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Matrix metalloproteinase 7, soluble Fas and Fas ligand serum levels for predicting docetaxel resistance and survival in castration-resistant prostate cancer

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Objective

To assess the predictive value of pre-chemotherapy matrix metalloproteinase 7 (MMP-7), soluble Fas (sFas) and Fas ligand (FasL) serum levels, as well as their changes during therapy.

Patients and Methods

Serum levels of MMP-7, Fas and FasL were determined by ELISA in 96 patients with castration-resistant prostate cancer (CRPC): 21 docetaxel-resistant patients who received one single series and 75 docetaxel-sensitive patients who received repeated series of docetaxel. In addition to the 96 pretreatment serum samples, 987 sera collected during chemotherapy were also analysed.

Results

Higher pretreatment serum MMP-7, sFas and prostate-specific antigen (PSA) levels were significantly associated with both docetaxel resistance ($P = 0.007$, $P = 0.001$, $P < 0.001$, respectively) and shorter cancer-specific survival ($P < 0.001$, $P = 0.041$, $P < 0.001$, respectively). High MMP-7 level remained an independent predictor of both docetaxel resistance (hazard ratio [HR] 2.298, 95% confidence interval [CI]: 1.354–3.899; $P = 0.002$) and poor cancer-specific survival (HR 2.11, 95% CI: 1.36–3.30; $P = 0.001$) in multivariable analyses. Greater increase in MMP-7 levels in the second treatment holiday and greater increase in PSA levels in the first and second treatment holidays were predictive of survival.

Conclusions

Pretreatment serum MMP-7 levels may help to select patients with CRPC who are likely to benefit from docetaxel chemotherapy. Furthermore, MMP-7 levels alone or in combination with PSA levels could be used for therapy monitoring. Correlative studies embedded in clinical trials are necessary to validate these biomarkers for clinical decision-making.

Keywords

MMP-7, Fas, serum, prognosis, docetaxel, #pcsm, #ProstateCancer

Introduction

Docetaxel is currently one of the standard first-line therapies for patients with castration-resistant prostate cancer (CRPC) [1–3]. While CRPC is generally a docetaxel-sensitive disease, there is a large variability in its response because of inherent or acquired docetaxel resistance. Patients who complete a first series of treatment without experiencing disease progression are considered docetaxel-sensitive, while those who experience disease progression are docetaxel-resistant. The recommended treatment is one single series of docetaxel containing 6 to 10 cycles in a 3-week schedule for both sensitive and resistant patients; however, docetaxel-sensitive patients might benefit from retreatment with further series of docetaxel as this can result in prolonged disease control [4,5]. Unfortunately, most docetaxel-sensitive patients will develop resistance to the chemotherapeutic drug during further treatment series. Thus, ideally docetaxel would be administered only to those patients

who are sensitive and therapy would only be continued until the development of resistance. In the last few years, several effective drugs, such as abiraterone-acetate, enzalutamide, cabazitaxel and alpharadin, with different therapeutic mechanisms of action have been approved for the treatment of CRPC before or after docetaxel therapy [6]. These therapeutic methods provide reasonable options for patients who are resistant to docetaxel.

Our ability to predict docetaxel sensitivity is limited. It is therefore important to explore predictive factors for the selection of patients who are likely to benefit from first-line docetaxel treatment. As drug resistance is an evolutionary process, monitoring of changes rather than a single snapshot of biomarker levels might be able to reflect the inherent sensitivity to docetaxel therapy, helping with timely detection or prediction of docetaxel resistance, and potentially leading to add-on or changes of therapies.

Matrix metalloproteinase 7 (MMP-7) has been associated with chemotherapy resistance. In head and neck cancer, Ansell et al. [7] performed comparative genome-wide transcript profiling and identified and validated MMP-7 as a predictive marker for chemotherapy resistance [7]. Similarly, Liu et al. [8] demonstrated that high MMP-7 tissue expression was associated with poor response and survival in patients with non-small-cell lung cancer treated with cisplatin-based chemotherapy [8]. In recent years, a growing body of evidence has been collected to support the hypothesis that MMP-7 is causally involved in therapy resistance by allowing tumour cells to escape from chemotherapy-induced apoptosis [9–14]. Interaction between the death receptor Fas, and its ligand FasL, plays a central role in chemotherapy-induced cell death. The degrading and downregulating effects of MMP-7 on receptor Fas and FasL have been identified as a key mechanism responsible for MMP-7-related chemotherapy resistance. In addition, we have previously shown that high MMP-7 serum levels are independently associated with worse cancer-specific survival in localized prostate cancer (PCa) [15].

These data and the unmet need for predictive markers in docetaxel-treated patients with CRPC led us to determine the pre-therapy levels of and changes in MMP-7, soluble Fas (sFas) and FasL during docetaxel treatment with the aim of assessing their predictive value.

