A further promising prognostic serum marker is matrix metalloproteinase-7 (MMP7), which have been identified by our group as a potential preoperative prognostic factor in non-metastatic PCA [8].

The three available studies on the prognostic relevance of serum CGA levels in clinically localized PCA, revealed controversial results [9–11]. Importantly, these studies applied different study end-points such as postoperative (pathological) tumor stage, Gleason score and time to biochemical (PSA) progress, making a direct comparison difficult. In addition, since none of these end-points can be considered as surrogate for survival, the use of disease-specific survival (DSS) as study end-point is mandatory for the valid evaluation of the prognostic effect of any marker candidates. To date, no study has been published analysing the prognostic value of serum CGA levels in clinically localized PCA with an end-point of DSS.

Therefore, we correlated the blood concentrations of CGA with disease-specific death in two independent PCA cohorts. The prognostic value of CGA alone or in combination with PSA and MMP7 was evaluated also in the subgroup of clinically localized PCA cases.

Methods

Clinical Samples

This study included 237 PCA patients divided in two groups; (1) serum samples of 127 patients obtained between 1990 and 1994 and (2) plasma samples of 110 patients collected between 2003 and 2004. All samples were collected preoperatively from patients who were surgically treated for PCA (radical prostatectomy with curative intent or palliative transurethral resection of the prostate [TURP]) at the Department of Urology at the University of Duisburg-Essen.

Entry criteria were histopathological diagnosis of PCA, surgical treatment (radical prostatectomy or palliative TURP) because of PCA and no history of other malignancies. Patients' characteristics are given in Table 1. The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approved by the ethical board of the hospital. Nuroendocrine histology was not reported in any patients. The primary endpoint of our study was disease-specific death. Cause of death was obtained from death certificates. Patient information relating to age (range: 39–88 years, median: 64 years for serum samples and range: 49–86 years, median: 66 years for plasma samples), clinical and pathological data and preoperative PSA level were noted. TURP specimens were collected from patients with locally progressed or metastatic PCA who underwent androgen deprivation therapy and TURP for local palliation of obstructive lower urinary tract symptoms. All TURP specimens were histologically confirmed to contain PCA.

In 10 cases of serum samples and in 11 cases of plasma samples, preoperative PSA levels were missing. In addition, Gleason score and postoperative PSA follow-up were not routinely available.

CGA and MMP7 Analysis by the KRYPTOR Method

CGA and MMP7 levels were measured on the fully automated B.R.A.H.M.S. KRYPTOR® instrument (Thermo Scientific B.R.A.H.M.S. GmbH, Hennigsdorf/Berlin, Germany) using a homogeneous sandwich fluoroimmuno-assay for both antigens as described previously [12–14].

Statistical Analysis

We used the non-parametric two-sided Wilcoxon rank sum test (Mann-Whitney test) for independent group comparisons. Univariable DSS analyses were done using both Kaplan-Meier curves with log-rank tests and univariable Cox regression analysis. For multivariable analysis, the Cox proportional hazards regression model was used. To determine the optimal cut-off value with the highest sensitivity and specificity for the detection of PCA and metastasis, we used the nonparametric receiver operating characteristics (ROC) curves in which the value for sensitivity is plotted against false-positive rate (1-specificity) were generated. The concentration with the highest specificity and sensitivity value was defined as a cut-off. Finally, we derived concordance indices as generalization of area under the curve (AUC) estimator for more than one predictor variable for the survival data.[15] Statistical testing was performed at the 5% level throughout. *P*-values are to be understood as strictly descriptive. Statistical analyses were performed using the SPSS 21.0 (Chicago, IL) and SAS 9.4 (Cary, NC) softwares.

