

Circulating Tumor Cell Number and Prognosis in Progressive Castration-Resistant Prostate Cancer

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Abstract **Purpose:** The development of tumor-specific markers to select targeted therapies and to assess clinical outcome remains a significant area of unmet need. We evaluated the association of baseline circulating tumor cell (CTC) number with clinical characteristics and survival in patients with castrate metastatic disease considered for different hormonal and cytotoxic therapies. **Experimental Design:** CTC were isolated by immunomagnetic capture from 7.5-mL samples of blood from 120 patients with progressive clinical castrate metastatic disease. We estimated the probability of survival over time by the Kaplan-Meier method. The concordance probability estimate was used to gauge the discriminatory strength of the informative prognostic factors. **Results:** Sixty-nine (57%) patients had five or more CTC whereas 30 (25%) had two cells or less. Higher CTC numbers were observed in patients with bone metastases relative to those with soft tissue disease and in patients who had received prior cytotoxic chemotherapy relative to those who had not. CTC counts were modestly correlated to measurements of tumor burden such as prostate-specific antigen and bone scan index, reflecting the percentage of boney skeleton involved with tumor. Baseline CTC number was strongly associated with survival, without a threshold effect, which increased further when baseline prostate-specific antigen and albumin were included. **Conclusions:** Baseline CTC was predictive of survival, with no threshold effect. The shedding of cells into the circulation represents an intrinsic property of the tumor, distinct from extent of disease, and provides unique information relative to prognosis.

The ability to detect, isolate, and characterize circulating tumor cells (CTC) in patients with progressive castration-resistant prostate cancer is now a clinical reality (1–4). Studying the molecular features of these cells in patients with progressive disease has the potential to guide treatment selection, assess pharmacodynamic effects, and study mechanisms of resistance

to therapy. In addition, prior studies in patients with metastatic breast, colon, and prostate cancer have shown that CTC enumeration before and after therapy is both prognostic and treatment predictive (5–10). As a result, the routine use of these technologies is likely to increase. Crucial to the optimal development of this biomarker is to understand which patients in the continuum of an illness are most likely to be shedding cells and at what frequency.

In this report, we evaluated CTC number in patients with progressive castration-resistant metastatic prostate cancer who were being considered for different hormonal and cytotoxic therapies. Specifically, we explored the relationship between CTC number and patterns of metastatic spread, along with other measures of disease burden including the level of prostate-specific antigen (PSA) and extent of disease in bone (11). Separately, we describe the strength of association between CTC number and survival, alone and in conjunction with patient clinical characteristics previously incorporated in prognostic nomograms (12–15).

We show that higher CTC numbers were present in patients with bone metastases relative to those with metastases limited to soft tissue sites; and in patients who had progressed after cytotoxic therapy relative to those who had not. Important was that higher cell number was not simply a matter of an increasing disease burden because the associations with baseline PSA and, separately, the extent of bone marrow involvement by tumor were modest. Noteworthy was that the association between baseline CTC number and overall survival

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Table 1. Patient characteristics (n = 120)

Age, y	
Median (range)	69.7 (41-87)
Primary therapy, n (%)	
Radical prostatectomy	33 (28)
Radiation therapy to the prostate	35 (29)
No primary treatment	52 (43)
Prior systemic therapy, n (%)	
Androgen depletion	120
Received first-line chemotherapy	32 (27)
Receiving second-line regimens	65 (54)
Sites of metastases disease, n (%)	
Soft tissue and no bone disease	12 (10)
Bone and soft tissue	67 (56)
Bone only	41 (34)
	Median (interquartile range)
Karnofsky performance status, %	80 (80-90)
Hemoglobin, g/dL	11.7 (10.3-12.6)
Albumin, g/dL	4.1 (3.9-4.4)
Alkaline phosphatase, units/L	107 (71-226.5)
PSA, ng/mL	117 (40.9-399)

did not have a threshold effect, and the discriminatory power of association was increased further by accounting for pretreatment PSA and albumin levels. The results suggest that the ability to shed cells into the circulation is an intrinsic property of the tumor and that it provides unique information, which, when properly applied, can significantly affect patient management and clinical trial design.

