RESEARCH COMMUNICATION

A Higher Number of Circulating Tumor Cells (CTC) in Peripheral Blood Indicates Poor Prognosis in Prostate Cancer Patients - A Meta-analysis

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Abstract

Objective: The prevalence of prostate cancer (PCa) is high and PCa is the most common cutaneous cancer in men worldwide. Despite extensive research efforts, very few biomarkers of PCa have been introduced to date in clinical practice. A meta-analysis was performed on the most recently reported CTC to assess its prognostic effect and to elucidate whether its detection in the peripheral blood of patients diagnosed with metastatic, castration-resistant prostate cancer (CPRC) and Hormone Refractory Prostate Cancer (HRPC) can be used as a prognostic factor for survival. Methods: We searched Science Direct, EMBASE, PubMed, and Cell Research databases for studies that assessed the prognostic relevance of the presence number of circulating tumor cells (CTC) detection in the peripheral blood (PB). A fixed effects model with relative risk (RR) and 95% confidence interval (95% CI) is used for analysis. Results: A total of 4 studies, including 486 patients, were eligible for final analysis. Pooled analysis indicated the presence number of CTC per 7.5 ml peripheral blood is associated with a poor survival rate (RR=2.51, 95% CI 1.96-3.21). Conclusion: The unfavorable count (presence of 5 or more CTCs per 7.5 ml peripheral blood) was associated with poor overall survival in patients with PCa. CTC counts can be used as an accurate and independent predictor of survival rate in patients with PCa.

Keywords: Circulating tumor cells - prostate cancer - prognosis - meta-analysis

Asian Pacific J Cancer Prev, **12**, 2629-2635

Introduction

The first descriptions of tumor cells with solid malignancies in the peripheral circulation of patients date from the 19th century (Ashworth, 1999). In old literature, these cells were frequently referred to as"carcinocythemia"(Carey et al.,1976), but they are now commonly known as circulating tumor cells (CTCs). Epithelial cancers initially arise as an organ-confined lesion, but eventually spread to distant sites (eg. lung, liver, bone, and/or brain) through the bloodstream, generating metastases that are mainly responsible for their lethality. The mechanism of how human tumor cells exit from their primary site and intravasate into the vasculature and distal organs is not well understood. Information gained from CTC characterization is likely to be useful for exploring mechanisms that underlie metastases and drug sensitivity (Sleijfer et al., 2007).

Isolation and enumeration of CTC have been reported by several groups, but controversy exists on the optimal approach to enumerate them. Only the cell search assay (Immunicon, Huntingdon, PA) is approved by the Food and Drug Administration (FDA). The primary studies established that CTC can be used as a tool of diagnosis in various cancers (Stewart et al., 1995; Ignatiadis et al., 2008; Shen et al., 2009; Mudan et al., 2010) or as a marker of metastatic breast cancer (Berruti et al., 2005; Lobodasch et al., 2007). Unfortunately, the lack of data and the inconsistent study design and results limit their individual clinical value, and the prognostic effects of CTC presence in patients with PCa in fact therefore remains debatable.

A recently published combined analysis showed that recurrence of CTC was associated with poor outcome in patients with metastatic breast cancer (Cristofanilli et al., 2004; Budd et al., 2006). Nevertheless, a pooled analysis of available studies that will provide a more precise estimate on the prognostic effect of detectable unfavorable numbers (>5 CTC/7.5ml PB) of CTC in blood stream in patients with PCa has not yet been performed.

The aim of this study is to use a meta-analytic approach to clarify whether the detection CTC in the peripheral blood of patients diagnosed with prostate cancer can be used as a prognostic factor.

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Materials and Methods

Search strategy and selection criteria

We systematically searched (through March 2010) in the following databases: PubMed, Science Direct, EMBASE, and Cell Research. Key words used were: "circulating tumor cell(s)," "prognosis," "prostate cancer," with limits of "cohort" and "10 years." Table1 shows the detailed search strategies used and results found in such databases respectively. To browse the identified articles, the accepted detection method is immunologic, immunocytochemistry and flow cytometric detection techniques.

