Chromogranin A and neurone-specific enolase serum levels as predictors of treatment outcome in patients with metastatic castration-resistant prostate cancer undergoing abiraterone therapy

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Objective

To determine the impact of elevated neuroendocrine serum markers on treatment outcome in patients with metastatic castration-resistant prostate cancer (mCRPC) undergoing treatment with abiraterone in a post-chemotherapy setting.

Patients and Method

Chromogranin A (CGa) and neurone-specific enolase (NSE) were determined in serum drawn before treatment with abiraterone from 45 patients with mCRPC. Outcome measures were overall survival (OS), prostate-specific antigen (PSA) response defined by a PSA level decline of ≥50%, PSA progression-free survival (PSA-PFS), and clinical or radiographic PFS.

Results

The CGa and NSE serum levels did not correlate ($P = 0.6$). Patients were stratified in to low- (nine patients), intermediate- (18) or high-risk (18) groups according to elevation of none, one, or both neuroendocrine markers, respectively. The risk groups correlated with decreasing median OS (median OS not reached vs 15.3 vs 6.6 months; $P < 0.001$), decreasing median clinical or radiographic PFS (8.3 vs 4.4 vs 2.7 months; $P = 0.001$) and decreasing median PSA-PFS (12.0 vs 3.2 vs 2.7 months; $P = 0.012$). In multivariate Cox regression analysis the combination of CGa and NSE (≥1 marker positive vs both markers negative) remained significant predictors of OS, clinical or radiographic PFS, and PSA-PFS. We did not observe a correlation with PSA response (63% vs 35% vs 31%; $P = 0.2$).

Conclusion

Chromogranin A and NSE did not predict PSA response in patients with mCRPC treated with abiraterone. However, we observed a correlation with shorter PSA-PFS, clinical or radiographic PFS, and OS. This might be due to an elevated risk of developing resistance under abiraterone treatment related to neuroendocrine differentiation.

Keywords

castration-resistant prostate cancer, abiraterone, chromogranin A, neuron-specific enolase, neuroendocrine prostate cancer, #ProstateCancer, #PCSM

Introduction

Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease with enormous variability of prognosis and treatment response between patients. In the background of evolving therapeutic options, both prediction of prognosis and treatment response pose a major challenge.

In mCRPC the androgen receptor (AR) has been shown to be still active and novel agents have been tailored to inhibit AR signalling [1]. One of these agents is abiraterone, a CYP17-inhibitor that suppresses intra- and extragonadal synthesis of androgens. Abiraterone was approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) following randomised phase III trials in the pre- and post-chemotherapy setting for mCRPC (COU AA 301/302) [2,3]. Most patients initially show a tumour response to abiraterone. However, about one-third of patients exhibit primary resistance without showing any decline in PSA

levels [4]. Furthermore, all patients who initially respond to abiraterone will eventually develop secondary resistance [2–5]. One explanation for treatment resistance in AR-targeted therapy with androgen-insensitive tumour progression may involve the presence of neuroendocrine prostate cancer (NEPC).

In vitro, prostatic adenocarcinoma cell lines treated with androgen-deprivation therapy (ADT) and novel AR-targeted therapies have been shown to undergo transdifferentiation to NEPC cells [6–8]. It has been hypothesised that neuroendocrine transdifferentiation is caused by selective pressure under AR-targeted therapy and displays a mechanism of adaptive treatment resistance [6]. This hypothesis is supported by alterations found in NEPC cells including loss of AR and androgen-regulated protein expression, induction of neuroendocrine and neural programmes, loss of tumour suppressors (tumour protein p53 [TP53], retinoblastoma 1 [RB_1], phosphatase and tensin homolog [PTEN]), activation of mitotic programmes, as well as genomic instability [6].

The detection of NEPC in tumour biopsies of patients who have a prior history of prostatic adenocarcinoma and are undergoing AR-targeted therapy is also referred to as treatment-related NEPC (trNEPC) [9]. Clinical studies show an increasing number of trNEPCs in correlation with the length of ADT in patients with mCRPC [10–12]. The diagnosis trNEPC correlates with poor prognosis and the median time of survival is 7 months [9].

Neuroendocrine prostate cancer cells express neuroendocrine markers such as chromogranin A (CGa) and neurone-specific enolase (NSE), which can be measured in serum. Elevation of neuroendocrine markers in serum has shown concordant results with the detection of NEPC in tumour tissue [13]. A correlation between the CGa level and the length of ADT has been reported [14]. Moreover, both elevated CGa and NSE levels in serum were shown to be independent predictors of poor survival in patients with mCRPC in the pre-abiraterone era [15–17].