Materials and Methods
Patient Characteristics

This study included 96 patients with CRPC who were treated with docetaxel at a single tertiary care centre between April 2003 and February 2010. Patients who completed the planned docetaxel treatment course with a good PSA response and without experiencing radiographic progression were considered docetaxel-sensitive and underwent retreatment with docetaxel. After the first retreatment, further retreatments were offered based on the same criteria. Our institutional ethics committee approved the study protocol. PSA response was defined, according to the Prostate Cancer Clinical Trials Working Group Criteria I, as a PSA decline from baseline of ≥50% during the first chemotherapy series [16]. Radiographic progression was defined according to the Response Evaluation Criteria in Solid Tumours (RECIST) [17].

Of the 96 patients with CRPC, 21 received one single series of docetaxel with five to eight cycles. These single-treatment patients were considered docetaxel-resistant. The remaining 75 patients were treated with at least two series of docetaxel. As these patients experienced a PSA decline and no radiographic progression during the first series, we considered them to be initially docetaxel-sensitive.

Criteria for inclusion were: availability of serum samples at first docetaxel treatment; no more than two missing serum samples during treatment follow-up; no second tumours at baseline; and no further lines of therapies after docetaxel.

Sample Selection for Analysis

As we aimed to assess the value of MMP-7, sFas and FasL as pre-therapy predictive and/or therapy-monitoring markers, baseline concentrations and changes during docetaxel therapy were analysed. Serum samples from the first, third, seventh, 21st, 42nd and last day of the first series were assessed. For the patients who received a second docetaxel series, serum samples were obtained on day 1, day 42 and the last day of therapy. In any additional series, sera from the first and last day were analysed. In all cases, serum samples from the time of first radiographic progression on docetaxel treatment and treatment holiday were analysed. Finally, in all cases, the last available serum sample after therapy failure was assessed and considered as samples at time of disease progression. Overall, 1 083 serum samples from 96 patients were analysed.

ELISA Analysis

Patients' MMP-7, sFas and FasL serum levels were quantified using Quantikine ELISA kits (DMP700, DFS00, DFL00, respectively; R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. Dilutions were twofold for MMP-7 and FasL, and 20-fold for sFas.

Statistical Analysis

For paired comparisons between groups, the non-parametric, two-sided Wilcoxon rank-sum test was applied. Survival analyses were carried out using Kaplan–Meier curves, log-rank tests and univariable Cox proportional hazards

regression analysis. For multivariable analysis, Cox regression models were used. Variables with an effect on survival in univariable analysis ($P \leq 0.05$) were considered in the Cox proportional hazards regression models. In calculations where dichotomizations were required, the median values were used as thresholds. Pearson's correlation coefficient was used to assess the relationship between MMP-7, sFas and FasL serum concentrations. Finally, we calculated concordance indices for survival predictive models [18]. All statistical analyses were two-sided; P values ≤ 0.05 were taken to indicate statistical significance. All tests were carried out using the SPSS software package (19.0; SPSS, Chicago, IL, USA).

Results

Clinical Background

Overall, 131 cycles of docetaxel were administered to 21 patients with primary docetaxel resistance and 1 099 cycles were administered to 75 docetaxel-sensitive patients. The total number of rechallenges was 151. The median durations of the first, second and third treatment holidays were 18, 16 and 15 weeks, respectively (Table 1). A total of 19 patients received vinorelbine as first-line therapy and 27 patients received docetaxel in combination with estramustine phosphate.

Of the 96 patients with CRPC, 47 had metastases, while 22 patients were treated after failure of androgen deprivation therapy with rising PSA level but without metastasis on CT. The main patient characteristics are provided in Table 1.

The main endpoints of the analysis were overall and PCa-specific survival. Of 96 patients, 93 had died at last follow-up, of whom 84 died from PCa. The median survival time was 39 weeks in the single-treatment, docetaxel-resistant group compared with 117 weeks in the retreated and initially docetaxel-sensitive group. Fifty-seven of 75 retreated patients had radiographic progression according to RECIST criteria on docetaxel chemotherapy (during treatment series) and 56 during treatment holiday (Table 1).

Associations of Clinicopathological Variables and Therapy Type with Serum PSA, MMP-7, sFas and FasL Levels at Baseline

The associations of baseline serum levels of MMP-7, sFas and FasL with clinicopathological variables are shown in Table 2. Patients' age, Eastern Cooperative Oncology Group performance status (ECOG PS), presence of lymph node or visceral metastasis and previously performed radical prostatectomy had no significant impact on PSA, MMP-7, sFas or FasL levels. Pretreatment with other first-line therapy or with estramustine phosphate was correlated with decreased MMP-7 serum levels. PSA levels were fourfold higher in patients with bone metastatic CRPC.

Associations between Clinicopathological Variables, Baseline and Serum Marker Levels with Response to Docetaxel

MMP-7, sFas and PSA levels were significantly higher in docetaxel-resistant (single-treatment) patients compared with those who were initially docetaxel-sensitive (retreatment patients; $P = 0.007$, $P = 0.010$ and $P < 0.001$, respectively). In a multivariate logistic regression model for the prediction of response to docetaxel therapy we assessed: (i) ECOG PS; (ii) formerly performed radical prostatectomy; (iii) presence of metastasis at baseline; (iv) pretreatment PSA level; (v) sFas level; and (vi) MMP-7 level. This analysis revealed the presence of metastasis (odds ratio [OR] 2.445, CI: 1.216–4.147; $P = 0.010$), MMP-7 (OR 2.298, CI: 1.354–3.899; $P = 0.002$) and PSA (OR 2.681, CI: 1.612–4.458; $P < 0.001$) to be independently associated with treatment response.