Two reviewers screened the literature using the following exclusion criteria: less than 30 patients analyzed; insufficient original data to calculate the relative risk (RR),used to evaluate the overall survival (OS) statement ; patients were not divided into favorable and unfavorable units to compare the different prognosis appearance ; the studies focused on the several special antigens or over-expressed proteins instead of CTC to make indirect judgment on the prognosis effect. Sample sizes for survival analysis were estimated under the assumption of 50% median OS.

Data extraction

Two reviewers extracted descriptive and quantitative information from each article: first author, publication year, tested sample consistent, patient and cancer characteristics such as cancer stage, age, treatment formula (ie. resistant or unresistant to the hormone), tracking range, number of patients in the favorable (<5CTCs/7.5ml PB) and unfavorable (>5CTCs/7.5ml PB) groups, and the 2- or 5- year survival rate and RR. In all these studies using the cytometric approach (which hinges upon visualization of intact CTC, as opposed to PCR-based methods where normal peripheral blood mononuclear cell and potentially contaminating CTC are lysed to extract the genetic material and assess the expression of tumor related genes), immunomagnetic cell enrichment expressing the epithelial cell adhesion molecule and fluorescently label all nuclei with 4 and light microscopy was used to recognize CTC by morphology only. Disagreements were resolved by discussion.

Literature quality evaluation

To assess the quality of the retrieved studies, we evaluated the articles based on the principles of evidence-based medicine of validity, importance, and applicability. The quality judgment of each study was based on the six principles cited from international clinical epidemiology. Firstly to explore whether the studies put the characteristics of the research subjects in a detailed description and whether the sample was representative. In the retrieved studies, research objectives were defined accurately and subjects complied with inclusion and exclusion criteria. Secondly, to verify whether the subjects were all in the similar stage of the disease course and the starting point clearly stated. The starting point was mostly reported in all 4 studies, including the time after when the patients with biochemically or histologically confirmed PCa were included (progressive metastatic PCa) and at early stage of testosterone treated. Because following the patients at early stage is better for the cohort design. Thirdly, to ascertain whether the tracking time was adequate and reported for all the patients. It is necessary that articles report the time to observed disease progression throughout the observation period, calculate the various kinds of outcomes, and to analyze why some patients were lost or withdrawn during the follow-up period. The most recommended method is to lock on each patient in the cohort until recovery, death, or transfer to other diseases. However, in practice, the longer the tracking time, the more likely the patient drops out. As a result, one study did not follow up on all patients. The method to test the degree of withdrawal or lost of following is to calculate the failed following rate. If the rate was over 5%, it threatens the facticity of the result and further investigation of the withdrawal is necessary. However, the default rate may not link with prognosis. Fourthly, to inspect whether the object end point index was adopted, which could improve physician understanding and judgment that in turn reduces inconsistency on outcomes. In our review, the 4 articles used death as the end point, which eliminated the possibility of diagnostic suspicious bias and expectation bias. Finally, to estimate whether the designers of these studies took into account other factors that influence the prognostic result (Table 4).

Statistical Methods

To statistically estimate the prognostic outcomes of CTCs, we extracted RRs and their associated standard errors (SE) on OS from the included studies. All of the studies offer RR without the 95% CI (confidence interval), which was calculated using the SE (Microsoft Excel 2007, Windows XP, Microsoft, Redmond, WA). All analyses were performed using a statistical package (SPSS, SPSS Inc., Chicago, IL, version 13.0).Values of 95% CI were used for all analyses. Significance was set at $p<0.05$.

In accordance with the evidence-based medicine, RR is a prognosis index for hazard judgment. RR=1 demonstrate analysis factor is unconcerned with prognosis; RR>1 indicates analysis factor is harmful to prognosis outcomes; RR<1 manifests analysis factor own protective affect on prognosis. In our context, RR>1 implies a poor prognosis in the unfavorable group in comparison to the favorable

Table 1. Key Words and Other Details for the Search

Database (Host)	Time span	Key words	No. of citations in database
Science direct	2010.3.17-22	CTC, prognosis, cohort, prostate cancer	147
Science direct	2010.3.15-23	CTC, prognosis, cohort, breast cancer	229
Pubmed	2010.3.20-26	CTC, prognosis, prostate, limit in 10 years and full text	25
EMBASE	2010.3.26-30	CTC, radical prostaectomy, prognosis and cohort	126