Table 1 Patients characteristics and CGA levels

		Plasma CGA			Serum CGA		
		n	median (range)	p	n	median (range)	p
Age	≤ 65 yrs.	48	21.8 (7.9–90.6)	**0.043**	72	23.5 (5.2–592.3)	0.564
	> 65 yrs.	62	26.4 (9.8–382.5)		55	24.4 (4.1–269.0)	
Stage	cT1 - pT2	64	21.3 (9.8–171.5)	0.401	58	22.9 (4.1–76.3)	0.603
	pT3- pT4	30	21.8 (7.9–74.9)		42	22.7 (5.3–184.2)	
Grade	G1	7	22.7 (13.7–31.8)		18	24.2 (10.4–76.3)	
	G2	62	22.1 (9.8–171.5)		83	22.9 (4.1–592.3)	
	G3	41	25.2 (7.9–382.5)		25	24.6 (5.3–139.4)	
Low-grade	(G 1–2)	69	22.4 (9.8–171.5)	0.253	101	23.5 (4.1–592.3)	0.782
High-grade	(G 3)	41	25.2 (7.9–382.5)		25	24.6 (5.3–139.4)	
Missing grade		0			1		
PostOP Gleason Sum							
≤ 6		50	22.1 (12.9–85.6)	0.682	26	21.5 (8.1–62.2)	0.090
> 6		44	21.3 (7.9–171.5)		23	20.3 (5.3–184.2)	
Missing Gl. score		16			78		
LN status	N -	87	21.2 (7.9–171.5)	0.205	94	22.4 (4.1–184.2)	0.229
	N +	7	25.3 (15.5–74.9)		9	24.5 (11.4–70.6)	
missing LN status		16			24		
Distant metastasis							
M -		102	22.3 (7.9–171.5)	**0.002**	116	23.0 (4.1–195.6)	**<0.001**
M +		8	86.7 (12.0–382.5)		11	62.8 (22.9–592.3)	
Treatment	RPE	94	21.5 (7,9–171.5)	**<0.001**	103	22.8 (4.1–184.2)	**0.001**
	pTURP	16	40.6 (12.0–382.5)		24	44.5 (9.5–592.3)	
PSA	< 10 ng/ml	63	22.4 (7.9–382.5)	0.606	49	22.8 (5.2–184.2)	0.323
	≥ 10 ng/ml	36	24.7 (12.0–156.3)		68	25.6 (5.3–592.3)	
	unknown PSA	11			10		

Abbreviations: RPE - radical prostatectomy, pTURP - palliative transurethrale resection of the prostate

Results

Follow-Up Characteristics

Out of the serum cohort of 127 PCA patients, collected between 1990 and 1994, 70 patients (55%) died during the follow-up period, 37 (29%) of them PCA-related. The median survival time was 134 months. In 20 patients metastasis was detected at diagnosis (9× lymph node and 11× distant metastasis). In the subgroup of patients (serum cohort) who were treated with radical prostatectomy (n = 103) 49 (47%) died during the follow-up period, 21 (20%) of them PCA-related. The median survival time in this group was 167 months.

For the plasma cohort of 110 PCA patients, collected between 2003 and 2004, 23 (21%) died during the follow-up period, 18 (16%) of them PCA-related. The median survival time was 120 months. In 15 patients metastasis was detected at diagnosis (7× lymph node and 8× distant metastasis). In the subgroup of patients (plasma cohort) who were treated with radical prostatectomy (n = 94) 8 (9%) died during the follow-up period, 5 (5%) of them PCA-related. The median survival time in this group was 121 months.

Association of CGA Serum Levels with Clinicopathological Parameters

The main characteristics of study population and their correlations with CGA serum levels are shown in Table 1. Clinical or pathological tumor stage, pathological grade, Gleason score were not associated with preoperative CGA levels (Fig. 1, Table 1). In contrast, CGA concentrations were 3 to 4 fold elevated in PCA patients with distant metastasis (22.3 ng/ml vs. 86.7 ng/ml, p = 0.002 for plasma and 23.0 ng/ml vs. 62.8 ng/ml, $p < 0.001$ for serum) (Fig. 1a). ROC analysis for the detection of distant metastasis revealed an optimal cut-off value at 40.4 ng/ml CGA plasma concentration providing a sensitivity of 75% and a specificity of 90%. The AUC (area under the curve) value of 0.82 was significantly higher than