Associations between Patients' Survival and Serum Levels of PSA, MMP-7, sFas and FasL at Baseline

Univariable Cox analysis found previously performed radical prostatectomy to be weakly associated with favourable overall survival ($P = 0.049$) but not with PCa-specific survival ($P = 0.19$). Furthermore ECOG PS score > 0, presence of any metastasis, bone metastasis or visceral metastasis as well as MMP-7, sFas and PSA levels above the median were

Table 1 Patients' characteristics.

Total number of patients	96
Median (range) age at baseline, years	69 (47–86)
ECOG PS at enrolment	
0	65
1	22
2	9
PSA at baseline, ng/mL	83 (2–1 212)
Bone metastasis	68
Lymph node metastasis (>2 cm)	16
Soft tissue lesions (lung/liver)	6
Previous prostatectomy	52
Docetaxel therapy	
1st-line	77
2nd-line	19
Pretreatment	
No (docetaxel only)	69
Yes (EMP)	27
Median (range) number of docetaxel series	2 (1–10)
Median (range) number docetaxel cycles	11 (5–44)
Single (only one series)	21
Retreatment (at least two series)	75
Number of patients who died	93
PCa-specific deaths	84
Median (range) follow-up, weeks	85 (27–363)

ECOG PS, Eastern Cooperative Oncology Group performance status; EMP, estramustine phosphate; FasL, fas ligand; MMP-7, matrix metalloproteinase 7; PCa, prostate cancer; sFas, soluble Fas. Pretreated with EMP and/or vinorelbine.

Table 2 Correlations of baseline matrix metalloproteinase 7, soluble Fas and Fas ligand serum levels with clinicopathological variables.

	n	MMP-7 serum concentration, ng/mL Median (range)	P	sFas serum concentration, ng/mL Median (range)	P	FasL serum concentration, ng/mL Median (range)	P	PSA serum concentration, ng/mL Median (range)	P
Age									
≤69 years	53	4.40 (0.50–34.30)	0.765	11.64 (5.83–22.64)	0.079	60.10 (21.00–118.00)	0.684	67.0 (2.0–1124.0)	0.244
>69 years	43	4.80 (1.10–27.20)		13.19 (7.18–21.77)		57.20 (23.00–145.00)		112.5 (2.0–1212.0)	
Primary RP									
No	44	4.90 (0.50–34.30)	0.330	13.55 (7.18–21.77)	0.505	59.05 (23.00–145.00)	0.644	123.0 (3.0–1212.0)	0.731
Yes	52	4.30 (1.10–20.20)		11.37 (5.84–22.64)		59.10 (21.00–143.00)		46.5 (2.0–772.0)	
Docetaxel therapy									
1st-line	77	5.00 (1.60–34.30)	**0.009**	12.43 (5.83–22.64)	0.292	54.00 (23.00–116.00)	0.063	84.0 (2.0–1212.0)	0.728
2nd-line	19	3.30 (0.50–14.60)		11.64 (7.46–20.68)		60.10 (23.00–145.00)		76.0 (14.0–311.0)	
Docetaxel therapy									
Docetaxel. only	69	5.30 (1.60–34.30)	**0.001**	13.08 (5.84–22.64)	0.162	50.50 (23.00–145.00)	**0.022**	85.0 (2.0–1212.0)	0.997
EMP/docetaxel	27	3.40 (0.50–27.20)		11.15 (7.18–19.75)		52.70 (21.00–118.00)		71.5 (14.0–772.0)	
Number of docetaxel series									
Single treatment	21	6.20 (2.70–20.20)	**0.007**	14.09 (7.51–21.77)	**0.010**	60.50 (23.00–145.00)	0.674	215.0 (22.0–1212.0)	**<0.001**
Retreatment	75	4.30 (0.50–34.30)		11.64 (5.84–22.64)		58.90 (21.00–143.00)		61.0 (2.0–772.0)	
ECOG PS									
0	65	4.30 (0.50–34.30)	0.230	12.43 (7.64–22.64)	0.240	59.30 (21.00–145.00)	0.931	79.5 (2.0–1124.0)	0.342
1–2	31	5.00 (2.40–27.20)		11.76 (5.84–20.88)		58.60 (23.00–116.00)		99.0 (3.0 –1212.0)	
Any metastasis									
No	22	4.30 (0.50–20.20)	0.214	11.68 (6.98–22.64)	0.395	60.70 (33.00–143.00)	0.408	26.0 (2.0–560.0)	**<0.001**
Yes	74	4.70 (1.60–34.30)		12.15 (6.98–22.64)		58.65 (21.00–145.00)		117.5 (2.0 –1212.0)	
Lyph node status									
N –	80	4.45 (0.50–34.30)	0.662	12.31 (5.84–22.64)	0.395	59.10 (21.00–145.00)	0.906	76.0 (2.0–1212.0)	0.321
N +	16	3.80 (1.60–14.60)		11.51 (7.51–20.68)		62.10 (28.00–91.00)		113.0 (21.0–500.0)	
Bone metastasis									
No	28	4.10 (0.50–20.20)	0.065	11.68 (5.84–21.41)	0.314	60.70 (30.00–143.00)	0.350	31.0 (2.0–560.0)	**<0.001**
Yes	68	4.85 (1.80–34.30)		12.16 (6.98–22.64)		58.40 (21.00–145.00)		126.5 (2.0–1212.0)	
Visceral metastasis									
No	90	4.40 (0.50–34.30)	0.229	12.16 (5.84–22.64)	0.964	58.80 (21.00–145.00)	0.449	75.0 (2.0–1212.0)	0.144
Yes	6	6.60 (2.80–13.30)		11.35 (9.93–20.46)		63.00 (40.00–110.00)		159.0 (69.0–560.0)	

