Detection of circulating tumor cells in different stages of prostate cancer

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Abstract

Purpose To explore circulating tumor cell (CTCs) counts in different stages of prostate cancer (PC) in association with tumor burden, metastatic pattern and conventional serum biomarkers. Overall survival (OS) analyses were conducted with respect to optimized CTC cutoff levels.

Methods Circulating tumor cell counts were assessed in healthy controls ($n = 15$) as well as in locally advanced high risk (LAPC, $n = 20$), metastatic castration resistant (mCRPC, $n = 40$) and taxane-refractory (mTRPC, $n = 15$) PC patients. CTCs were detected using the CellSearch^{TM} System.

Results In metastatic PC (mPC), CTC counts were significantly increased compared to LAPC ($p < 0.001$). In LAPC, CTCs were at control level ($p = 0.66$). Patients with both bone and visceral lesions revealed the highest median CTC count ($p = 0.004$), whereas patients with sole soft tissue metastases displayed CTC counts comparable to controls ($p = 0.16$). No correlation was observed between CTC counts and osseous tumor burden assessed by bone lesion count ($p = 0.54$) or bone scan index ($p = 0.81$). CTC counts revealed a positive correlation with alkaline phosphatase ($p < 0.001$) and lactate dehydrogenase ($p < 0.001$) as well as a negative association with hemoglobin ($p = 0.004$) and PSA-doubling time ($p = 0.01$). Kaplan–Meier analyses demonstrated a cohort adjusted cutoff level of 3 CTCs with a shorter OS in case of ≥ 3 CTCs compared to <3 CTCs ($p = 0.001$), a cutoff level applicable in mCRPC ($p = 0.003$) but not in mTRPC patients ($p = 0.054$).

Conclusions Circulating tumor cell counts are applicable as a prognostic molecular marker, especially in mCRPC patients harboring bone metastases with or without visceral metastases. For clinical practice, mPC patients with elevated CTC counts in combination with short PSA-DT, high alkaline phosphatase and lactate dehydrogenase levels as well as low hemoglobin levels are at high risk of disease progression and limited OS.

Keywords Biomarkers · CellSearch^{TM} System · Circulating tumor cells · CTCs · Prostate cancer · Immunomagnetic capture

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Introduction

In prostate cancer (PC), there is no reliable and approved serum derived molecular marker with the potential of clinical application for diagnosis and staging along different stages from localized to metastatic disease. Although prostate-specific antigen (PSA) is the routinely used serum marker for about 35 years, PSA displays a low positive predictive value in localized PC and is unable to

differentiate pathological stages (Stamey et al. 1987; Brawer et al. 1999; Davis et al. 2008). In metastatic PC (mPC), incoherence of PSA values with imaging results during staging procedures for treatment monitoring is frequently observed (De Bono et al. 2008; Olmos et al. 2009). Hence, PSA values often do not reflect accurately tumor burden and cancer progression leading to therapeutic dilemmas and the demand for more reliable serum derived surrogate markers (De Bono et al. 2008; Olmos et al. 2009; Davis et al. 2008).

Among different potential new molecular markers, the last decade has focused on the detection of circulating tumor cells (CTCs) in peripheral blood as a prognostic tool (Sun et al. 2011). It is widely accepted that the spread of CTCs into blood circulation is an essential mechanism of cancer metastases during the leukemic phase of solid tumors indicating CTC counts as a reliable index of tumor burden and activity (Panteleakou et al. 2009). With the development of new and reliable tools for CTC capturing, CTCs were detected in venous blood of locally confined and advanced malignancies (Moreno et al. 2001; Allard et al. 2004; Nagrath et al. 2007).

The first standardized and automated CTC quantification device with the potential of clinical routine use is the CellSearch^{TM} System (CSS). The method combines immunomagnetic enrichment for epithelial cell adhesion molecule (EpCAM) expressing cells out of peripheral venous blood with an automated fluorescent staining for cytokeratin and nucleic acid (De Bono et al. 2008; Olmos et al. 2009; Davis et al. 2008). A strong association with prognosis was found for CTCs detected by the CSS leading to approval by the US Food and Drug Administration (FDA) for treatment monitoring in metastatic breast, colon and prostate cancer (Cristofanilli et al. 2005; De Bono et al. 2008; Cohen et al. 2009). In mPC, a threshold of $\geq$5 CTCs per 7.5 ml venous blood was determined as prognostic significant for overall survival (OS) (Moreno et al. 2005; De Bono et al. 2008; Olmos et al. 2009).