Materials and Methods

Study design. Patients with histologically confirmed progressive metastatic prostate cancer and castrate levels of testosterone <50 ng/dL, treated at Memorial Sloan Kettering Cancer Center, were considered. All underwent a history that included details of treatment of the primary tumor at the time of diagnosis and all subsequent systemic therapies. A physical examination, including Karnofsky performance status, and laboratory studies, including complete blood count and chemistry panel (albumin, alkaline phosphatase, and PSA), were also done at the time of CTC draw. Disease status was determined to be progressing following the recommendations of the Prostate Specific Antigen Working Group (16) based on PSA, with a minimum of three increasing levels at least 1 week apart, or by radiographic criteria as new lesions by bone scintigraphy or as new or enlarging soft tissue lesions by computed tomography or magnetic resonance imaging. The study was conducted under Institutional Review Board-approved protocols with informed consent.

To determine the distribution of soft tissue disease, computed tomography and/or magnetic resonance imaging scans were reviewed and scored for the presence of lymph nodes or viscera (liver and/or lung; L.S.). Radionuclide bone scans were evaluated first for the presence or absence of metastatic bone disease. For those with metastases, extent of disease was estimated using the bone scan index, which assesses the proportion of the bony skeleton involved by tumor. The latter was done by an independent and blinded review of baseline bone scans (S.L.; ref. 11).

CTC counts. Blood samples for CTC counts were drawn from patients with progressing disease before the start of the new chemotherapy regimen. CTC number was determined in the Memorial Sloan-Kettering Clinical Laboratories as previously described (3). In brief, one 7.5-mL sample of blood was collected into a CellSave tube containing cell preservatives (Immunicon). Using immunomagnetic

isolation, epithelial cells were captured based on expression of epithelial cellular adhesion molecule with CellTracks Autoprep (17, 18). Enriched epithelial cells were identified by immunofluorescence staining with Cell Track Analyzer II. Cells were scored as CTC when 4',6-diamidino-2-phenylindole-stained nucleated cells expressed cytokeratin, excluding WBC contamination by negative selection with CD-45 staining. Automatically selected images were reviewed by the operator for identification. Quality controls were maintained via standard procedures. The CellSearch System is available from Veridex LLC.

Statistics. The Kaplan-Meier method was used to estimate the probability of survival over time. Univariate comparisons were based on the score test derived from the Cox proportional hazards model. The hazard function for the survival time t of the i th subject with covariate vector z_i is assumed, under the proportional hazards specification, to have the form

$$h_i(t) = g(t)\exp(\beta z_i)$$

where β is the vector of regression coefficients to be estimated and $g(t)$ is the baseline hazard function. To depict the relationship between survival time and the continuous covariates PSA and CTC, a smoothed Kaplan-Meier estimate of the median survival conditional on the continuous covariate was generated (19). This approach avoids the arbitrary grouping of a continuous covariate (e.g., CTC ≤ 5 and CTC > 5) and enables the estimation of the median survival time for any CTC value.

To assess the discriminatory power of the baseline factors on survival, the factors were jointly entered into a proportional hazards model and the concordance probability estimate (CPE) was computed (20). The CPE measures the level of concordance between the survival time and the prognostic index based on the linear combination of covariates βz_i in the Cox model. A strong concordance would indicate that the baseline factors in the Cox model are highly informative in understanding the relative risk of death between any two patients at time t . The concordance estimate ranges between 0.5 and 1.0, with 1.0 representing perfect concordance between the prognostic index and survival time and an estimate of 0.5 representing no relationship between the prognostic index and survival time. Kendall's τ was used to assess the level of association between CTC and markers of tumor burden.

Results

Clinical characteristics of the patient population. The clinical characteristics of the 120 patients evaluated are detailed in Table 1. Sixty-eight (57%) had received prior treatment with curative intent for localized prostate cancer by radical surgery (33 cases) or radiation therapy (35 cases), whereas 52 (43%) had metastatic disease at presentation. The patterns of metastatic spread included disease limited to soft tissue with no bone disease in 12 (10%) patients, in bone and soft tissue in 67 (56%), and in bone only in 41 (34%). Overall, 23 (19%) had CTC drawn after failing only hormonal manipulations, whereas 65 progressed after second-line chemotherapy regimens. The

Table 2. CTC number in patients with progressive metastatic castration-resistant disease

CTC no.	n.a.	0-2	3-4	5-9	10-50	≥ 51
No. patients	8	30	13	15	25	29
Percent	(7)	(25)	(11)	(12)	(21)	(24)

Abbreviation: n.a., not available because of uninterpretable data.