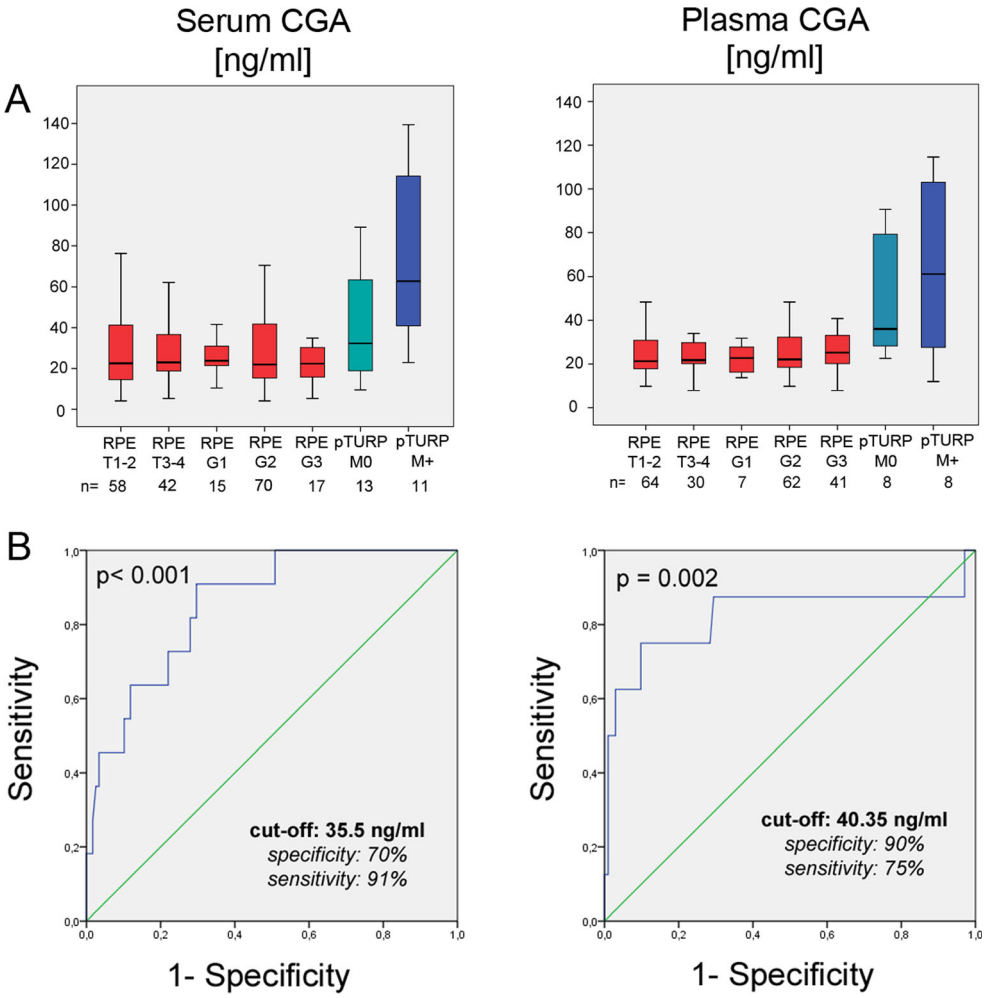

Fig. 1 **a** CGA serum and plasma concentrations in PCA patients and controls. **b** Receiver operating characteristics (ROC) analysis of CGA serum and plasma concentrations for the detection of distant metastasis in PCA

the reference of 0.50 ($p = 0.002$) (Fig. 1b). The same analysis for serum CGA levels identified a cut-off concentration of 35.5 ng/ml with a sensitivity of 91% and a specificity of 70%. The AUC of 0.86 was significantly ($p < 0.001$) above 0.50 (Fig. 1b). Correlation of MMP7 serum levels with clinical and pathological parameters were formerly published [8].

Univariable and Multivariable Survival Analysis

Results of univariable analyses and DSS are listed in Table 2 and Fig. 2. Survival analyses were performed in serum and plasma cohorts with all PCA patients (including those with localized and progressed PCA). In addition, since the prediction of clinically localized PCA is of major clinical importance, we performed a second analysis including only patients who were treated with RPE. As in the RPE subgroup of the plasma cohort only 5 PCA-related deaths occurred, results of the univariable analysis for this subgroup have to be handled with critical caution. Because of the same reason, multivariable analysis for the RPE cohort could only be performed in the serum but not in the plasma cohort.