In the present study, we retrospectively analysed CGa and NSE serum levels in patients with mCRPC before undergoing treatment with abiraterone in a post-chemotherapy setting. We hypothesised that elevation of CGa and NSE might predict treatment outcome. We determined their impact on overall survival (OS), clinical or radiographic progression-free survival (clinical or radiographic PFS), PSA level decline of ≥50%, and PSA PFS (PSA-PFS).

Patients and Methods

All patients with mCRPC initially were diagnosed with adenocarcinoma of the prostate and had progressive disease as defined by Prostate Cancer Working Group 2 (PCWG2) criteria at inclusion [18]. They were scheduled for systemic treatment with abiraterone and had previously been treated with chemotherapy (45 patients). Patients were treated at the Department of Urology, Klinikum rechts der Isar, Technical University of Munich in Germany between 2011 and 2012. All patients signed Institutional Review Board (IRB)-approved consent before participation. Their serum samples were prospectively collected according to an IRB-approved biorepository protocol.

Treatment response under abiraterone was determined according to the institutional standard procedure including PSA levels at ≤1 week before and every 4 weeks after treatment initiation, as well as imaging procedures (CT and bone scan) at ≤4 weeks before and every 3 months after treatment initiation.

The study endpoints included: OS, PSA response defined by a PSA level decline of ≥50%, PSA-PFS according to PCWG2 criteria [18], and clinical or radiographic PFS. Clinical or radiographic PFS was defined by worsening disease-related symptoms or new cancer-related complications, radiographic progression based on Response Evaluation Criteria in Solid Tumors (RECIST) [18] or two or more new bone lesions on bone scan or death, whichever occurred first.

Serum samples were collected in 7.5-mL tubes at ≤1 week before treatment initiation and were centrifuged and stored at −80 °C within 4 h. The CGa and NSE concentrations were determined retrospectively from the collected sera. The CGa was measured on a BRAHMS Kryptor compact plus device (BRAHMS GmbH, Hennigsdorf, Germany) and NSE and PSA on a Cobas e602 module (Roche, Mannheim, Germany).

Data were analysed using IBM SPSS Statistics version 22.0. Association analyses between CGa, NSE and PSA were performed with the use of Pearson correlation. A receiver operator characteristics analysis (ROC) for prediction of death at ≤12 months was performed and the areas under the curve (AUC) were calculated. Optimal prognostic threshold levels for CGa and NSE were set to yield a maximum Youden index (= sensitivity + specificity − 1). Association analysis with a PSA level decline of ≥50% was performed with the use of Spearman's rank correlation. Correlations with PSA-PFS, clinical or radiographic PFS, and OS were assessed using Kaplan–Meier curves with log-rank statistics. Furthermore, uni- and multivariate Cox regression analyses were used to calculate their respective hazard ratios (HRs) and 95% CIs. We included neuroendocrine marker status, lactate dehydrogenase (LDH) level, alkaline phosphatase (AP) level, Eastern Cooperative Oncology Group (ECOG) performance status and presence of visceral metastasis in each univariate analysis. Only factors significant in univariate analyses were included in the subsequent multivariate analyses.

Results

The characteristics of the patients with mCRPC are summarised in Table 1. Of the 45 patients, 13 (29%) patients had received two or more lines of systemic treatment before undergoing abiraterone therapy. At enrolment 44 (98%) patients had bone metastases and 14 (32%) patients had visceral metastases. At a median (range) follow-up of 12.1 (0.4–33.4) months, 36 (80%) patients had died. The median OS was 12.7 months (95% CI 7.2–18.2) and median clinical or radiographic PFS was 3.7 months (95% CI 2.5–5.0). PSA follow-up was available in 38 (84%) patients. PSA response with a decline of ≥50% was achieved in 15 (39%) patients and the median PSA-PFS was 3.2 months (95% CI 2.4–4.0).