Table 2. Baseline Characteristics ofthe Included Studies

Reference	Detection method	Target antigen	Tracking months	No .of patients		Survival rate after 2 / 5 years		Hazord risk(HR)
				Favorable*	Unfavorable*	Favorable*	Unfavorable*	
Moreno et al., 2005	Immunomagnetically	CD45	More than 48 mo	14	23	85%	28%	7.37
de Beno et al., 2008	Immunomagnetically	CD45	30 mo	100	131	49%	21%	4.5
Danila et al ., 2007	Immunomagnetically	CD45	25 mo	30	69	46.6%	14.5%	Not given
Olmos et al ,. 2009	Immunomagnetically	CD45	More than 36 mo	59	60	71%	30%	3.25

*<5CTC/7.5ml PB

Table 3. Overview of the Study Design Variables

Reference	Year of issued	No. of patients	Age(mean/median)	Sample site	Types of PCa
Moreno et al .,2005	2005	37	(64-84) 75	PB	Matastatic PCa
de Beno et al., 2008	2008	231	(61-79) 70	PB	Castration resistant PCa
Danila et al ., 2007	2007	120	NR (41-87)	PB	Castration resistant PCa
Olmos et al., 2009	2009	119	NR 67.5	PB	Castration resistant PCa

Table 4. Assessment of Risk of Bias

Reference	A	B	C	D	E	F
Moreno et al .,2005	Yes	Confirmed before CTC evaluation	Yes	Yes	No	Yes
de Beno et al .,2008	Yes	Confirmed before chemotherapy	Yes	Yes	No	Yes
Danila et al., 2007	Yes	Confirmed and castrate levels of testosterone <50 ng/dl	Yes	Yes	No	Yes
Olmos et al., 2009	Yes	Confirmed and castrate levels of testosterone <50 ng/dl	Yes	Yes	No	Yes

A, Were the patients selected properly? Was the sample representative and unbiased? B, Were the samples collected in the same stage of the disease course? C, Was the follow-up complete and adequate in duration? D, Adopted an objective prognostic indicators? E, Was blindness apply in resulting judgment? F, Was confounding adequately controlled for? whether correction other factors which affect the prognosis?

group. We pooled the extracted RRs with the use of the generic inverse variance method available in the Revman 4.2 software (Copenhagen: The Nordic Cochrane Centre; The Cochrane Collaboration, 2008). Then we applied the fixed effect model and use I2 statistics instead of sampling error to describing the proportion of total variation attributable to differences among these studies.

Sensitivity analysis, Assessment of Risk of Bias and Publication Bias

The aim of analyzing the sensitivity is to test the stability and reliability of the conclusion, the specific measure is re-analyzing the studies to inspect the changes after excluding some studies or information. Because all of the systemic reviews involved in the subjective judgments, the sensitivity analysis attempts to find how the variety of subjective assessments influence the review conclusion. We performed sensitivity analysis by excluding one study with biggest test population>200, (RR=2.42, 95% CI 1.86 -3.15, n=3). Funnel plot analyses were used to evaluate publication bias (Egger et al., 1997). The publication bias is due to the fact that positive results are easier to issue than negative results, and some published studies contain small test populations but show dramatic results. The publication bias contributes to unreliable conclusions and causes improper direction in clinical practice. The RevMan 4.2-generated funnel plot (Figure 3) shows there is no public bias in these four studies.

Heterogeneity evaluation

Based on the statistical principle, combining varied data to make a statistical analysis should be dependent on homogeneity studies. Using the Chi square test, P>0.1 indicates the studies are homogenous, hence we choose the fixed effect model. P=0.1 indicates heterogeneity in the data; hence the random effect model is applicable.