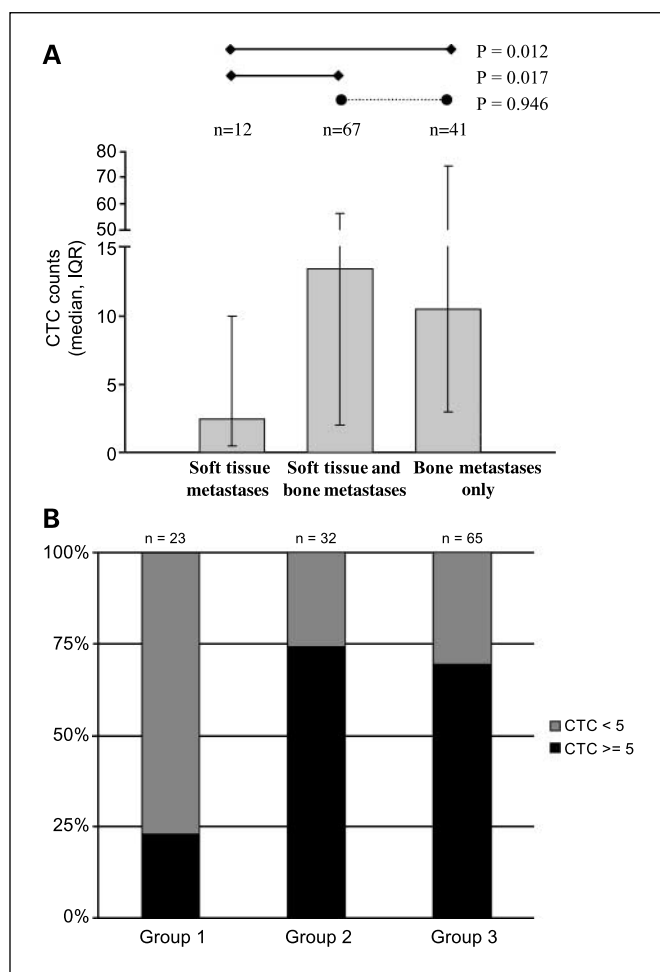


Fig. 1. CTC number in progressive castrate metastatic disease. *A*, CTC by metastasis site. *B*, CTC by treatment. Group 1, patients received noncytotoxic therapy; Group 2, patients are about to receive first-line chemotherapy; Group 3, patients progressed on one or more cytotoxic therapies.

baseline Karnofsky performance status, hemoglobin, albumin, alkaline phosphatase, and PSA are listed by median and interquartile ranges.

CTC counts. In this cohort of 120 patients, 112 had evaluable CTC counts, with a median of 9 cells/7.5 mL of blood. The distribution of CTC counts is shown in Table 2. Sixty-nine (57%) patients had five or more CTC, whereas 30 (25%) had cell counts of two or less. Samples in 8 (7%) patients failed to produce an interpretable result. This was due to an insufficient amount of blood or an error in the processing of the sample.

Associations with disease distribution and disease extent. Higher CTC numbers were detected in patients with bone metastasis alone (median, 10.5 cells) or with bone and soft tissue involvement (median, 13.5 cells) relative to those with soft tissue only disease (median, 2.5 cells; Fig. 1A). Patients treated with noncytotoxic therapies had a significantly lower median CTC number of 2.5 (interquartile range, 1, 4) than those about to receive first line (median, 10; interquartile range, 4.25, 47.75) or second line (median, 20; interquartile range, 3, 91) of cytotoxic agents. The distribution of patients with five or more CTC by treatment group is shown in red in Fig. 1B and those

patients with four or fewer CTC are represented in blue. In the group receiving second-line therapy, 58% of patients had 10 or more cells. As expected, higher CTC numbers were found in patients failing multiple chemotherapeutic regimens.