Pathological tumor stage (>pT2) was associated with shorter DSS, however, this correlation was significant only in the serum cohort ($p = 0.005$) but slightly missed the significance level in the plasma cohort ($p = 0.055$). Postoperative Gleason score was available only in a limited number of patients ($n = 94$ for plasma and 49 for the serum cohort) and showed an insignificant trend to be associated with poor survival in the RPE subgroup of both serum and plasma cohorts (Table 2). When including all patients (both with local and progressed PCA) CGA was associated with poor DSS. The hazard ratios with the lowest p-values for DSS were registered when combining CGA with PSA or with MMP7. In the subgroup of patients treated with RPE, only these combinations but not CGA or PSA alone were associated with DSS (Table 2).

The cut-off values for dichotomization were 10 ng/ml for PSA, 3.4 ng/ml for MMP7 (as formerly published) and 44.4 ng/ml for CGA (highest 20% of values).

As the possible consequence of a preoperative risk assessment may be the avoidance of surgery, our multivariable models included only those factors available preoperatively (clinical tumor stage and serum marker levels). CGA and PSA when used as separate variables in addition to clinical

Table 2 Cox univariable and multivariable analyses

		Univariable analysis					
		Serum			Plasma		
		HR	95% CI	P	HR	95% CI	P
clinically localized PCAs (RPE)			*n* = 103			*n* = 94	
Age	> 65 yrs.	0.900	0.363–2.230	0.819	3596	0.402–32.172	0.252
Stage	pT3 - pT4	4.381	1.574–12.193	**0.005**	8511	0.951–76.162	0.055
Grade	G3	0.550	0.127–2.372	0.422	3945	0.659–23.613	0.133
PostOP Gleason	>6	3472	0.673–17.912	0.137	4533	0.507–40.562	0.176
LN status	N +	1567	0.461–5.321	0.472	21,282	3.540–127.95	**0.001**
PSA	≥ 10 ng/ml	2486	0.932–6.634	0.069	3588	0.599–21.482	0.162
CGA	high	1753	0.640–4.801	0.274	1690	0.189–15.127	0.639
CGA/PSA	at least one high	3.525	1.157–10.737	**0.027**	2182	0.365–13.061	0.393
CGA/MMP7	both high	3.006	1.001–9.030	**0.050**	0.046	0–1,056,243	0.721
all patients (RPE + advanced)			*n* = 127			*n* = 110	
PSA	≥ 10.0 ng/ml	3.893	1.680–9.024	**0.002**	3813	1.430–10.165	**0.007**
CGA	> 44.4 ng/ml	3.664	1.900–7.066	**<0.001**	4229	1.666–10.736	**0.002**
MMP7	3.4 ng/ml	2.986	1.558–5725	**0.001**	3022	1.192–7.662	**0.020**
CGA/PSA	at least one high	6.167	2.156–17.639	**0.001**	4214	1.386–12.807	**0.011**
CGA/MMP7	both high	6.007	3.030–11.910	**<0.001**	5216	1.951–13.944	**0.001**
		Multivariable analysis					
		Serum					
		HR	95% CI	p			
Model 1							
Clinical tumor stage (T2)	2.345		0.510–10.787	0.247			
PSA level (>10 ng/ml)	2.809		0.921–8.567	0.069			
CGA level > 44.4 ng/ml	2.725		0.838–8.867	0.096			
Model 2							
Clinical tumor stage (T2)	2.150		0.474–9.757	0.321			
PSA or CGA or both high	4.884		1.347–17.707	**0.016**			
Model 3							
Clinical tumor stage (T2)	4.214		0.747–23.763	0.103			
PSA level (>10 ng/ml)	3.062		0.996–9.410	0.051			
CGA and MMP7 level high	7.458		1.654–33.625	**0.009**			

tumor stage showed a tendency to be independent predictors for DSS ($p = 0.096$ and $p = 0.069$) (Table 3). Their combination revealed to be a significant prognostic factor for DSS independent of clinical tumor stage ($p = 0.016$). In addition, the most accurate prognostication was reached when combining CGA with MMP7. This combination proved to be independent from both PSA and clinical tumor stage ($p = 0.009$) (Table 3).

Harrell's Concordance Index (c-Index) Analysis

We performed Harrell's c-index analysis to further assess whether CGA in addition to or in combination with PSA provide a better prognostic accuracy for patients with clinically localized PCA. The low Harrell's c-value of clinical tumor stage of 0.514 increased to 0.612 and 0.635 when sequentially adding PSA and CGA. The improvement of the prognostic model was more obvious when adding CGA/PSA as a combined factor. By doing so, the AUC value increased to 0.677, showing the benefit of combining these two markers instead of use them as separate variables (Table 3). The highest prognostic accuracy with the highest AUC value of 0.716 could be reached when including clinical tumor stage, PSA and CGA in combination with MMP7.