Results

Baseline study characteristics

The whole literature search yielded a sum of 4 studies comprising 486 patients for final analysis (Figure 1).The sample size of the included studies ranged from 37 to 231 with a median sample size of 121. The studies were conducted in two countries (United States and United Kingdom) and issued between 2004 and 2008. All 4 studies applied the Cell Search System to detect the tumor cells by testing for anti-cytokeratin antibodies. The patients from all the studies were divided into an unfavorable group and a favorable group based on the numbers of CTC in peripheral blood >5CTC in 7.5ml

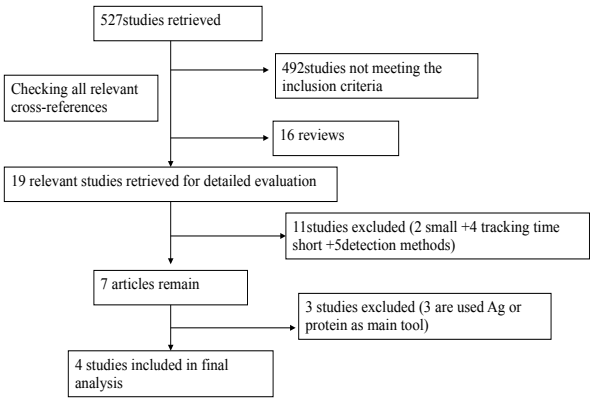

Figure 1. Selection of Studies

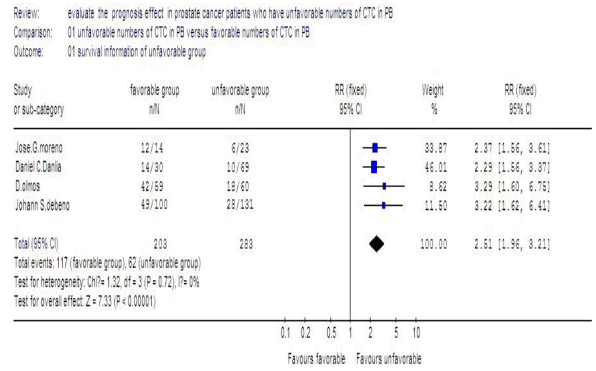

Figure 2. Summary Estimates of Relative Risk (RR) for Prognosis Outcomes Associated with CTCs Detection in the Peripheral Blood of Prostate Cancer Patients

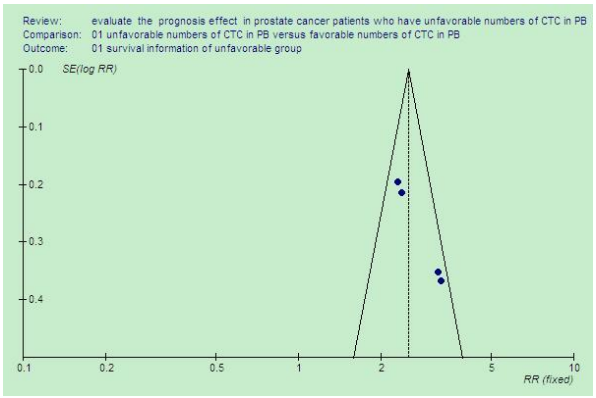

Figure 3. Funnel Plot

PB and <5CTC in 7.5ml PB, respectively). The baseline characteristics of included studies are summarized in Table 2, the study design variables are presented in Table 3, and the assessment of retrieved studies quality in Table 4. Analysis of CTC/DTC detection as prognostic factor was confirmed in univariate and multivariate analyses in all 4 studies, and the OS and hazard ratios (HR) were retrieved. Calculation of the fail-save number showed that there was no relevant publication bias.

Overall analysis

The meta-analysis of all studies yielded a lower OS in the unfavorable group than the favorable group, and the unfavorable group is associated with worse outcome (RR =2.51, 95% CI 1.96-3.21) (Figure 2). To avoid intra-study patient redundancies, study arms that could not be combined were prioritized and described in "Materials and Methods." Sensitivity analyses by systematically removing the study with the largest patient sample size (de Bono et al., 2008) changed this result marginally (lowest effect: RR=3.25, 95% CI 2.01-5.24, n =26, I2 =78%). The test for heterogeneity was insignificant (P>0.10), implying that the included study populations are statistically homogeneous and were suitable for the fixed effect model, which assumes that all the studies share the same common prognostic effect.