The median bone scan index, a measure of percent of bony skeletal involvement with tumor, was 4.61 (interquartile range, 1.56, 18.35). Unexpected was the modest correlation between CTC and extent of disease as assessed by PSA (Kendall's $\tau = 0.32$; Fig. 2A) and bone scan index (Kendall's $\tau = 0.35$; Fig. 2B).

Associations with survival. The median survival time for all patients was 13.2 months (95% confidence interval, 10.5-16.2 months; Fig. 3A). At the time of analysis, 58 of the 120 (48%) patients remained alive. In univariate analysis, both baseline CTC number and PSA level were strongly associated with survival from the time of the blood draw ($P < 0.001$; Table 3A). The risk of death increased with higher CTC number and higher PSA level. As a result, the relationship between CTC (Fig. 3B) or PSA (Fig. 3C) and median survival time was produced as a continuous function using nonparametric smoothing. As shown, there was no threshold effect for either CTC or PSA and survival. In addition to CTC and PSA, the univariate tests of association showed that Karnofsky performance status, number of prior cytotoxic therapies, as well as baseline hemoglobin, albumin, alkaline phosphatase, and bone scan index were all

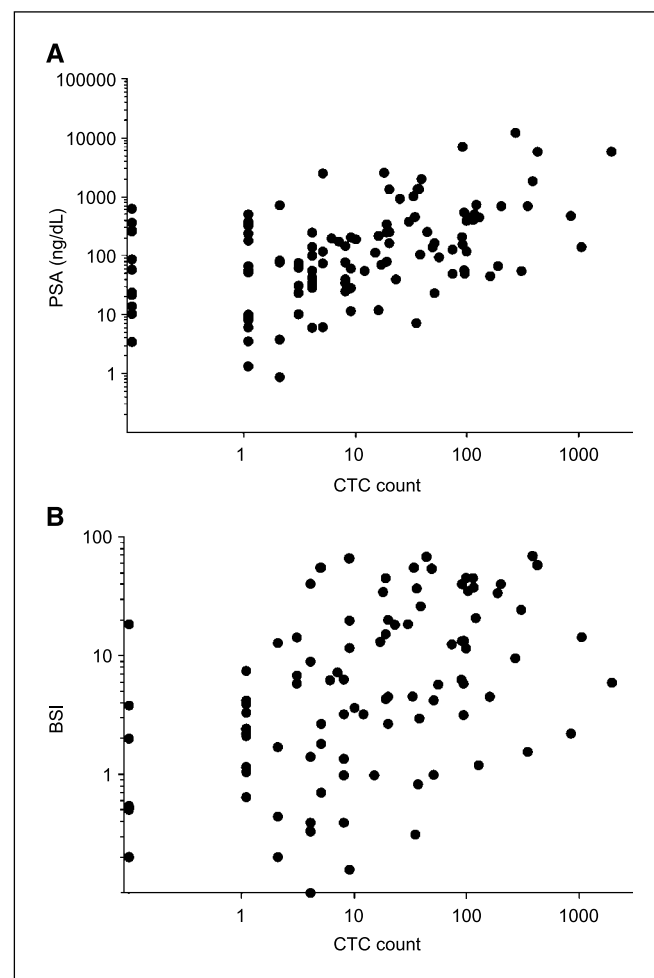


Fig. 2. CTC count relationship with markers of tumor burden. Association of CTC with PSA (Kendall's $\tau = 0.32$; *A*) and bone scan index (*BSI*; Kendall's $\tau = 0.35$; *B*).