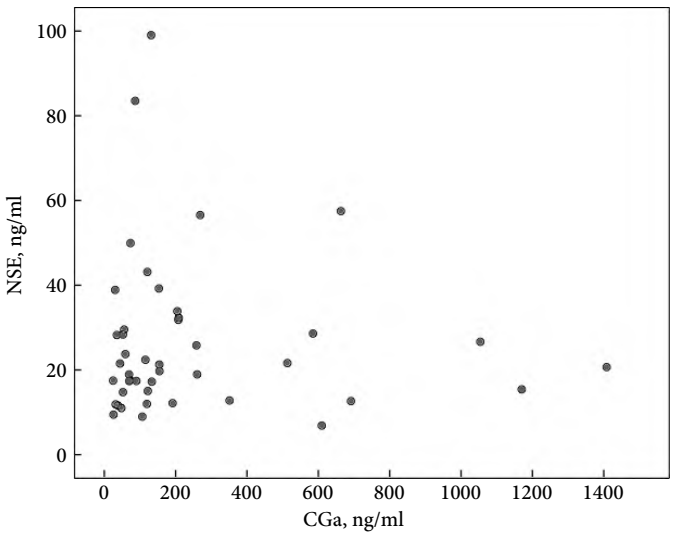
Correlation of CGa, NSE and PSA Levels

The median (range) serum level of PSA was 328 (0–4 077) ng/mL, of CGa was 122 (25–1 408) ng/mL and of NSE was 21 (7–2 905) ng/mL before treatment initiation with abiraterone. Neither, CGa and NSE levels showed a correlation (Pearson $r = 0.07$, $P = 0.6$; Fig. 1), nor were PSA levels correlated with any of the neuroendocrine serum markers (PSA and CGa Pearson $r = -0.06$, $P = 0.7$; PSA and NSE Pearson $r = -0.12$, $P = 0.4$).

Table 1 Characteristics of the patients with mCRPC.

Characteristic	Value
Number of patients	45
Median (range)	
Age, years	67 (50–87)
Serum chemistry	
PSA, ng/mL	328 (0–4 077)
AP, U/L	147 (50–1 899)
LDH, U/L	284 (136–1 749)
CGa, ng/mL	122 (25–1 408)
NSE, ng/mL	21 (7–2 905)
ECOG performance status	0 (0–2)
N (%)	
Prior systemic treatments for mCRPC	
Docetaxel	44 (98)
Abiraterone	0 (0)
Cabazitaxel	8 (18)
Other	8 (18)
Prior lines of systemic treatment for mCRPC	1 (1–3)
1	32 (71)
2	11 (24)
3	2 (4)
Site of metastasis	
Bone	42 (98)
Visceral	14 (32)
Deceased, *n* (%)	36 (80)
Median (range) follow-up, months	12.1 (0.4–33.4)
Median (95% CI) OS, months	12.7 (7.2–18.2)
Median (95% CI) PSA-PFS, months	3.2 (2.4–4.0)
Median (95% CI) clinical or radiographic PFS, months	3.7 (2.5–5.0)

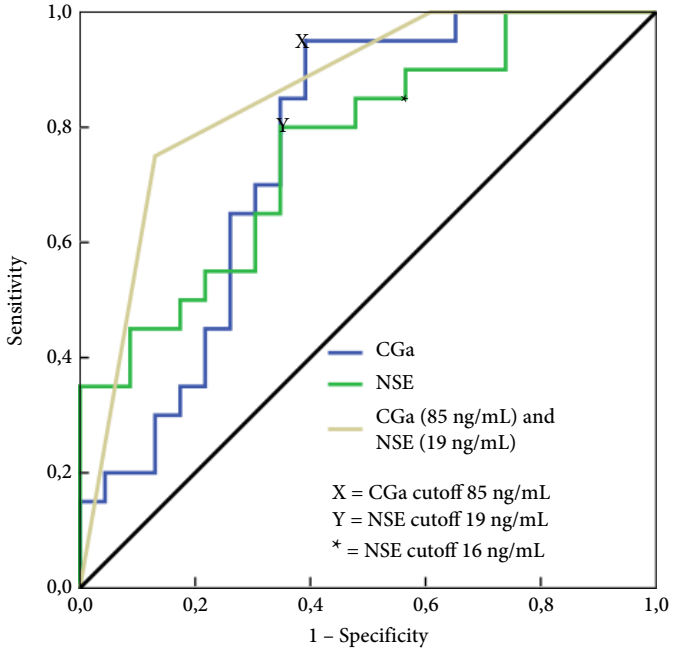
Fig. 1 Scatter plot of serum concentrations of CGa and NSE. One patient with a CGa concentration of 407 ng/mL and a NSE concentration of 2 905 ng/mL are not shown.



Prognostic Threshold Levels for CGa and NSE

According to the thresholds supplied by the manufacturers of the respective assays (CGa ≤85 ng/mL, NSE ≤16 ng/mL) CGa was elevated in 29 (64%) and NSE in 32 (71%) patients. Prognostic thresholds were optimised in a ROC analysis for prediction of death at ≤12 months (Fig. 2). We calculated an AUC of 0.76 (95% CI 0.62–0.91) both for CGa and for NSE

Fig. 2 ROC analysis for prediction of death at ≤12 months.