CTC prognostic value

Correlation with survival

Survival analysis according to CTC status was done in all these 4 studies. The mean follow up was 42.5 months (range 36- 49 months). All of them used the overall survival time as the evaluating index for the prognosis assess. The mean of the median survival time in all 4 unfavorable groups was 12.5 months (range 8.4-19.5 months), compared to the favorable group of 29.5 months (range 21.7-48 months). The mean of 2 years survival rate of the former group is 23.4% (range 14.5%-30%), and for the later group is 62.9% (range 46.6%-85%).

Correlation with disease stage

To investigate whether other factors influence the conclusion, all 4 articles are included the Cox model to test the prognostic effect of CTC. Cox proportional hazards regression was used to determine univariate and multivariate hazards ratios for OS. Based on the multivariate analysis, RR is applied as a useful index to reflect the mono value in the patients of unfavorable number CTC in PB. However, among these relative factors, the Biopsy Gleason score (BGS) and baseline lactate dehydrogenase (LDH) have a comparatively powerful effect on the final outcomes and prognosis judgment. So all researchers adopted univariate test to calculate the RR of any other related factors especial the LDH and Biopsy Gleason score, the HR of BGS =1.67 and P value<0.05 in Moreno's research (Moreno et al.,2005) ; the HR of LDH=1.0 and P<0.001 between the positive and negative group in de Bono's research (de Bono et al., 2008) whereas the P=0.990 in Danlia's research (Danlia et al.,2007); the HR of LDH=2.24 and P<0.0001 between the normal and elevated group in Olmos' research (Olmos et al., 2009). Changes in levels of LDH may also offer additional prognostic information to that offered by CTC count because they have been shown to have independent prognostic relevance in our series. Based on forward analysis, we know there is synergetic effect between the CTC numbers and some relative factors co-influence the prognosis outcomes in prostate cancer patients. Nevertheless, the number of CTC in PB is definitely the most convincing and powerful factor in predicting the prognosis outcomes in patients.

Discussion

The previous studies had provided evidence that the presence of CTCs in circulating blood is a strong adverse prognostic factor in various kinds of prostate cancer patients (Berruti, 2005; John et al., 2008). However, in those researches just focus on comparison between the outcome of survival quality in patients with and without CTC in peripheral blood, there is a lack of the investigating the relationship between the numbers of CTC in PB and the relevant survival years in prostate cancer patients. Therefore, the prognostic value of unfavorable numbers of CTC in PB needs further analysis. The present analysis that is based on a pool of cohort studies differs from the other published meta-analysis (Rahbari et al., 2010; Mocellin et al.,2006) which considered that the presence of CTC was a harmful factor in survival, OS. Our results support the hypothesis that CTC could play a prognostic role in patients with prostate cancer. In fact, a lot of studies reported a significant correlation between

CTC detection and patients' survival (Stott et al., 2010; de Bono et al.,2008), however a few of them showed CTC independent value of prognosis within multivariate factors as age and biopsy Gleason score(Bastian et al., 2007; Moreno et al., 2001; Wang et al., 2000).

In the survival analysis, the progression period of chronic diseases always involved in long term disease evolution, accumulation and disease reversion; the course of disease spans months and years, and so does the progression observations of final outcomes. Therefore, use normal statistic methods may lose some information then the survival analysis is required (Buyse et al., 2000; Baker et al., 2007; Burzykowski et al., 2006). In our retrieved four articles, the Kaplan-Meier survival rate plot is applied as an effective and objective way to evaluate the prognosis significant of research factors. All four articles offered the medium survival time or overall survival time as important indexes. However, the medium survival time or overall survival time are not normal distribution, the data are not measurement data, so we can not use the WSD or SMD as effective statistical index to undergoing weight analysis, so we had to absorb 5 years or 2years survival rate from the survival rate plot then put these data into heterogeneity test (Allan et al., 2010; Stewart et al., 1993; Tibbe et al., 2007). In our realistic situation, all the four studies undergoing the multivariate analysis so all of them offer the HR as an objective index to evaluate the independent effect of CTC in caner patients but without 95% confidence interval of HR, therefore, in our case, we adopted the RR (relative risk) to evaluate the hazard degree of research prognosis factors, the meaning of RR is proportionality between the probability rate of some outcomes in high exposure(risk) group and low exposure group, which often used in etiology to demonstrate the causal relationship between tested factors and disease outcomes. As we describe the RR concept in method, in fact, when RR=1 implies the tested factors are independent of disease progression, so only the RR owns a statistic significant when the 1 is not contained in 95% CI of RR. As showed in forest plot in Figure 2, each RR of the four studies are more than 1, as formal mention in method, elucidate deaths occurred rate in unfavorable group is significant higher than the favorable group.