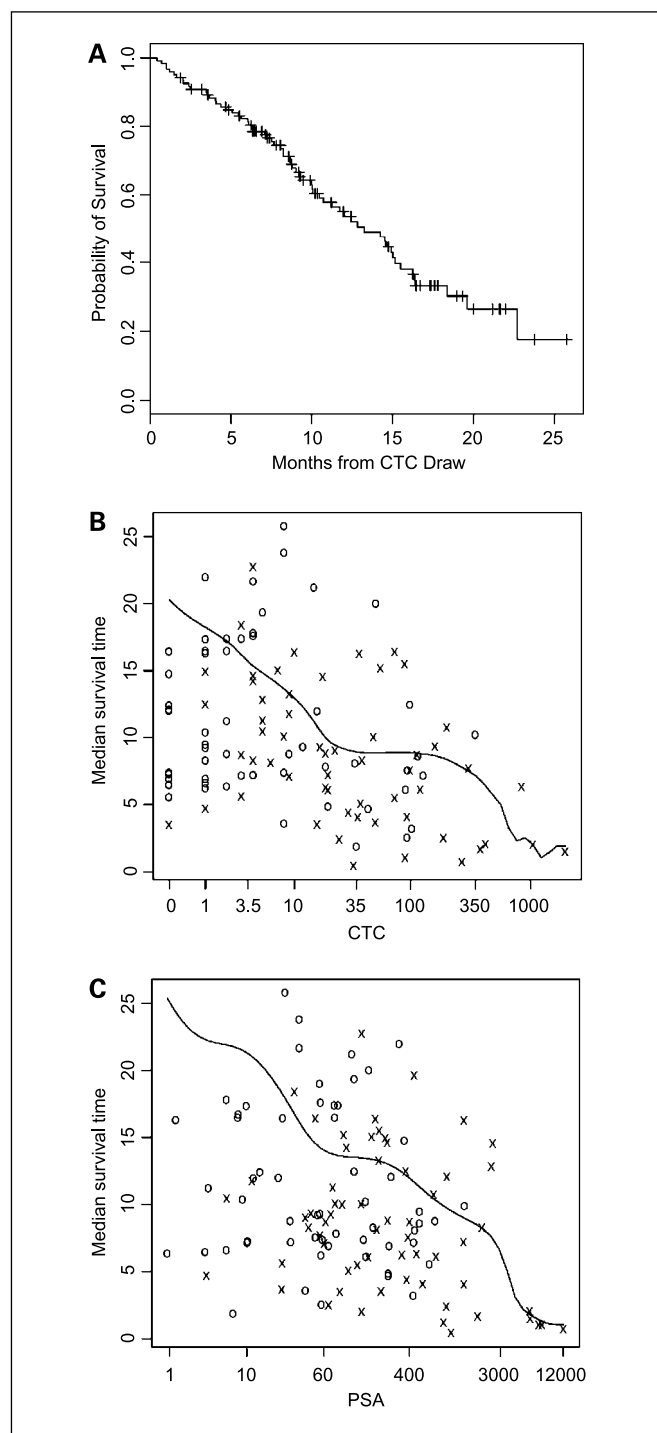


Fig. 3. Survival data. Kaplan-Meier estimate of survival, calculated from time of CTC draw (A). Estimated median survival time based on baseline CTC counts (B) and PSA (C). The symbol (x) represents death times and (o) represents the last follow-up times.

strongly associated with survival time. Gleason score at the time of diagnosis was not associated with survival time (Table 3A). In the group of patients receiving noncytotoxic therapies, CTC counts of five or more had a significantly lower median survival (10.4 months; $n = 28$) compared with those with four cells or less (median, 18.4 months; $n = 25$). Patients who progressed on one or more cytotoxic therapies with five or fewer cells detected

had a median survival of 22.7 ($n = 18$) months, and 9 months if five or more cells were detected ($n = 41$). CTC results were not available in two patients in the first group and in six patients in the second group.

To determine the subset of factors that provided independent information on survival time, a Cox proportional hazards model was developed. The predictive strength of the Cox model was assessed using the CPE (Table 3B). The factors that jointly produced the strongest association with survival time were CTC, PSA, and albumin ($P < 0.01$ for each factor). The model indicated that the risk of death increased with higher CTC number and PSA level and lower albumin level. Considering all three variables, the Cox model produced a CPE equal to 0.779 (SE, 0.024). Noting that the concordance metric ranges between 0.50 and 1.00, the proposed model presents a moderately high ability to discriminate between patient survival times based on their CTC, PSA, and albumin levels.

Eight subjects had missing CTC values. To determine if these missing values had an effect on our results, a sensitivity analysis was done. The missing CTC values were replaced with either the minimum CTC value observed (0) or the maximum CTC value observed (1958). In each of these additional analyses, the three factors found in the multivariate Cox model (CTC, PSA, and albumin) remained strongly associated with survival time and the lower bound for the CPE in these analyses was 0.754. Thus, the sensitivity analysis showed that the missing CTC values had minimal effect on the association analysis with survival time.