Besides the results of the first PC studies, detailed systematic CTC analyses according to different PC stages and clinical characteristics are still missing, and the literature is partly controversial. In particular, the prognostic value of CTCs for locally advanced high risk PC (LAPC) is still not answered satisfactory. Therefore, we performed a systemic analysis investigating CTC counts, CTC detection rates and the proportion of the unfavorable CTC count ($\geq$5 CTCs) in the continuum of different PC stages from LAPC to metastatic castration resistant PC (mCRPC) and finally in metastatic taxane-refractory PC (mTRPC). We analyzed the association of CTC counts with the metastatic osseous tumor volume, different metastatic patterns and clinical routine serum markers. Finally, the prognostic value of CTC counts for the presence of metastatic PC and OS was analyzed, with respect to the optimal CTC cutoff level.

Patients and methods

Study design

The institutional review board approved the prospective trial, and all subjects gave informed consent. The study was performed in accordance with the ethical standards of the Declaration of Helsinki. In total, 75 men with histological confirmed adenocarcinoma of the prostate, and 15 healthy volunteers (8 males, 7 females) were accrued from June 2008 to November 2010 at the Department of Urology, Rechts der Isar Medical Center, Technische Universität München. Patients with a second malignancy within 5 years were excluded.

The first group presented locally advanced PC patients (LAPC, $n = 20$) with no evidence of metastases and a high risk of progression despite intended radical prostatectomy (RP) depicted by the preoperative nomogram by Kattan (Graefen et al. 2002). In LAPC patients, prior androgen deprivation or radiation therapy was not allowed. The metastatic castration refractory patient group (mCRPC, $n = 40$) was defined according to the EAU guidelines with rising PSA values or progression of metastases despite ongoing androgen deprivation (Heidenreich et al. 2010). All mCRPC patients were planned for first line chemotherapy with docetaxel in a 3-week schedule. Metastatic taxane-refractory patients (mTRPC, $n = 15$) experienced a progression during a previous chemotherapy with docetaxel and were further treated by best supportive care and continuing androgen deprivation.

Blood sampling and CTC isolation

Venous blood was collected at the clinical site into CellSave^{TM} Preservative Tubes (Veridex, Raritan, NJ, USA) containing cellular preservatives and EDTA as an anticoagulant. Samples were stored to a maximum of 96 h at room temperature before being processed (Shaffer et al. 2007; Allard et al. 2004; De Bono et al. 2008). In LAPC patients, blood was collected before radical prostatectomy, in mCRPC patients before the first cycle of docetaxel chemotherapy and in mTRPC patients at the diagnosis of progression during supportive care. In addition, routine laboratory analyses were conducted including PSA, lactate dehydrogenase (LDH), alkaline phosphatase (AP), hemoglobin (Hb) and calcium. PSA-doubling time (PSA-DT) was assessed by all available PSA values of the last 3 months using the calculator of the Memorial Sloan-Kettering Cancer Center (http://nomograms.mskcc.org).

Isolation and enumeration of CTCs were performed at the University of Munich, Germany. CTCs were isolated and processed from 7.5 ml peripheral venous blood using the FDA-approved CellSearch^{TM} System (Veridex,

Raritan, NJ, USA) according to the manufacturer's protocol (De Bono et al. 2008). In brief, for CTC enrichment, whole blood was centrifuged and the plasma layer discarded. Automated immunomagnetic capture of CTCs using ferroparticles coated EpCAM antibodies with an immunocytochemical fluorescent staining was performed using the CellTracks® AutoPrep System and the CellSearch® Epithelial Cell Reagent Kit. Cells were stained for cytokeratin 8, 18, 19 and for nucleic acid. The processed cells were transferred into the CellSpotter® Analyzer for an automated imaging capture. Two independent operators evaluated the selected cells in a blinded fashion. CTCs were identified by nuclear and cytokeratin staining and the absence of the leukocyte typical CD45 epitope (Allard et al. 2004; Cristofanilli et al. 2005; Davis et al. 2008; Rink et al. 2011).