As the above results mentioned, our data suggest that CTC may provide a more sensitive marker give an important indication of long-term outcome and in monitoring disease status during treatment. Because of CTC counts being proved to be a surrogate of outcome, these could also potentially assist in guiding earlier discontinuation of ineffective treatment (Cristofanilli et al., 2005; Wang et al., 2000; Moreno et al.,2001). It would be a significant advance for making therapeutic decisions is frequently a major challenge for both patient and physician. Importantly, the earlier cessation of ineffective treatment could also potentially increase the survival time and quality of patients or made them available for clinical trials investigating novel agents. This method may additionally have potential advantages in accessing tissue for molecular analysis. CTCs thus hold immense potential as improved biomarker of response and to accelerate evaluation of emerging novel therapies.

Although the heterogeneity test indicated that, among these related studies, most data were basically homogeneity but still a number of differences exists, such as the stage of disease (from stage one to stage four), the timing of blood withdrawal (before versus during versus after treatment, the statistical analysis method (univariate versus multivariate survival analysis, different covariates investigated at multivariate analysis). Moreover, intra-study variability (e.g. enrollment of patients treated with different regimens or blood samples withdrawn at different time points of the patients' treatment schedule) made it virtually impossible to assess the effect of one of the most important variables potentially affecting CTC detection. In the other hand, the detection method are the same cytometric instead of PCR-based; the technical features contains the isolation and enrichment of CTC based on the immunomagnetical beads with monoclonal antibodies specific for leukocytes (CD45-Allophycocyan), in fact, three of them adopted the Cell search and Cell Tracks systems. In regard to the type and number of tumor markers analyzed, definition of risk are exactly the same which as describe before is favorable numbers (<7) of CTCs in 7.5ml PB versus unfavorable numbers (>7) of CTCs in 7.5ml PB, the unfavorable numbers are deservedly classified as the higher risk group. About the clinic end point, in survival study, set the mortality as the terminal outcomes is recommended and generally accepted by most observers. Generally, in our meta-analysis, the heterogeneity among these data could be accepted but the soundness of the conclusion is not completely steady due to the small number of included documents. More than that, one has to consider that none of the studies included in our analyses provided separate data on patients' prognosis depending on preoperative versus postoperative and metastatic versus pre-metastatic tumor cell detection, though our pooled analysis showed tumor cell detection in the PB as a prognostic marker, further studies with preoperative and postoperative samplings within the same patient populations are thus required to evaluate and confirm postoperative tumor cell detection as the most accurate predictor of prognosis. The other drawback in our study is incompleteness on the kinds of prostate cancer was involved. Three of studies focused on the Castration -Resistant Prostate Cancer (CRPC) (Davis et al., 2008; Olmos et al., 2009; Rahbari et al., 2010), the rest is hormone resistant prostate cancer (HRPC)(de Bono et al., 2001). However, because of the numbers of studies were too small to perform a subgroup analysis, this will affect the pertinence of the significant which the CTCs detected in PB in prostate cancer patients. Then the criterion setting which divided the patients by the number of CTCs in 7.5ml PB also deserves debatable. In metastatic breast, prostate and other cancers more than 5 CTC are often detected using the Cell Search System, and may correlate with prognosis. However, in the setting of localized prostate cancer the number of detectable CTC was low, with findings comparable to those in men who were biopsy negative for cancer. There is no correlation between the number of CTC and known prognostic factors in this population (Jose et al., 2005). In fact, this number (5 CTC /7.5ml PB) is more likely to be a somewhat

arbitrary number with more bases in statistical significance between prognosis groups than actual clinical or biological relevance. From a biological perspective, it is logical to hypothesize that the greater the number of CTCs presents in a patient's blood, the more aggressive the disease and the poor the outcome will be. However, reanalysis of the data from Cristofanilli et al. (2004) demonstrated that median survival does not decrease further when greater than 5 CTCs (ie. 6-100 CTCs) versus 5 CTCs are detected (Allan et al., 2010). This was somewhat surprising and suggests that CTCs need to be present at a certain concentration in order to be detected at any level. As a corollary, these considerations should prompt investigators to validate the prognostic power of CTC by studying large homogeneous series of patients enrolled in multicenter prospective studies adopting standardized technical protocols.