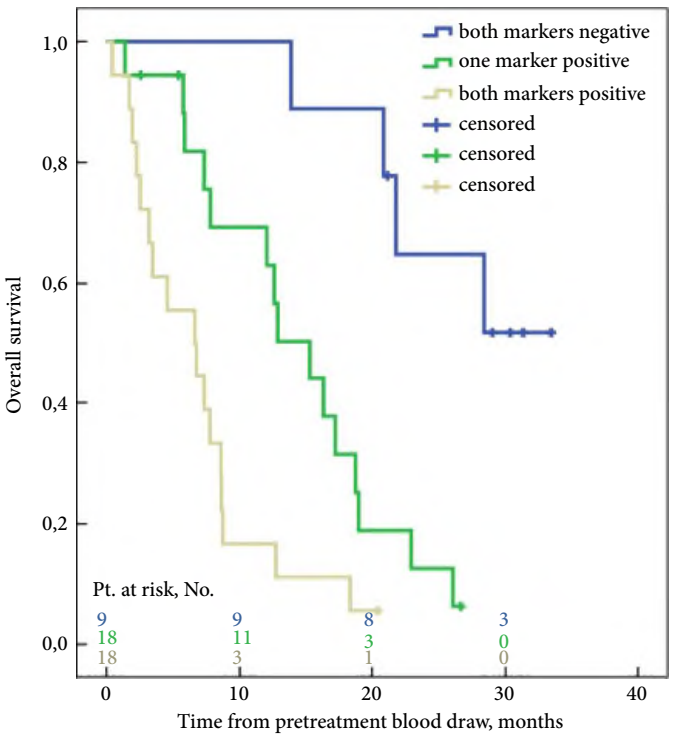
($P = 0.003$, respectively). The threshold of 85 ng/mL used for CGa performed well and was consistent with a point on the ROC curve that reached the maximum Youden index of 0.559. The threshold of 16 ng/mL used for NSE performed poorly and reached a Youden index of only 0.285. A threshold of 19 ng/mL enhanced the prognostic ability for NSE reaching a Youden index of 0.452. The thresholds 85 ng/mL for CGa and 19 ng/mL for NSE were therefore used for further analyses.

Both markers were negative in nine (20%), CGa alone was positive in 11 (24%), NSE alone was positive in seven (16%), and both markers were positive in 18 (40%) patients. We used a combination of CGa and NSE markers to stratify patients in to low- (both markers negative), an intermediate- (one marker positive) or a high-risk (both markers positive) groups. This risk group stratification resulted in an improved AUC of 0.86 (95% CI 0.75–0.97) for prediction of death at ≤12 months ($P = 0.001$; Fig. 2) and was therefore used for further correlations with treatment outcome.

Association of Neuroendocrine Markers with OS

In the Kaplan–Meier analysis, the median OS was not yet reached in the low-risk group, whereas the intermediate- and high-risk groups decreased to 15.3 months (95% CI 10.2–20.5) and 6.6 months (95% CI 2.1–11.1 months), respectively ($P < 0.001$; Fig. 3). In a multivariate Cox regression analysis together with clinical variables (LDH, AP and ECOG performance status) elevation of at least one of the neuroendocrine markers (≥1 marker positive vs both markers negative) independently predicted survival (HR 7.2. 95% CI 1.6–31.7; $P = 0.009$) (Table 2).

Fig. 3 Kaplan–Meier curves for the association of CGa and NSE with OS.



Association of Neuroendocrine Markers with Clinical or Radiographic PFS

The median clinical or radiographic PFS was 8.3 months (95% CI 5.6–10.9) in the low-risk group and in the intermediate- and high-risk groups was 4.4 months (95% CI 2.5–6.4) and 2.7 months (95% CI 2.2–3.2), respectively ($P = 0.001$; Fig. 4). In multivariate Cox regression analysis together with clinical variables (LDH and ECOG performance status) the combination of CGa and NSE (≥1 marker positive vs both markers negative) remained significant (HR 2.9, 95% CI 1.2–7.3; $P = 0.022$) (Table 3).

Association of Neuroendocrine Markers with PSA Response and PSA-PFS

A waterfall plot for the best relative PSA response showed a rather random distribution of the different risk groups (Fig. 5). Any decline in PSA level was seen in six of eight of patients in the low-risk group, in 10 of 17 (59%) patients in the intermediate-risk group, and in 10 of 13 patients in the high-risk group. A PSA response with a decline of ≥50% was achieved in five of eight patients in the low-risk group, in six of 17 (35%) patients in the intermediate-risk group, and in four of 13 patients in the high-risk group. On statistical evaluation the observed difference was not significant (Spearman's $r = -0.2$; $P = 0.202$).