Radiographic evaluation

Prostate cancer patients were concomitantly staged by computed tomography (CT) and bone scan. In LAPC, local tumor staging was additionally performed by magnetic resonance imaging (MRI) of the pelvis with endorectal coil (D'Amico et al. 1998). Radiologists and nuclear medicine specialists reviewed the imaging assessments independently. The metastatic pattern was classified into patients with bone lesions, with bone and visceral lesions and patients with soft tissue metastases (lymph node ± visceral metastases). Metastatic bone lesions were counted (BLC), and the percentage of skeleton involvement was defined as bone scan index (BSI) (Imbriaco et al. 1998).

Statistical analysis

The aim of the study was to compare CTC counts between the control group and LAPC, mCRPC as well as mTRPC patients. Furthermore, the prognostic value of CTC counts for the presence of metastatic PC was assessed. For metastatic patients (mCRPC and mTRPC), CTC counts were further analyzed in association with BLC, BSI, metastatic pattern and routine laboratory results. Additionally, the prognostic value and optimal threshold of CTCs for OS were investigated in mPC. OS was defined as the elapsed time from CTC collection to death or the last follow-up. CTC counts were presented as number per 7.5 ml of venous blood. According to literature, an unfavorable CTC count was defined as ≥5 CTCs and the detection rate, as the number of patients with detectable CTCs.

Linear trend analyses were performed with Kruskal–Wallis tests. Continuous measures were compared using the Mann–Whitney U test. Association analyses were performed with the Spearman's rank correlation (R). Receiver operator characteristic curves (ROC) were used for threshold determination with a 95 % confidence interval (CI). Estimates of survival were analyzed using the Kaplan–Meier method, and differences were calculated using the log-rank test. For all tests, $p < 0.05$ was considered statistically significant. Analyses and figures were performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA).

Results

CTC counts in different stages of PC

The first aim was to assess CTC counts and CTC detection rates between controls and different stages of PC. The clinical characteristics of the 75 PC patients are depicted in Table 1. In the control group including healthy volunteers ($n = 15$) with a median age of 28 years (range 23–83), no CTCs were found. The LAPC group ($n = 20$) had a median risk of tumor recurrence of 90 % (range 45–95 %) within 5 years according to the Kattan nomogram. Despite an elevated preoperative median PSA level of 21 ng/ml and a tumor stage of ≥cT3a in 95 % of LAPC cases, only one subject (5 %) presented a characteristic CTC. Hence, LAPC patients displayed no difference in CTC counts compared to controls ($p = 0.66$).

In contrast, patients with mPC (mCRPC and mTRPC, $n = 55$) displayed a median number of 9 CTCs (range 0–2,347) and a detection rate of 84 %. Thus, CTC levels in metastatic disease were significantly elevated compared to non-metastatic PC patients ($p < 0.001$) and to controls ($p < 0.001$). Analyzing the predictive value of 1 CTC for the presence of metastatic PC disease, a sensitivity of 78 % and a specificity of 95 % (AUC 0.9; $p < 0.001$, 95 %CI 0.83–0.97) were found.

Metastatic patients were further stratified in a mCRPC group prior to first line docetaxel chemotherapy and a mTRPC group refractory to taxane-based therapy. mCRPC patients ($n = 40$) displayed a CTC detection rate of 80 % and a median CTC count of 7.5 (range 0–225). In total, 57 % of mCRPC patients showed an unfavorable CTC count of ≥5 CTCs. mTRPC patients ($n = 15$) presented a CTC detection rate of 93 % with a median number of 14 CTCs (range 0–2,437) while 60 % harbored ≥5 CTCs. Comparing mCRPC and mTRPC patients, CTC counts increased from mCRPC to mTRPC stage, but missed statistical significance ($p = 0.38$) (Fig. 1a).

Association of CTC counts with metastatic pattern

In the cohort of metastatic PC (mCRPC and mTRPC; $n = 55$), 25 patients harbored both visceral and bone metastases, 23 bone metastases and 7 a soft tissue involvement only. Analyses of patients with both visceral