ECOG PS, Eastern Cooperative Oncology Group performance status; EMP, estramustine phosphate; FasL, fas ligand; RP, radical prostatectomy; sFas, soluble Fas. Significant correlations are marked by bold letters.

Table 3 Univariable Cox analysis.

Variables	Overall survival			Disease-specific survival		
	HR	95% CI	P	HR	95% CI	P
Age (years)						
≤65	Ref.			Ref.		
>65	1.466	0.916–2.348	0.111	1.406	0.866–2.284	0.168
Primary RP						
No	Ref.			Ref.		
Yes	0.658	0.434–0.998	**0.049**	0.749	0.483–1.159	0.194
Docetaxel therapy						
1st line	Ref.			Ref.		
2nd line	0.586	0.342–1.003	0.051	0.607	0.348–1.057	0.078
Docetaxel therapy						
EMP/docetaxel	Ref.			Ref.		
Docetaxel only	1.099	0.696–1.736	0.685	0.995	0.626–1.584	0.985
ECOG PS						
0	Ref.			Ref.		
1–2	1.816	1.158–2.847	**0.009**	1.841	1.154–2.935	**0.010**
Any metastasis						
No	Ref.			Ref.		
Yes	2.791	1.631–4.774	**<0.001**	3.209	1.789–5.754	**<0.001**
Lymph node status						
N −	Ref.			Ref.		
N +	1.359	0.788–2.345	0.270	1.400	0.796–2.462	0.243
Bone metastasis						
No	Ref.			Ref.		
Yes	2.656	1.634–4.316	**<0.001**	2.849	1.702–4.768	**<0.001**
Visceral metastasis						
No	Ref.			Ref.		
Yes	3.331	1.431–7.754	**0.005**	3.558	1.523–8.313	**0.003**
MMP-7 serum concentration						
≤4.40 ng/mL (median)	Ref.			Ref.		
	2.268	1.466–3.508	**<0.001**	2.444	1.549–3.856	**<0.001**
sFas serum concentration						
<12.035 pg/mL (median)	Ref.			Ref.		
	1.690	1.111–2.571	**0.014**	1.578	1.020–2.440	**0.041**
FasL serum concentration						
<59.1 pg/mL (median)	Ref.			Ref.		
	0.935	0.617–1.418	0.751	0.917	0.594–1.415	0.696
PSA serum concentration						
<83 ng/mL (median)	Ref.			Ref.		
	2.905	1.862–4.534	**<0.001**	3.145	1.975–5.008	**<0.001**

ECOG PS, Eastern Cooperative Oncology Group performance status; EMP, estramustine phosphate; FasL, fas ligand; RP, radical prostatectomy; sFas, soluble Fas. Significant correlations are marked by bold letters.

significantly associated with both poor overall and disease-specific survival (Table 3).

Multivariable analyses showed presence of any metastases, and high MMP-7 and PSA serum levels as independent and unfavourable prognostic factors for both overall and PCa-specific survival (Table 4). MMP-7 and PSA levels remained significant risk factors when included as continuous variables in the multivariable models (MMP-7: hazard ratio [HR] 1.067, 95% CI: 1.024–1.113, $P = 0.002$; PSA: HR 1.002, 95% CI: 1.001–1.003, $P < 0.001$). In addition, because pretreatment with other chemotherapeutic agents and presence of metastasis were observed to influence MMP-7 baseline levels, we performed multivariable analysis excluding patients without metastasis who were pretreated with other chemotherapeutic agents ($n = 49$). Also in this model, high MMP-7 baseline level remained a significant and independent