Although elevation of neuroendocrine markers did not predict PSA response, there was an association with PSA-PFS. The median PSA-PFS was 12.0 months (95% CI 0.0–28.4) in the low-risk group, 3.2 months (95% CI 2.7–3.6) in the intermediate-risk group, and 2.7 months (95% CI 2.2–3.3) in the high-risk group ($P = 0.012$; Fig. 6). In multivariate Cox regression analysis together with ECOG performance status the combination of CGa and NSE (≥1 marker positive vs both markers negative) remained significant (HR 2.8, 95% CI 1.1–7.1; $P = 0.028$) (Table 4).

Discussion

In this retrospective study we determined serum levels of CGa and NSE in 45 patients with mCRPC before treatment with abiraterone in a post-chemotherapy setting. In immunohistochemistry, the neuroendocrine markers CGa and NSE are often combined for detection of NEPC in prostate cancer tissue to account for a heterogeneous marker expression [12]. In our present study, CGa and NSE levels in

Table 2 Cox regression analysis for associations of elevated CGa, elevated NSE, and further clinical and laboratory variables with OS.

Variable	Categories	Univariate analysis			Multivariate analysis		
		N	HR (95% CI)	*P*	*N*	HR (95% CI	*P*
Combination of CGa and NSE	≥1 positive vs both negative	36 vs 9	7.82 (2.33–26.23)	**0.001**	35 vs 7	7.17 (1.62–31.70)	**0.009**
LDH, U/L	Continuous	45	1.00 (1.00–1.00)	**<0.001**	42	1.00 (1.00–1.00)	**0.049**
AP, U/L	Continuous	42	1.00 (1.00–1.00)	**0.019**	42	1.00 (1.00–1.00)	0.672
ECOG PS	2 vs 0–1	5 vs 40	9.37 (3.20–27.48)	**<0.001**	5 vs 37	9.83 (2.83–34.13)	**<0.001**
Visceral metastasis	Yes vs no	14 vs 29	1.13 (0.55–2.32)	0.745			

ECOG PS, ECOG performance status. Bold indicates significant P values.

Fig. 4 Kaplan–Meier curves for the association of CGa and NSE with clinical or radiographic PFS.

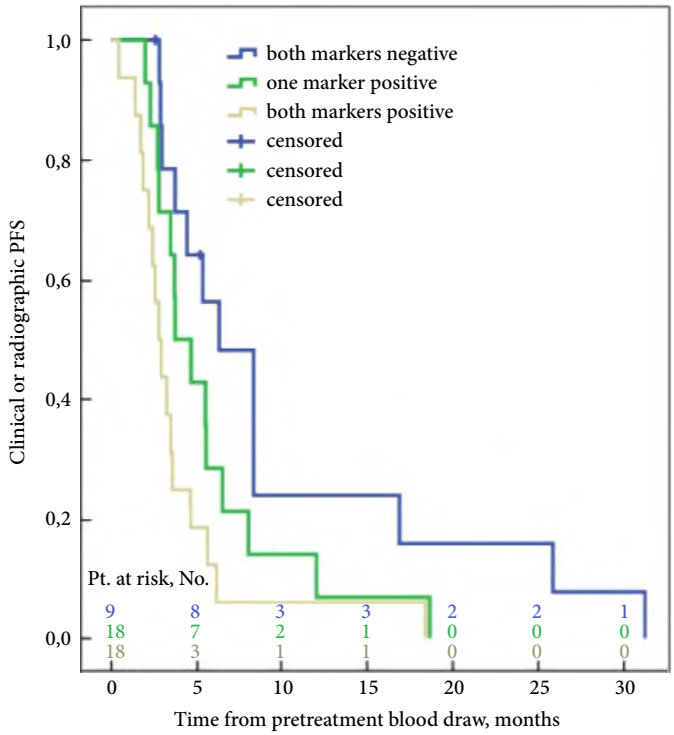
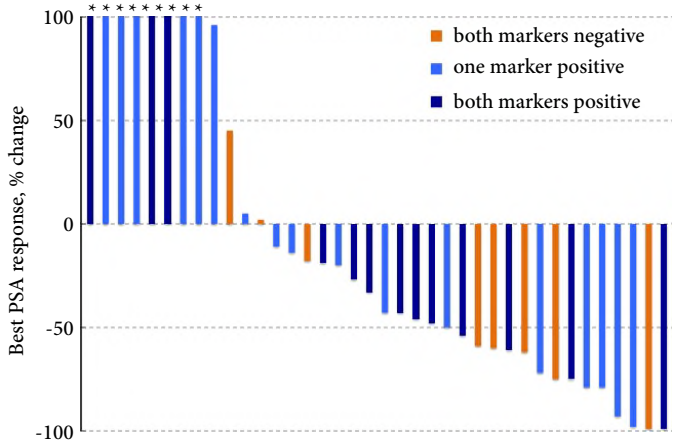


Fig. 5 Waterfall plot for best relative PSA response according to the combination of CGa and NSE in serum. Asterisks indicate an increase of >100% in best PSA response.



serum also displayed a heterogeneous distribution. We did not detect a correlation in their serum concentrations, which is consistent with previous studies [16,19]. Therefore, we combined CGa and NSE levels to account for heterogeneous marker secretion in serum and stratified patients in to low-, intermediate- and high-risk groups according to the elevation of none, one, or both neuroendocrine markers, respectively.