Table 1 Clinical and pathological characteristics of PC patients

	LAPC[b]	mCRPC	mTRPC	Metastatic PC (mCRPC + mTRPC)
Patients (*n*)	20	40	15	55
Age (years)				
Median (mean)	70 (68.1)	70 (69.3)	70 (67.3)	70 (68.8)
Range	52–77	52–82	44–82	44–82
ECOG				
Median	0	1	2	1
Range	0	0–2	0–3	0–3
Gleason score at diagnosis				
Median	7.5	8	8	8
Range	6–10	5–9	5–10	5–10
Primary therapy (*n*) (%)				
RP	–	12	6	18
EBRT	–	3	1	4
Palliative	–	25	8	33
Systemic therapy (*n*)				
Androgen depletion	–	40	15	55
Docetaxel	–	0	15	15
Metastatic sites (*n*)				
Soft tissue	–	6	1	7
Bone	–	16	7	23
Bone + visceral	–	18	7	25
Bone scan[a]				
BSI (%)	–	16.9 (25.0) 0.3–67.2	20 (22.9) 0.03–45.6	19.45 (25.5) 0.03–67.2
BLC (*n*)	–	55 (101) 4–280	82 (97.9) 1–220	63 (103.9) 1–280

PC prostate cancer, *ECOG* eastern cooperative oncology group performance status, *RP* radical prostatectomy, *EBRT* external beam radiotherapy, *BSI* bone scan index, *BLC* bone lesion count, *PSA* prostate-specific antigen, *CT* computed tomography

[a] Values given as median (mean); [b] CTC collection before radical prostatectomy

and bone lesions revealed the highest median CTC count of 26 (range 0–207), whereas patients with only bone lesions showed a median CTC count of 6 CTCs (range 0–2,437), without a statistical difference between the latter ($p = 0.12$). For patients with only soft tissue disease, the median CTC number was 0 (range 0–28) being significantly lower compared to the group with both visceral and bone metastases ($p = 0.001$) and to only bone metastatic patients ($p = 0.029$) (Fig. 1b). Notably, patients with a sole metastatic soft tissue disease displayed no statistical difference to the control level ($p = 0.16$).

The highest CTC positive rate was found in the group of osseous and visceral metastatic patients (96 %) followed by only osseous (83 %) and by only soft tissue disease (43 %). The proportion of patients with an unfavorable CTC count of ≥5 CTCs was 14 % in case of only soft tissue disease increasing in case of bone metastases as well as in the group of bone and visceral metastases to 57 and 72 %, respectively.

A consecutive performed Kaplan–Meier analysis for OS in dependency of the metastatic pattern demonstrated a significant linear trend ($p = 0.02$). Patients with bone and visceral metastases showed the shortest median OS of 148 days compared to 463 days in patients with bone metastases and finally to patients with only soft tissue metastases (median OS not defined).

Association of CTC counts with metastatic osseous tumor burden

Metastatic patients with only bone metastases (mCRPC and mTRPC; $n = 19$) were further evaluated concerning the osseous tumor burden. No correlation of CTC counts with the BSI ($R = 0.06$; $p = 0.81$) or the BLC was found ($R = 0.15$; $p = 0.54$).

Association of CTC counts with blood-derived markers in metastatic PC

Analyses of CTC counts with blood-derived markers in mPC patients (mCRPC and mTRPC; $n = 55$) revealed a significant positive correlation with AP ($R = 0.54$;