Discussion

In the present study, analyzing the prognostic value of CGA alone and in combination with PSA and MMP7 in PCA

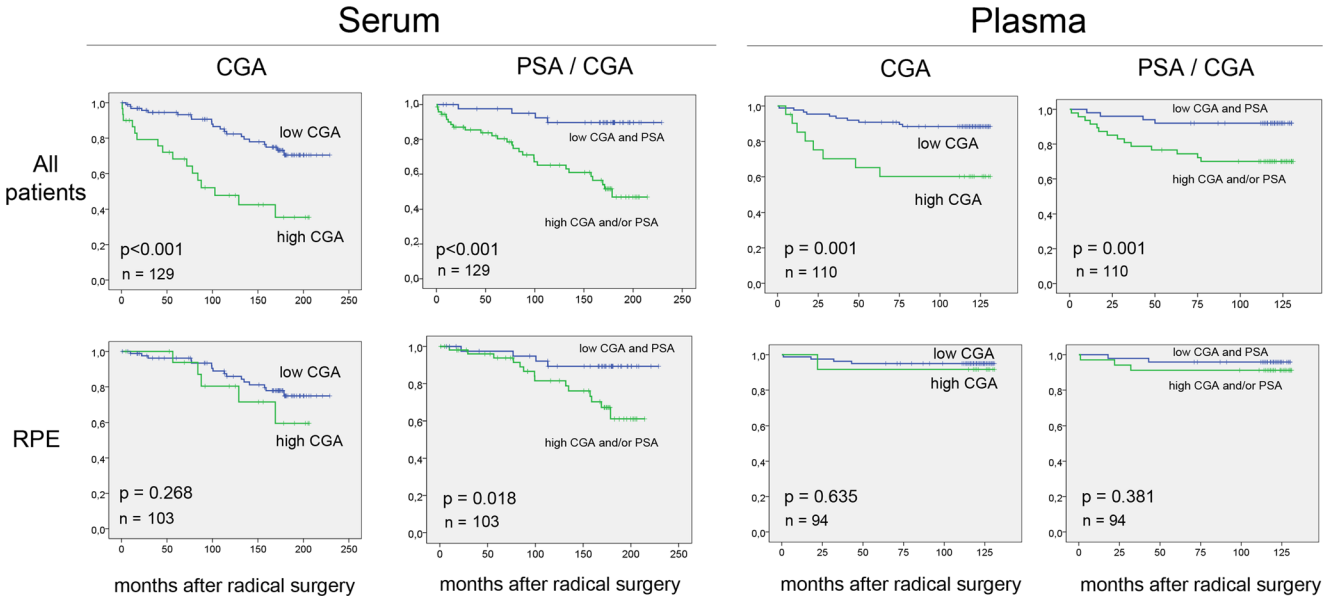

Fig. 2 Kaplan–Meier curves of disease-specific survival stratified by serum / plasma CGA and PSA levels. Kaplan-Meier analysis was performed in all PCA cases (including both clinically localized and progressed cases) and in a subgroup of patients with clinically localized PCA treated with radical prostatectomy (RPE). * patient number within the RPE serum cohort is reduced from 103 to 96, because of 7 cases with missing PSA values

patients, we found a strong prognostic value for all the three serum markers, suggesting their potential in disease monitoring. When restricting the analysis to clinically localized PCA, none of the single serum markers remained significant as a predictor of survival. Most importantly, the combination of CGA with PSA or with MMP7 found to be an independent prognostic factor in clinically localized PCA.

Former studies assessing the prognostic role of serum CGA in clinically localized PCA found contrary results. Sciarra et al. analysed preoperative serum PSA and CGA levels in RPE-treated patients and found both PSA and CGA but not preoperative Gleason score as significant predictors for extracapsular tumor growth (stage pT3) [9]. In a subsequent analysis on 264 RPE-treated patients, the same authors identified high CGA levels (> 60 ng/ml) as independent predictors for PSA-recurrence [10]. In the third study, serum CGA levels at diagnostic biopsy were found to be significantly higher in patients with high-grade compared to low-grade PCAs, however, these correlations proved not to be independent from other variables. Therefore, the authors concluded that serum CGA should not be considered as a predictive marker of poorly differentiated PCA. Importantly, the endpoints used in these studies can only be considered as soft endpoints, since Gleason grade provide only a limited prognostic value and the biochemical (PSA) progress is only loosely associated with DSS. Therefore, these parameters cannot replace the valid endpoint of DSS.