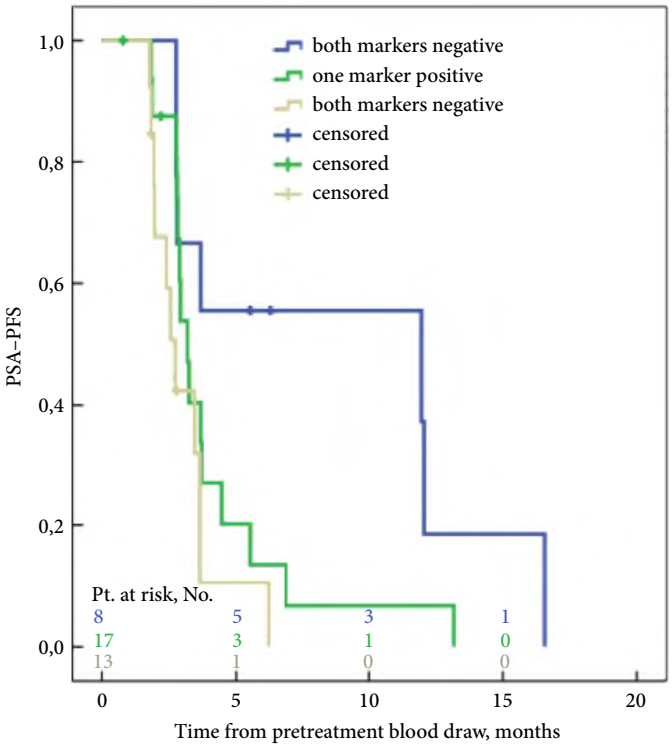
We hypothesised that elevation of neuroendocrine markers, as a surrogate of neuroendocrine differentiation, might correlate with worse treatment outcome under abiraterone. In our present study, patients in the low-risk group had the longest PSA-PFS, clinical or radiographic PFS, and OS, which decreased in patients in the intermediate-risk group and were

Table 3 Cox regression analysis for associations of elevated CGa, elevated NSE, and further clinical and laboratory variables with clinical or radiographic PFS.

Variable	Categories	Univariate analysis			Multivariate analysis		
		N	HR (95% CI)	*P*	*N*	HR (95% CI)	*P*
Combination of CGa and NSE	≥1 positive vs both negative	36 vs 9	3.65 (1.51–8.82)	**0.004**	35 vs 7	2.92 (1.17–7.28)	**0.022**
LDH, U/L	Continuous	45	1.00 (1.00–1.00)	**0.006**	42	1.00 (1.00–1.00)	0.053
AP, U/L	Continuous	42	1.00 (1.00–1.00)	0.216			
ECOG PS	2 vs 0–1	5 vs 40	10.88 (3.51–33.72)	**<0.001**	5 vs 37	9.74 (3.05–31.12)	**<0.001**
Visceral metastasis	Yes vs no	14 vs 29	1.87 (0.94–3.74)	0.075			

ECOG PS, ECOG performance status. Bold indicates significant P values.

Fig. 6 Kaplan–Meier curves for the association of CGa and NSE with PSA-PFS.



the shortest in the high-risk group. In multivariate Cox regression analyses the combination of both markers (≥1 marker positive) independently predicted PSA-PFS, clinical or radiographic PFS, and OS. Notably, we did not find an association of high neuroendocrine marker levels with PSA response, suggesting that their elevation does not indicate primary resistance to abiraterone. Nonetheless, our present results indicate that in patients with mCRPC with elevated neuroendocrine markers treatment resistance develops faster. This observation might support the role of neuroendocrine differentiation in androgen-insensitive tumour progression.