risk factor for patients' overall (HR 2.417, CI: 1.179–4.958; $P = 0.016$) and disease-specific survival (HR 2.774, CI: 1.282–6.005; $P = 0.010$). The concordance index for the base model (ECOG PS score > 0, presence of any metastasis, PSA > 83 ng/mL) was 0.708, which increased to 0.731 when MMP-7 (>4.4 ng/mL) was added.
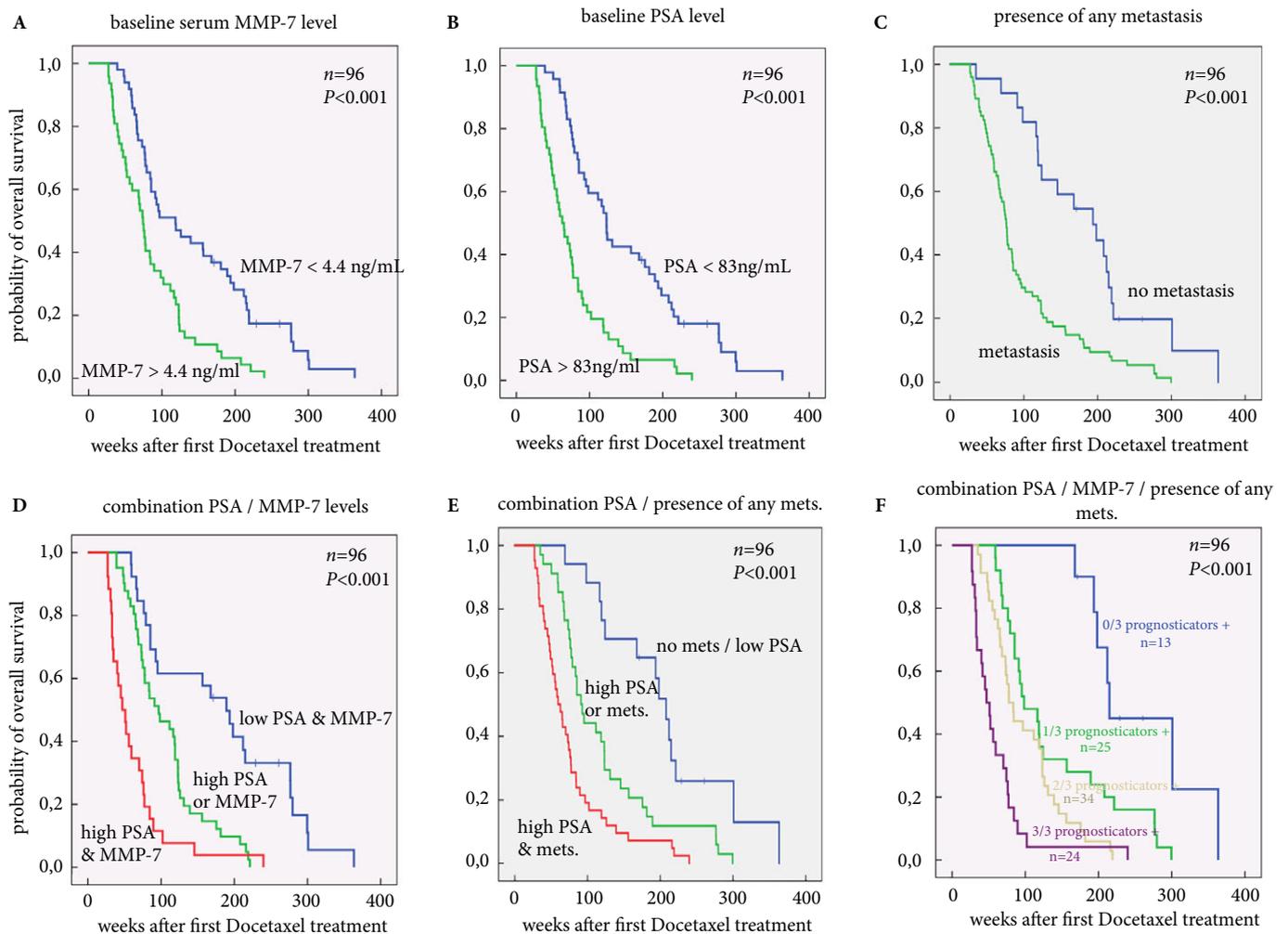
To show the independent prognostic value of PSA, MMP-7 and the metastatic status (Fig. 1A–C), we combined these three prognostic factors for the prediction of patients' overall survival in Kaplan–Meier analysis (Fig. 1D–F) By combining PSA, MMP-7 and metastatic status, patients with low PSA and MMP-7 levels as well as absence of metastatic lesions had a survival probability of 100% after 150 weeks in contrast to the high-risk group (high PSA, high MMP-7 levels and metastatic lesions) in which only ~5% of patients survived 150 weeks (Fig. 1F). Also in the subgroups of patients with

Table 4 Multivariable Cox analysis.

Variables	Overall survival			Disease-specific survival		
	HR	95% CI	P	HR	95% CI	P
ECOG PS						
0	Ref.			Ref.		
1–2	1.630	1.023–2.598	**0.040**	1.669	1.028–2.710	**0.038**
Any metastasis						
No	Ref.			Ref.		
Yes	2.087	1.180–3.692	**0.011**	2.178	1.189–3.990	**0.012**
MMP-7 serum concentration						
≤4.40 ng/mL	Ref.			Ref.		
>4.40 ng/mL	2.114	1.356–3.296	**0.001**	2.134	1.372–3.476	**0.001**
PSA serum concentration						
≤83 ng/mL	Ref.			Ref.		
>83 ng/mL	2.384	1.512–3.759	**<0.001**	2.270	1.621–4.216	**<0.001**

ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; MMP-7, matrix metalloproteinase 7. Significant correlations are marked by bold letters.