Discussion

Our study provides additional evidence of the importance of CTC number, determined in a clinical laboratory setting, as a prognostic biomarker in patients with progressive castration-resistant disease (21). The difference in the frequency of CTC shedding in patients with bone relative to soft tissue disease and the modest association between CTC number and PSA and bone scan index show that the information provided is distinct and not simply related to an increased disease burden. This,

Table 3. Association between baseline characteristics and survival

(A) Univariate analysis

Factor	P
Karnofsky performance status	<0.001
Gleason score	0.990
Line of chemotherapy	0.003
Hemoglobin	<0.001
Albumin	<0.001
Log (alkaline phosphatase)	<0.001
Bone scan index	0.002
Log (PSA)	<0.001
Log (CTC)	<0.001

(B) CPE

Factor	CPE (SE)
CTC	0.711 (0.027)
PSA	0.701 (0.029)
CTC + PSA	0.747 (0.026)
CTC + PSA + albumin	0.779 (0.024)

together with the strong association between baseline CTC and survival, without a threshold effect, shows that this biomarker reflects the intrinsic biology of the tumor.

Higher numbers of CTC were detected in patients with bone metastases relative to those with no osseous spread, although the number of patients was small. This is consistent with the known routes of spread by which osseous sites are seeded hematogenously and those in soft tissue disease predominantly via a lymphatic route. The finding, which will need confirmation in larger cohorts, supports the recent Prostate Cancer Clinical Trials Working Group 2 report that, for the first time, defined distinct clinical subtypes based on pattern of spread, suggesting that each may be uniquely sensitive or resistant to a particular type of therapy (22).

Not surprisingly, higher CTC numbers were observed in patients with later state (postchemotherapy) as opposed to those treated in a prechemotherapy setting. This does not simply reflect an increasing disease burden but more of an intrinsic property of the tumor because, within each clinical setting, only a proportion of patients had high cell numbers isolated. It may also be a reflection of the clinical impression of the treating physician that the patient's disease progression has worsened to the point that a more aggressive cytotoxic approach was required. In this regard, it is of note that the treating physicians were blinded to the CTC results. Alternatively, the higher frequency of cell shedding postchemotherapy may be secondary to the cytotoxic treatment itself, representing partially damaged, yet viable, tumor cells.

More significant was the power of the baseline CTC to discriminate between low and high survival times (CPE, 0.71) and the increase of this power with the addition of baseline PSA and albumin (CPE, 0.78). Previous work by others in breast (6, 9) and colorectal (5) cancers and by ourselves and others in castration-resistant prostate cancer (8, 10, 23) have shown its prognostic importance without quantitation of its

predictive strength. Although it is a factor in a prognostic nomogram (13), baseline Gleason score was not predictive of overall survival in our study. In addition, median survival time was best explained as a continuum function of shedding without a clear threshold. This argues against an arbitrary dichotomous interpretation of CTC numbers in prediction of survival.

Patient-tailored therapy requires the ability to identify the putative target of interest in a sample that reflects the patient's tumor at the time treatment is being considered. Ideally, the sample can be obtained easily and assessed reproducibly, quantitatively, and with a rapid turnover in a clinical laboratory setting, so that it can be used for patient management. CTC number has the potential to fulfill this important unmet need for a significant proportion of patients. In addition to providing pretreatment prognostic information, specific biomarkers can be characterized in CTC at the DNA, RNA, and protein levels (1, 3, 18, 24–31). As one example, we have set a cutoff of 10 or more CTC in a patient sample as the minimum to be characterized by fluorescence *in situ* hybridization. In this extended cohort, 58% of patients receiving second-line chemotherapy had 10 or more cells at baseline. This cutoff, and any future determinant, will need validation and prospective evaluation in discrete clinical contexts evaluating specific drugs. The Oncology Biomarker Qualification Initiative provides a road map for these investigations, which, if followed, will facilitate the incorporation of these types of assays into clinical decision making. Also important, the road map will allow the description and identification of additional markers for those patients with tumors that do not shed cells into the circulation.

Prospective studies, designed around the biomarker itself and the specific clinical context for which it is applied, will need to be conducted to assess the role of these and future markers for pretreatment stratification in large-scale trials.

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