Our present results are supported by previous reports with novel AR-targeted therapies. Burgio et al. [20] retrospectively measured CGa levels in serum samples of 48 patients with mCRPC before treatment initiation with abiraterone in a post-chemotherapy setting. Elevation of CGa in serum correlated significantly with shorter clinical PFS and showed a trend to shorter OS in a multivariate Cox regression analysis. A correlation with PSA response was also not observed. From the same group, Conteduca et al. [21] determined the impact of elevated CGa on treatment outcome in 35 patients with mCRPC who were treated with the new AR-antagonist, enzalutamide, in a post-chemotherapy setting. While abiraterone inhibits AR signalling indirectly via depletion of androgens, enzalutamide acts as a competitive antagonist of the AR, prevents its translocation into the nucleus, and impairs transcriptional activation of androgen-responsive target genes [4,22,23]. Conteduca et al. [21] reported in their study a correlation of elevated CGa with shorter OS and a strong trend towards shorter clinical PFS in a multivariate analysis. A correlation with PSA response was also not observed. The study group hypothesised that neuroendocrine differentiation is only present in a subclone of prostate cancer cells. Thus, PSA decline could be achieved by activity of abiraterone or enzaludamide in the non-neuroendocrine carcinoma component but the progressing neuroendocrine component may have impaired overall clinical outcome.

In contrast to our present study, the investigators Burgio et al. [20] and Conteduca et al. [21] only assessed one marker (CGa in serum), used a different kit for CGa measurement with an upper limit of normal values of 120 ng/mL instead of 85 ng/mL, and they determined prognostic thresholds arbitrarily (<120 vs 120–360 vs >360 ng/mL). In addition to their studies, we assessed optimal prognostic thresholds in a ROC analysis for prediction of death at ≤12 months and observed improved patient stratification by a combination of CGa and NSE. Moreover, we determined PSA-PFS and observed a clinically relevant and significantly longer PSA-PFS in patients without elevation of one of the neuroendocrine markers.

Determination of CGa and NSE in serum is a cost-effective and widely accessible test, which could easily be used for counselling patients with mCRPC who are eligible for treatment with novel AR-targeted therapies. However, based on our present study results, a clinical application of CGa and NSE as a treatment-guided strategy cannot be

Table 4 Cox regression analysis for associations of elevated CGa, elevated NSE, and further clinical and laboratory variables with PSA-PFS.

Variable	Categories	Univariate analysis			Multivariate analysis		
		N	HR (95% CI)	*P*	*N*	HR (95% CI)	*P*
Combination of CGa and NSE	≥1 positive vs both negative	30 vs 9	2.92 (1.17–7.30)	**0.022**	30 vs 9	2.80 (1.12–7.05)	**0.028**
LDH, U/L	Continuous	39	1.00 (1.00–1.00)	0.163			
AP, U/L	Continuous	37	1.00 (1.00–1.00)	0.162			
ECOG PS	2 vs 0–1	2 vs 37	11.71 (2.11–65.00)	**0.005**	2 vs 37	9.70 (1.74–53.96)	**0.009**
Visceral metastasis	Yes vs no	12 vs 26	1.52 (0.69–3.33)	0.302			

ECOG PS, ECOG performance status. Bold indicates significant P values.

recommended, yet. All reported studies including our present data are hampered by retrospective study design, small sample size, and lack of external validation of the applied neuroendocrine serum marker kits and thresholds. The growing evidence in this field therefore merits prospective validation in a larger sample size.

Recently, AR splice variant 7 (AR-V7) mRNA in circulating tumour cells was shown to be predictive for primary resistance to treatment with abiraterone and enzalutamide [4]. AR-V7 lacks the ligand-binding domain of the AR but remains constitutively active as a transcription factor. Although most patients with AR-V7-negative status yielded tumour response to treatment with abiraterone or enzalutamide, all of them eventually develop secondary resistance. Combined detection of AR-V7 status and neuroendocrine markers might therefore enhance patient stratification in future studies.

The patients in our present cohort had shorter OS and PSA-PFS when compared with data from the COU-AA-301 study that led to approval of abiraterone in the post-chemotherapy setting [2]. This might be due to more advanced disease in our present cohort, which is reflected by higher PSA levels (median PSA 328 vs 129 ng/mL) and a higher rate of visceral metastases (32% vs 11%).

In conclusion, elevated CGa and NSE levels in serum did not predict PSA response in patients with mCRPC treated with abiraterone. However, in our present cohort increased neuroendocrine marker levels were associated with shorter PSA-PFS, clinical or radiographic PFS, and OS. This might be due to an elevated risk of rapidly developing resistance under abiraterone related to neuroendocrine differentiation. These findings merit further prospective validation in a larger cohort.

Acknowledgements

None.

Conflicts of Interest

None.