Fig. 1 Kaplan–Meier curves of overall survival according to (**A**) matrix metalloproteinase 7 (MMP-7) and (**B**) PSA levels and (**C**) presence of any metastasis (mets). The risk-stratification improves when MMP-7 (**D**) or metastatic status (**E**) are combined with PSA. The combination of three prognostic factors (MMP-7, PSA and the presence of any metastasis) results the most accurate risk assessment (**F**).

high PSA level and metastatic disease and with low PSA level without metastasis, high MMP-7 levels were significantly associated with shorter patient survival time (Fig. S1).

Compared with baseline levels, MMP-7, sFas and FasL levels were not significantly higher at progression (Table 1). In contrast, MMP-7 and sFas levels were significantly elevated after docetaxel failure and in the last samples before patients' deaths. PSA levels were mildly but not significantly elevated at the time of radiographic progression during treatment holiday ($P = 0.093$), but were significantly higher at PCa progression on docetaxel treatment (median 69 vs 129 ng/mL; $P = 0.027$). Finally, PSA levels were fourfold elevated after docetaxel failure in comparison with baseline levels (median 440 ng/mL vs 123 ng/mL; $P < 0.001$).

Changes in MMP-7, sFas and FasL levels in response to docetaxel treatment

PSA levels decreased during treatment series and increased in treatment holidays. MMP-7 levels showed no significant changes during docetaxel treatment if all patients were considered (Fig. 2). The subgroup analysis including only patients with high baseline MMP-7 levels (>8 ng/mL), however, revealed decreasing MMP-7 levels during treatment and increasing MMP-7 levels in treatment holidays (Fig. 2). In contrast, sFas and FasL levels exhibited no significant variations between different stages of docetaxel treatment.

Predictive Value of Changes in MMP-7, sFas and FasL Serum Levels during Docetaxel Therapy

Changes in PSA, MMP-7, sFas and FasL levels during treatment series were not predictive for overall or PCa-specific survival. In contrast, PSA increase in the first and second treatment holidays as well as MMP-7 increase in the second holiday proved to be predictive for both overall and PCa-specific survival independently of whether PSA and MMP-7 levels were calculated as continuous or categorized (median of change as cut-off) variables (Fig. S2). PSA and MMP-7 levels characteristically increased before or at the time of radiographic progression (Table S1 and Fig. S3).

Correlations between MMP-7, sFas, FasL and PSA Levels

Pearson's correlation analyses between marker levels showed a significant and linear correlation between sFas and MMP-7 serum concentrations ($r = 0.437$, $P < 0.001$), and between MMP-7 and PSA levels ($r = 0.217$, $P = 0.037$).
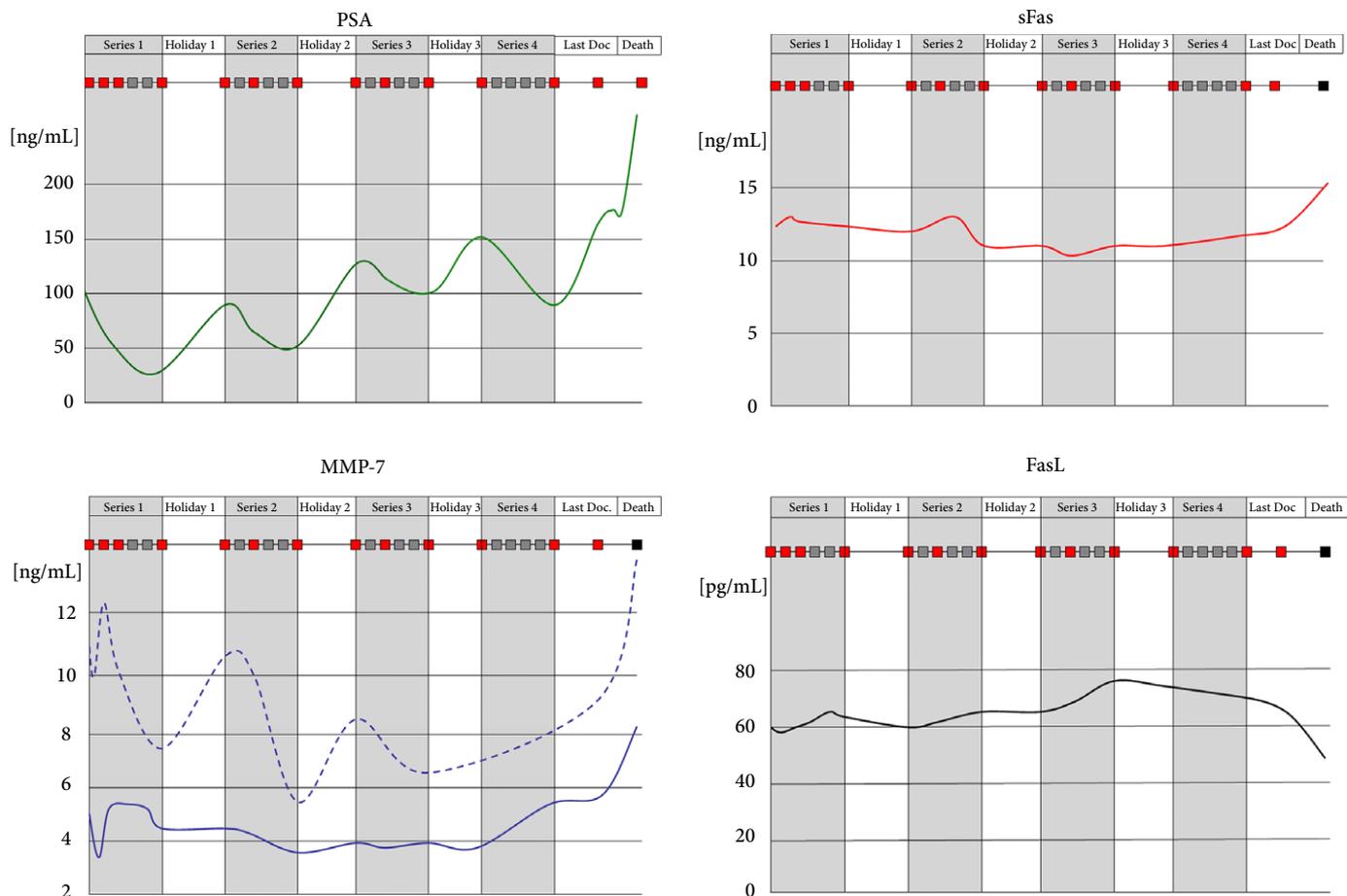
Discussion