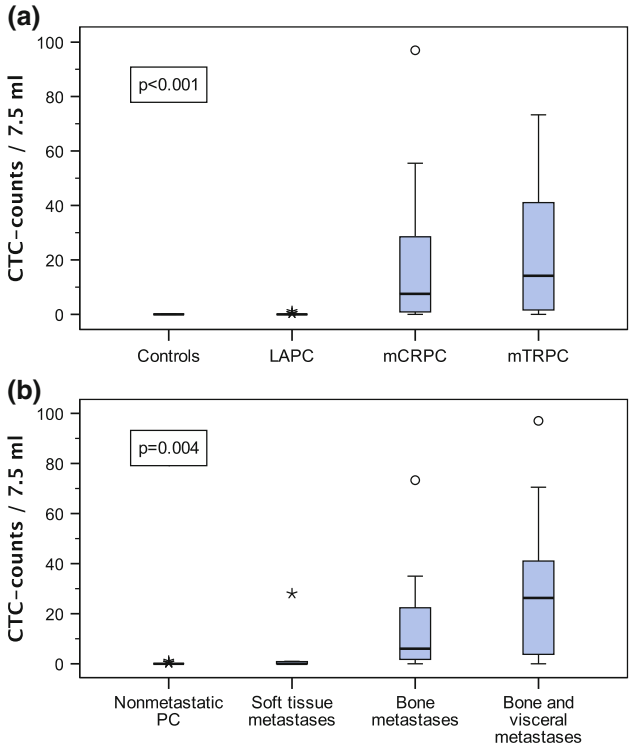

Fig. 1 *Boxplot diagram* showing CTC findings in venous blood **a** in controls ($n = 15$), locally advanced (LAPC; $n = 20$), metastatic castration refractory (mCRPC; $n = 40$) and metastatic taxane-refractory (mTRPC; $n = 15$) patients (Kruskal–Wallis test on linear trend: $p < 0.001$) **b** in association with metastatic pattern comparing non-metastatic PC patients ($n = 20$) with mPC patients harboring only a soft tissue involvement (LN ± visceral metastases; $n = 7$), only bone metastases ($n = 23$) or visceral and bone metastases ($n = 25$), (Kruskal–Wallis test on linear trend: $p = 0.004$); *o* outliers, *Asterisks* extreme outliers, extreme outliers for mCRPC, bone, bone and visceral metastases not shown ($n = 3$)

$p < 0.001$) and LDH levels ($R = 0.54$; $p < 0.001$) as well as a negative correlation with hemoglobin levels ($R = -0.39$; $p = 0.004$) and PSA-DT ($R = -0.37$; $p = 0.01$). No correlation was found for absolute PSA ($R = 0.06$; $p = 0.67$) and calcium values ($R = 0.02$; $p = 0.91$) (Table 2).

Association of CTC counts with OS in metastatic PC

Association analyses of CTC counts with OS were performed in the whole group of metastatic patients (mCRPC and mTRPC; $n = 55$). The median estimated survival time was 284 days (95 % CI 187–381) for the complete cohort of mPC. At the time of analysis, 31 (56 %) mPC patients were dead after a median OS of 143 days (range 6–469) harboring a median CTC count of 22 (range 0–2,437; 97 % detection rate; 77 % ≥ 5 CTCs) compared to 1 CTC (range 0–225; 67 % detection rate; 33 % ≥5 CTCs) in patients who were still alive. A consecutive performed Cox regression analyses revealed a significant association of CTC counts with survival ($p = 0.021$).

Investigating the described threshold of ≥5 CTCs according to literature, a sensitivity of 74 % and a specificity of 67 % were found. Kaplan–Meier analyses demonstrated in metastatic patients with <5 CTCs a significantly ($p = 0.003$) longer OS (median OS not defined; $n = 23$) compared to patients harboring ≥5 CTCs (median 158 days; $n = 32$) (Fig. 2a). Subgroup analyses investigated the threshold of <5 CTCs versus ≥5 CTCs with respect to OS in the mCRPC and mTRPC groups. Significance was reached in the mCRPC group ($p = 0.026$), but was missed in mTRPC patients ($p = 0.054$).

In order to enhance the prognostic significance of a CTC threshold applicable in both the mCRPC and the mTRPC subgroup, we investigated different cutoff levels for OS. In our cohort, an optimized threshold of ≥3 CTCs was found predictive with a sensitivity of 87 % and a specificity of 67 % (AUC 0.78; $p < 0.001$; 95 % CI 0.6–0.9) (Fig. 3). Kaplan–Meier analyses demonstrated a significant shorter OS for patients with ≥3 CTCs (median 178 days; $n = 35$) compared to those having <3 CTCs (median OS not defined; $n = 20$) ($p = 0.001$) (Fig. 2b). Investigating the cutoff level of ≥3 CTCs in the mCRPC and mTRPC group significance was found for mCRPC ($p = 0.003$) but was missed in mTRPC patients ($p = 0.054$) (Fig. 4a, b).

Discussion

The detection of CTCs with the CSS was approved in mPC as a prognostic marker for OS (De Bono et al. 2008). Nevertheless, a systemic analysis investigating CTCs as a biomarker in different stages from localized to mPC is still fragmentary. Therefore, we explored CTC counts in venous

Table 2 Correlation of CTC counts with blood-derived markers in mPC patients

Serologic value (unit)	Patients (*n*)	Median (mean) range	Correlation (R) with CTC counts	Significance (*p*)
PSA (ng/ml)	55	155.2 (329.7) 0.1–3,378	$R = 0.059$	$p = 0.667$
PSA-doubling time (months)	47	1.96 (3.39) −13.5 to 20.7	$R = -0.372$	$p = 0.010$
Lactate dehydrogenase (U/l)	51	365 (531.5) 179–1,900	$R = 0.536$	$p < 0.001$
Alkaline phosphatase (U/l)	55	211 (321.6) 50–1,466	$R = 0.540$	$p < 0.001$
Hemoglobin (g/dl)	55	11.0 (11.3) 7–16.1	$R = -0.385$	$p = 0.004$
Calcium (mmol/l)	53	2.2 (2.19) 1.59–2.52	$R = 0.016$	$p = 0.907$