Technology development and interest in the area of CTC analysis is advancing rapidly, CTCs have great potential as surrogate markers for cancer progression and treatment, the CTCs detected in Pca patients whose application in clinical practice is either current or expected in the near future need. Stott.SL hold the believe that CTCs will facilitate the application of noninvasive tumor sampling to direct targeted therapies in advanced prostate cancer (Daniel et al., 2007). A study discussed several fields concerning the application of CTC in PB, in the metastatic setting, a new clinical tool that can accurately track disease progression and/or predict response to therapy would be particularly useful (Allan et al., 2010). The majority of evidences supporting the use of CTCs as clinical decision making tools in patients with metastatic cancer have been obtained using the Cell Search system and analysis of 7.5 ml blood samples. Then in the early stage disease, currently, the use of well-established prognostic indicators (such as tumor size or grade) to predict outcome is helpful but imperfect, owing mainly to tumor plasticity and the reliance on subjective assessment criteria. Similarly, although some specific molecules are currently used as prognostic markers such as HER-2 for breast cancer, they are too imperfect because of tumor heterogeneity. A few studies have demonstrated that CTCs can be observed in 10% of early-stage patients using the Cell Search (Fehm et al., 2002; Kelly et al., 2003; Hara et al., 2004). However, some studies in localized prostate cancer did not show such a relationship, where analysis of CTCs by either the Cell Search system or RT-PCR for various transcripts (PSA, KLK2 [kallikrein-related peptidase 2], and PSCA [prostate stem cell antigen]) demonstrated that CTCs were rarely observed in patients with localized prostate cancer (de la Taille et al., 1999; Allen et al., 2007). In a word, at present, there is no enough evidence available regarding how CTC detection and enumeration might be useful for making clinical decisions in the early-stage/adjuvant setting (Kelly et al., 2003). As the prognosis aspect, more numbers of CTCs in PB can be correlated with poor outcome with regards to both progression-free and overall survival, regardless of nodal status or adjuvant therapy (de la Taille et al., 1999; Millon et al., 1999; Helo et al., 2009).As a corollary, we believe that many technical and statistical issues remain to be resolved before CTC analysis can be considered for widespread application to the clinic. The success of CTC detection and enumeration is influenced by many parameters including quality of the starting sample, frequency of CTCs, sample preparation, specificity and expression level of the chosen markers, robustness of the assay, and objective and reproducible readouts, including intrareader and interlaboratory variability. All these factors will contribute to the statistical probability of accurately detecting and quantifying rare events such as CTCs, and therefore are important to consider when designing and interpreting CTC assays for clinical use.

In conclusion, the results of our study suggest that presence unfavorable numbers of CTCs is associated with a relatively shorter survival in patients with PCa. Although these data do not establish CTC as a true surrogate of outcome, they do support this claim (de Bono et al., 2008; Shannon et al., 2010). Demonstrating true surrogacy remains complex and controversial with evolving statistical methodology. Establishing that CTC can be used as a surrogate for survival benefit will now require evaluation in multiple prospective, randomized phase 3 therapeutic trials, powered on survival end points and CTC as a biomarker, with meta-analytic analyses (Bastian et al., 2007). Our findings also might pave the way to the design of more informative studies.

Acknowledgements

The authors thank members of their laboratory and their collaborators for their research work, in particular Guo Yi, Chief director in the Epidemiology department of Wuhan University, Shen XingFu, graduate student in the Epidemiology department, and Liao Han Lin, graduate student in public health.This work was supported by National Natural Science Foundation of China (grant No. C30901387). The authors declare that they have no competing interests.

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