In the present study, we determined MMP-7, sFas, FasL and PSA concentrations in over 1 000 serum samples collected

before and during docetaxel treatment from 96 patients with CRPC. Marker concentrations at baseline as well as their changes during docetaxel treatment were assessed for their predictive value for therapy effectiveness value. High pretreatment MMP-7, sFas and PSA levels were significantly associated with the presence of docetaxel resistance. Furthermore, MMP-7 and PSA baseline levels proved to be independent and unfavourable predictive factors for survival in patients with CRPC treated with docetaxel chemotherapy. Changes in MMP-7 and PSA levels during treatment holidays were able to predict patients' prognosis.

MMP-7 is a member of the matrix metalloproteinase family capable of degrading extracellular matrix components and thereby promoting metastasis formation. In addition, MMP-7, based on its large substrate specificity, is involved in the regulation of several tumour-supporting cellular processes such as angiogenesis, apoptosis and epithelial-to-mesenchymal transition [19]. In PCa, MMP-7 has been shown to be focally expressed by the tumour cells [20]. A genome-wide screening study analysing >200 000 single nucleotide polymorphism loci in 25 000 patients with PCa and controls identified a region within the MMP-7 promoter that was associated with PCa risk [21]. In addition, Lynch et al. [22], using a rat PCa metastasis model, analysed the gene expression profiles of PCa cells from the centre of the metastasis compared with cells originated from the tumour-bone interface. They found MMP-7 to be one of the most significantly upregulated genes at the tumour–bone interface, suggesting its involvement in PCa bone metastasis formation. The causal involvement of MMP-7 in bone metastasis formation was confirmed using an *in vivo* MMP-7-deficient mouse model [22]. In accordance with that study, we recently found elevated serum MMP-7 levels in serum samples of PCa patients harbouring bone metastases compared with those without bone metastatic lesions [15]. These data together strongly suggest the causal involvement of MMP-7 in both formation and progression of PCa, and make MMP-7 a promising marker for monitoring tumour burden.

In addition, independent studies in different cancers including breast, lung and colon cancer confirmed the causal involvement of MMP-7 in the formation of chemotherapy resistance [9–14]. Elevated MMP-7 levels and decreased Fas expression was found in oxaliplatin-resistant colon cancer cells as compared with parental, oxaliplatin-sensitive cells. Sensitivity to chemotherapy could be restored by inhibition of MMP-7 which was accompanied by enhanced Fas expression, clearly indicating the causal involvement of MMP-7 in platinum resistance [11]. Similarly, in Ewing's sarcoma cells, MMP-7 was shown to proteolytically degrade FasL, resulting in decreased doxorubicin sensitivity [9]. MMP-7-expressing breast cancer cells exhibited reduced sensitivity to mitomycin-induced apoptosis, and this effect was related to lower FasL levels in MMP-7 transgenic mice [14]. In lung

Fig. 2 Changes in PSA, matrix metalloproteinase 7 (MMP-7), soluble Fas (sFas) and Fas ligand (FasL) serum levels during docetaxel treatment and treatment holidays. Red squares represent treatment days when serum samples were analysed. Curves were drawn by using the median values of marker concentrations. In case of MMP-7 a separate curve was drawn including only those patients with high MMP-7 serum levels (>8 ng/mL) at baseline (dashed line).

adenocarcinoma cells, exogenous exposure to MMP-7 significantly enhanced cisplatin resistance [13]. In accordance with this, MMP-7 immunostaining was predictive of therapy response and survival in patients with lung cancer treated with platinum-based chemotherapy [8]. These data suggest that MMP-7 is causally involved in apoptosis inhibition, enabling tumour cells to escape chemotherapy. Our results show that pretreatment MMP-7, sFas and PSA serum levels are higher in patients who are resistant to docetaxel. Accordingly, high pretreatment MMP-7 and PSA levels proved to be independent risk factors for survival in patients with CRPC treated with docetaxel chemotherapy. Based on these data, MMP-7 in combination with selected clinicopathological variables and PSA level might help to select patients for effective docetaxel treatment. Docetaxel-sensitive patients might benefit from repeated docetaxel treatments until the development of docetaxel insensitivity. Conversely, men with docetaxel-resistant tumours might be spared from ineffective therapy and unnecessary toxicity. In

the last few years, the landscape of treatment options for CRPC has significantly changed as active, life-prolonging agents such as cabazitaxel, abiraterone-acetate and enzalutamide have become available for patients who failed docetaxel treatment. Men with docetaxel-resistant tumours, independently of having initial or acquired resistance, may benefit from the timely use of these new treatments.