References

1 **Longo DL**. New therapies for castration-resistant prostate cancer. *N Engl J Med* 2010; 363: 479–81

2 **de Bono JS, Logothetis CJ, Molina A et al**. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011; 364: 1995–2005

3 **Ryan CJ, Smith MR, de Bono JS et al**. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med* 2013; 368: 138–48

4 **Antonarakis ES, Lu C, Wang H et al**. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014; 371: 1028–38

5 **Heck MM, Höppner M, Horn T, Thalgott M, Gschwend JE, Retz M**. [Compassionate use of abiraterone and cabazitaxel: first experiences in docetaxel-pretreated castration-resistant prostate cancer patients.]. *Urologe A* 2012; 51: 390–7

6 **Beltran H, Tomlins S, Aparicio A et al**. Aggressive variants of castration-resistant prostate cancer. *Clin Cancer Res* 2014; 20: 2846–50

7 **Dang Q, Li L, Xie H et al**. Anti-androgen enzalutamide enhances prostate cancer neuroendocrine (NE) differentiation via altering the infiltrated mast cells → androgen receptor (AR) → miRNA32 signals. *Mol Oncol* 2015; 9: 1241–51

8 **Lin D, Wyatt AW, Xue H et al**. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. *Cancer Res* 2014; 74: 1272–83

9 **Wang HT, Yao YH, Li BG, Tang Y, Chang JW, Zhang J**. Neuroendocrine Prostate Cancer (NEPC) progressing from conventional prostatic adenocarcinoma: factors associated with time to development of NEPC and survival from NEPC diagnosis-a systematic review and pooled analysis. *J Clin Oncol* 2014; 32: 3383–90

10 **Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N**. Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* 2004; 45: 586–92

11 **Ito T, Yamamoto S, Ohno Y et al**. Up-regulation of neuroendocrine differentiation in prostate cancer after androgen deprivation therapy, degree and androgen independence. *Oncol Rep* 2001; 8: 1221–24

12 **Komiya A, Yasuda K, Watanabe A, Fujiuch Y, Tsuzuki T, Fuse H**. The prognostic significance of loss of the androgen receptor and neuroendocrine differentiation in prostate biopsy specimens among castration-resistant prostate cancer patients. *Mol Clin Oncol* 2013; 1: 257–62

13 **Berruti A, Mosca A, Porpiglia F et al**. Chromogranin A expression in patients with hormone naïve prostate cancer predicts the development of hormone refractory disease. *J Urol* 2007; 178: 838–43

14 **Sasaki T, Komiya A, Suzuki H et al**. Changes in chromogranin a serum levels during endocrine therapy in metastatic prostate cancer patients. *Eur Urol* 2005; 48: 224–30

15 **Berruti A, Mosca A, Tucci M et al**. Independent prognostic role of circulating chromogranin A in prostate cancer patients with hormone-refractory disease. *Endocr Relat Cancer* 2005; 12: 109–17

16 **Hvamstad T, Jordal A, Hekmat N, Paus E, Fosså SD**. Neuroendocrine serum tumour markers in hormone-resistant prostate cancer. *Eur Urol* 2003;44:215–21

17 **Taplin ME, George DJ, Halabi S et al**. Prognostic significance of plasma chromogranin a levels in patients with hormone-refractory prostate cancer treated in Cancer and Leukemia Group B 9480 study. *Urology* 2005; 66: 386–91

18 **Scher HI, Halabi S, Tannock I et al**. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol* 2008; 26: 1148–59

19 **Kamiya N, Akakura K, Suzuki H et al**. Pretreatment serum level of neuron specific enolase (NSE) as a prognostic factor in metastatic prostate cancer patients treated with endocrine therapy. *Eur Urol* 2003; 44: 309–14

20 **Burgio SL, Conteduca V, Menna C et al**. Chromogranin A predicts outcome in prostate cancer patients treated with abiraterone. *Endocr Relat Cancer* 2014; 21: 487–93

21 **Conteduca V, Burgio SL, Menna C et al**. Chromogranin A is a potential prognostic marker in prostate cancer patients treated with enzalutamide. *Prostate* 2014; 74: 1691–6

22 **Beer TM, Armstrong AJ, Rathkopf DE et al**. Enzalutamide in metastatic prostate cancer before chemotherapy. *N Engl J Med* 2014; 371: 424–33

23 **Scher HI, Fizazi K, Saad F et al**. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med* 2012; 367: 1187–97

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Abbreviations: ADT, androgen-deprivation therapy; AP, alkaline phosphatase; AR, androgen receptor; AR-V7, AR splice variant 7; AUC, area under the curve; CGa, Chromogranin A; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; IRB, Institutional Review Board; LDH, lactate dehydrogenase; mCRPC, metastatic castration-resistant prostate cancer; (tr)NEPC, (treatment-related) neuroendocrine prostate cancer; NSE, neurone-specific enolase; OS, overall survival; PCWG2, Prostate Cancer Working Group 2; PFS, progression-free survival; ROC, receiver operating characteristic.