Using DSS as the end-point, our results - in two independent patient cohorts – consequently show a significant prognostic value for CGA when including both clinically localized and progressed stages of PCA. Based on this, CGA may have a potential in disease monitoring. Considering the often slow progression of PCA, periodical measurements rather than one single analysis at diagnosis seems to be feasible for the early detection of PCA progression. In the subgroup of clinically localized PCA, neither PSA nor CGA alone were able to predict disease-specific death. As PSA and CGA are released from different cell clones of PCA, it can be assumed that they may reinforce each other in the prediction of PCA. Our present data confirm this assumption by revealing a significant and independent prognostic effect for the combination of CGA with PSA both in the preoperative and postoperative multivariable model. In accordance, the low prognostic accuracy of clinical tumor stage as shown by the c-index of 0.514 increased

Table 3 Harrel's concordace index (c-index)

Variables	C-index	95% CI
Clinical tumor stage	0.514	0.400–0.635
Clinical tumor stage + PSA	0.612	0.433–0.816
Clinical tumor stage + CGA	0.553	0.473–0.682
Clinical tumor stage + PSA/CGA	0.677	0.553–0.825
Clinical tumor stage + PSA + CGA/MMP-7	0.716	0.606–0.848

to 0.612 and 0.635 when adding PSA and CGA sequentially, but increased to 0.677 when adding PSA and CGA in one step as a combined variable suggesting that combining CGA with PSA may provide an additional prognostic effect over the separate inclusion of both markers in a prognostic model. Furthermore, the best performing prognostic model with the highest hazard ratio of 7.458 in the multivariable analysis and also the highest c-index value (0.716) was achieved when combining CGA with MMP7. The possible biological background, for this effect however, is not known and should be the subject of further research.

As the therapeutic decision based on the preoperative prognostication might be active surveillance instead of surgery, our results may have direct clinical relevance when confirmed in larger studies in independent patient cohorts. Furthermore, our results suggest that those patients who are eligible for active surveillance may also benefit from the combined analysis of PSA, CGA and MMP7 as it may improve the accuracy of disease monitoring.

We aimed to confirm the prognostic effect of the combination of CGA and PSA in plasma samples of an independent cohort of PCA patients. The prognostic value of CGA alone or in combination with PSA could be confirmed when including both clinically localized and progressed PCA cases. However, subgroup analysis including only clinically localized PCAs could not be performed in the plasma cohort because of the low number (n = 5) of disease-specific deaths, which is probably related to the sample collection period. Our serum PCA cohort from the early 1990s originates from the pre PSA-screening era while the plasma cohort was collected between 2003 and 2004 in the PSA era. This fact consequently leads to the lower presentation of high-risk cases in the later collected (plasma) cohort.

There are some potential limitations for this study. First, the preoperative model does not include the preoperative Gleason score as this information was not available from the archive files. To overcome this limitation, we retrieved available paraffinized tumor samples and reclassified these cases according to the current WHO criteria [15]. When including this postoperative (or pathological) Gleason score - which is more accurate as that of preoperative Gleason score - in the preoperative multivariable model, the prognostic value of combined CGA/PSA and CGA/MMP7 remained significant (data not shown). A further limitation is inherent from the low number of PCA-related deaths in the plasma cohort which prohibited the performance of any multivariable analysis. Therefore, further prospective analysis with a large number of PCA patients is necessary to confirm our results.

A further potential use of serum CGA analysis may be the prediction of novel hormonal therapies (abiraterone-acetate and enzalutamide). Recent studies showed that serum CGA levels may be able to predict the effectivity of these drugs [16–19].

In conclusion, serum CGA in addition to PSA and/or combined with MMP7 is a promising marker in monitoring PCA progression. In addition CGA in combination with PSA or MMP7 may be able to improve the identification of PCA patients with clinically localized PCA at high risk of disease progression and PCA-related death. Further studies are needed to validate these promising results.

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