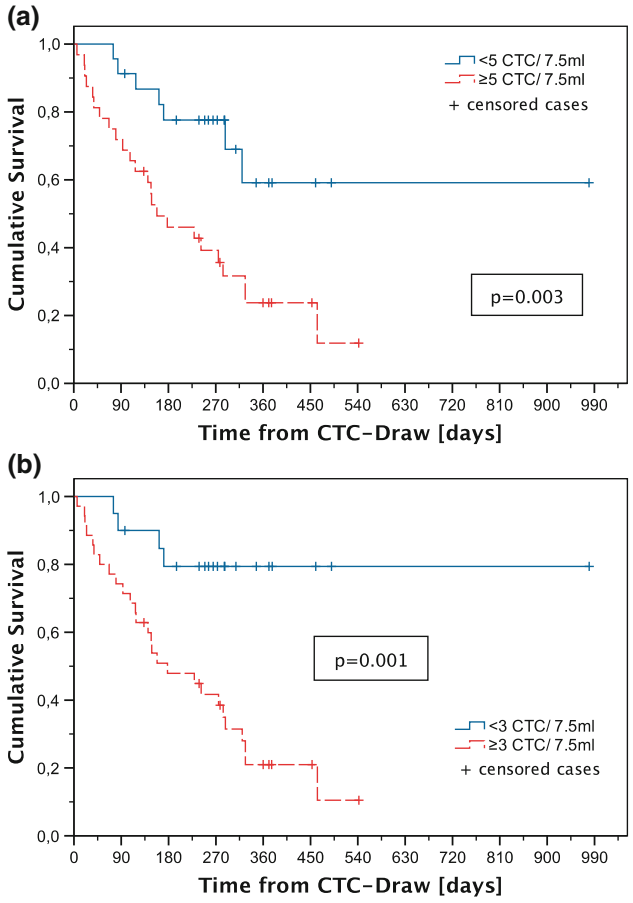

Fig. 2 *Kaplan–Meier plot* for survival in metastatic PC patients in dependency of CTC levels **a** comparing patients with <5 CTCs ($n = 23$) versus ≥5 CTCs ($n = 32$); **b** comparing patients with <3 CTCs ($n = 20$) versus ≥3 CTCs ($n = 35$)

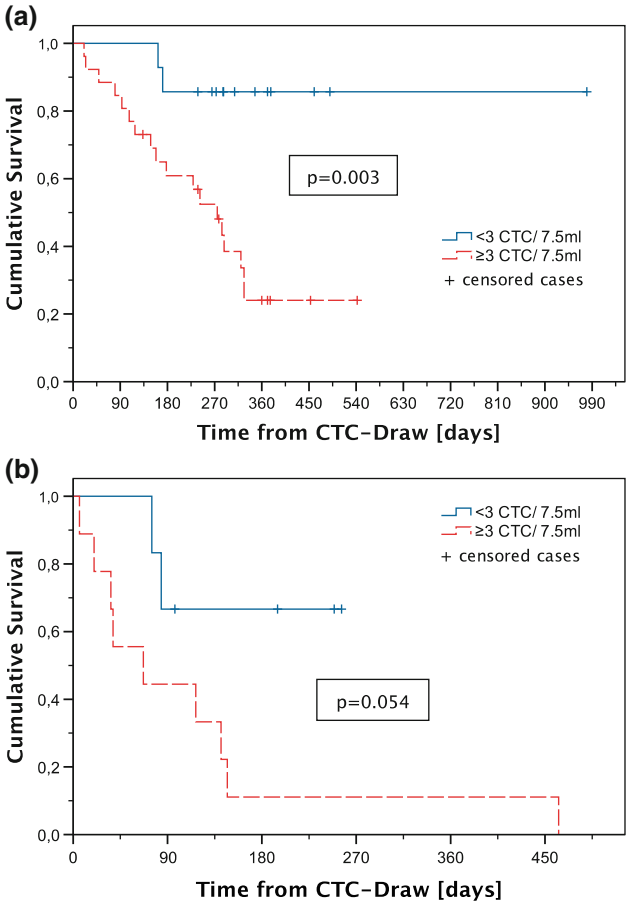

Fig. 4 *Kaplan–Meier plot* for survival in dependency of CTC levels comparing patients with <3 CTCs versus ≥3 CTCs **a** in mCRPC patients ($n = 14$ with <3 CTCs; $n = 26$ with ≥3 CTCs); **b** in mTRPC patients ($n = 6$ with <3 CTCs; $n = 9$ with ≥3 CTCs)