Currently, a large meta-analysis confirmed the role of alkaline phosphatase and lactate dehydrogenase and newly identified serum aspartate aminotransferase as a strong predictor of survival in docetaxel-treated patients with CRPC [23]. A proteomic approach showed that increasing serum MIC-1 levels following the first cycle of docetaxel treatment are associated with poor survival [24]. Others found circulating miRNAs to be associated with docetaxel resistance [25]. Circulating biomarkers may better reflect molecular heterogeneity and hold the potential to capture tumour heterogeneity; therefore, when combining with established

baseline clinical variables, circulating biomarkers may help to increase the accuracy of predictive models.

We found significantly lower MMP-7 levels in serum samples of men who were pretreated with estramustine phosphate and vinorelbine, suggesting that the anti-tumour effect of these agents might also have an impact on serum MMP-7 levels. Thus, MMP-7 might be used for monitoring treatment response or for the detection of developing therapy resistance. To test this hypothesis, we determined MMP-7 levels at various stages during docetaxel treatment; however, none of the changes in any of the assessed serum proteins during docetaxel treatment were associated with survival. By contrast, a PSA increase in the first and second treatment holidays, as well as an MMP-7 increase in the second holiday, were predictive for survival suggesting both serum proteins as candidates for therapy monitoring markers. In accordance, PSA levels showed characteristic changes with consequently decreasing levels during docetaxel treatment and increasing concentrations in treatment holidays. MMP-7 levels exhibited similar characteristics only when cases with high baseline levels were considered, suggesting the existence of an MMP-7-expressing docetaxel-resistant molecular subtype of PCa. This might give the clinical rationale to consider MMP-7 as a target for therapy. As the initial therapeutic concept that general, systemic inhibition of a broad spectrum of MMPs might significantly improve survival has failed, current research focuses on specific inhibition of single MMPs. The first MMP-7-specific inhibitor has recently been developed [26]. Our results suggest that a specific, MMP-7-targeted therapy might improve sensitivity of PCa to docetaxel treatment; however, despite a large body of evidence in several cancers showing that MMP-7 is causally involved in chemotherapy resistance, we cannot state with certainty that this is also the case in PCa. Further functional analyses are therefore needed to determine whether the association between MMP-7 and docetaxel resistance is a coincidence or a causal relationship. Because the anti-apoptotic impact of MMP-7 was related to its proteolytic effect on sFas and FasL, we sought correlation between their serum levels. MMP-7 serum concentrations were significantly and linearly correlated with those of the extracellular domain of receptor Fas (sFas). This may underline the role of MMP-7 in the degradation of Fas and suggest an anti-apoptotic effect for MMP-7 in PCa.

In conclusion, our data identify elevated MMP-7 and sFas serum levels as markers of a docetaxel-resistant PCa phenotype. In addition, pretreatment MMP-7 levels were independently associated with poor patient survival, making MMP-7 a promising therapy-predicting marker in CRPC. Finally, not only the baseline levels but also the changes in PSA and MMP-7 levels in treatment holidays were predictive for survival, suggesting these serum proteins could be used as therapy-monitoring markers. These results need to be validated in larger prospective studies before being proposed for everyday clinical practice.

Acknowledgements

Conflict of Interest

None declared.

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Abbreviations: CRPC, castration-resistant prostate cancer; ECOG, Eastern Cooperative Oncology Group; FasL, fas ligand; HR, hazard ratio; HR, hazard ratio; MMP-7, matrix metalloproteinase 7; OR, odds ratio; PCa, prostate cancer; RECIST, Response Evaluation Criteria in Solid Tumours; sFas, soluble Fas.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Fig. S1. Kaplan-Meier survival plots stratified by (A) PSA level and presence of metastasis, by MMP-7 in the subgroup of (B) patients with low PSA & and no metastases and (C) patients with high PSA and metastasis. MMP-7 is significantly associated with survival in both subgroups.

Fig. S2. Kaplan-Meier curves of overall survival according to (A) MMP-7-, (B) PSA-changes and (C) their combination during the 2nd treatment-free period (2nd holiday).

Fig. S3. Changes of PSA (blue lines) and MMP-7 levels (red lines) in representative cases of CRPC. Grey area represents treatment periods (series). Note that PSA and MMP-7 levels elevate prior or at the time of metastatic progression.

Table S1. MMP-7, sFas and FasL serum levels at baseline and at time of radiographic progression.