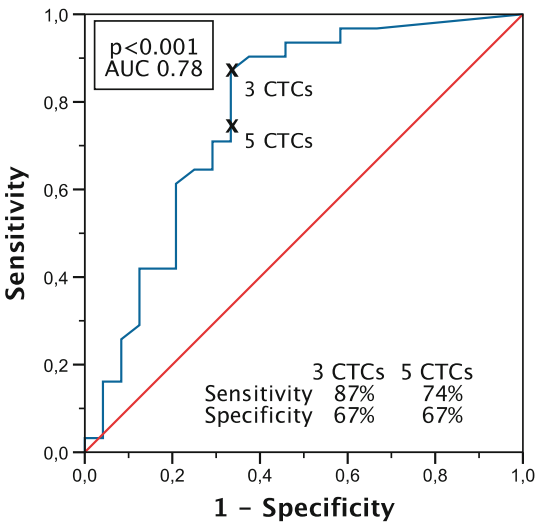

Fig. 3 Receiver operator characteristic *curve* demonstrating the significance and specificity for the cutoff values of ≥3 CTCs and ≥5 CTCs predictive for survival in metastatic prostate cancer

blood in the continuum of different PC stages. Association analyses to clinical staging parameters were performed concomitantly. The prognostic value of CTCs to predict metastatic PC was assessed, and threshold analyses for OS were performed.

In our study, patients with a LAPC displayed CTC counts at control level although the included patients presented a stage on the crossover to metastatic disease likely to harbor clinically unapparent metastases. A systematic operation error, resulting in negative results, can be excluded since assay controls were performed. Our results are similar to a larger series that demonstrated equal CTC counts in controls and in patients with localized PC (Davis et al. 2008). Thus, we and others could not confirm initial published data that demonstrated significant elevated CTC counts in localized PC (Moreno et al. 2001). In summary, the CSS seems not applicable for staging or diagnosis in non-metastatic PC. Whether the presence of CTCs in patients with non-metastatic PC is stochastic or rather predictive for survival and disease recurrence, as demonstrated for

localized bladder cancer, remains to be evaluated by larger prospective series (Rink et al. 2012). The low detection rate of CTCs in localized PC might be due to the selectively targeting of EpCAM, an adhesion molecule possibly underlying perdition during the epithelial to mesenchymal transition (EMT). Since a certain subpopulation of CTCs is characterized by absence of epithelial-specific markers, reported CTC counts are underestimated. Another pitfall of the CSS might be the low blood volume analyzed, although a high recovery rate, specificity and reproducibility were reported (Sun et al. 2011). The CSS is currently the only FDA-approved device for CTC detection although an abundance of new innovative different techniques for CTC detection and identification is under investigation including morphological and immunological approaches as well as nucleic acid- and cytometric-based methods. Nevertheless, these detection methods are still partly hampered by poor reproducibility and specificity (Sun et al. 2011; Panteleakou et al. 2009).

Focusing on mPC patients CTC counts were significantly increased compared to patients with LAPC. A tendency for increased CTC counts and detection rates was identified in metastatic taxane-refractory PC patients (mTRPC) compared to metastatic castration refractory patients (mCRPC), but significance was missed. Similar earlier reports demonstrated a correlation between increasing CTC counts and the number of cytotoxic therapy regimens (Danila et al. 2007; Goodman et al. 2009).

Evaluating CTC counts in dependency of the metastatic pattern, patients with bone and visceral metastases as well as patients with bone metastases presented significant higher CTC counts than patients with only soft tissue involvement. The latter displayed even no statistical difference to the control level. The low detection rate in patients with soft tissue involvement only is in accordance with the analysis of Goodman and co-workers (Goodman et al. 2009). Bone metastases seem to be preconditious for the occurrence of CTCs in several trials (Chen et al. 2005; Danila et al. 2007; Scher et al. 2009; Olmos et al. 2009; Goodman et al. 2009). Thus, in clinical practice, treatment guidance with CTCs seems to be especially applicable in mPC patients with bone metastases either with or without additional visceral metastases. The distribution of CTC counts and their prognostic value in dependency of the metastatic pattern is in accordance with a linear trend analysis for OS demonstrating the shortest OS in patients with bone and visceral metastases followed by bone metastatic and finally by soft tissue metastatic patients.

Despite the observed statistically significant dependency of high CTC numbers with the presence of bone metastases, no correlation of CTC counts with osseous tumor burden, assessed by the BSI or BLC, was found. To our knowledge, the association of CTCs to BLC was not described in the literature up to date. With respect to BSI, Danila and co-workers confirmed our data, demonstrating only a modest correlation with CTCs (Danila et al. 2007). In consequence, elevated CTC counts predict with high significance the presence of bone metastases but not the osseous tumor burden.

Association analyses of CTC counts with routine laboratory results showed a significant positive correlation of increased CTC counts with high AP and LDH levels as well as a negative correlation with hemoglobin levels. Our results in mPC patients are confirmed by earlier reports (Chen et al. 2005; Goodman et al. 2009). Focusing on the absolute PSA level, no correlation was found for CTC counts which is in accordance with Danila et al. who demonstrated only a modest correlation without significance (Danila et al. 2007). In contrast, studies by Chen or Goodman demonstrated a significant correlation of CTC counts with absolute PSA values (Chen et al. 2005; Goodman et al. 2009). These controversial results need further evaluation in randomized trials. Despite a missing correlation with the absolute PSA value, a significant negative correlation with the PSA-doubling time (PSA-DT) was found in our cohort. To our knowledge, the association of CTCs with PSA-DT is demonstrated for the first time. As a consequence for clinical practice, patients with high PSA-DT, AP and LDH levels as well as low levels for hemoglobin, in combination with elevated CTC counts, are at high risk of disease progression.

In mPC, the presence of CTCs is of prognostic significance for survival in multivariate analyses (Goodman et al. 2009; Okegawa et al. 2009; Olmos et al. 2009). Although CTCs were suggested as a continuous variable correlating with an increased risk of death (Danila et al. 2007; Scher et al. 2009), a threshold of $\geq$5 CTCs per 7.5 ml was established leading to FDA approval of the CSS in a mixed cohort of patients receiving several lines of chemotherapy (De Bono et al. 2008).

Our study confirms a significant association of CTC counts with survival. Analyzing the optimized sensitivity and specificity of a CTC threshold to predict OS in our cohort of metastatic patients, a cutoff value of $\geq$3 CTCs was found to be most predictive. Patients with $\geq$3 CTCs showed a significant shorter median OS of 178 days compared to patients with <3 CTCs (median OS not defined). Interestingly, the earlier described threshold of $\geq$5 CTCs was also applicable but demonstrated lower sensitivity. Analyzing the prognostic relevance of the CTC threshold for OS in the mCRPC and mTRPC subgroups, the threshold of $\geq$3 as well as $\geq$5 CTCs was statistically significant in mCRPC patients, while none of them was valid in the mTRPC group. Our results might be attributed to the small patient cohort and have to be taken with some caution. Nevertheless, our findings suggest a continuous

threshold function of CTC counts for OS in mPC confirming a study by Goodman et al. who demonstrated the variability between 2 and 5 CTCs as cutoff value in castration refractory patients, with an optimum of 4 CTCs (Goodman et al. 2009).

In conclusion, CTC counts are applicable as a prognostic molecular marker, especially in mCRPC patients harboring bone metastases with or without additional visceral metastases. In contrast, the prognostic benefit of CTC counts in patients with only soft tissue metastases or in progressive mTRPC patients seems to be limited. In LAPC patients, the detection of CTCs with the CSS is not applicable. Notably, CTC counts showed no correlation with bone tumor burden, but a significant association with PSA-DT, thus possibly reflecting the intrinsic tumor activity. Despite the prognostic value of CTCs, the medical need is to define a predictive marker for treatment efficacy of systemic therapies. Therefore, our group is planning a randomized trial with CTCs and additional molecular markers in order to validate a new diagnostic tool for treatment guidance.

Acknowledgments We thank Medistat GmbH, Kiel, Germany, for the statistical analyses. The work was supported by Sanofi-Aventis, Frankfurt, Germany, and scientific grants from Siegfried Gruber-Foundation, Munich, Germany.

Conflict of interest No actual or potential conflict of interest in relation to this article to declare.

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