Sequential use of primary malignant circulating prostate cells to detect prostate cancer

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Screening for prostate cancer remains controversial; the use of circulating prostate cells could be an ideal biomarker to detect prostate cancer. However, different methods have produced differing results. We review these methods and the published results, comparing with the use of standard immunocytochemistry and double immunomarcation with anti-PSA and anti-P504S to define a primary malignant circulating prostate cell. It’s use as a sequential test in men with an elevated total serum PSA and before a prostate biopsy. We review the results comparing with total serum PSA, PSA based parameters and nomograms.

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Screening for prostate cancer remains controversial, the two large studies published from the USA and Europe produced different results [1, 2]; as a consequence the American Urology Association guidelines do not recommend screening in men over 70 years or less than 10 years life expectancy [3], however they recognize that some elderly men who are healthy may benefit from screening. Why the controversy? Presently, a new diagnosis of prostate cancer nearly always in men with an elevated screening serum total PSA referred for a prostate biopsy. Serum total PSA is prostate specific, however it is increased in benign diseases, such as hyperplasia and prostatitis [4, 5], as such 10% to 20% of men aged 50 and 70 years will have a raised PSA. But, only 25% of those with a serum total PSA of 4-10ng/ml will be found to have a biopsy positive for cancer [6]. However, the frequency of men with an elevated PSA and benign biopsy is country dependent [7], and may be significantly different between rural and metropolitan populations in the same country [8]. To complicate matters further, not all prostate cancers need treatment, it has been estimated that of screen detected prostate cancers 23-42% are over-treated [9]. Thus for every 100 with an elevated PSA between 4-10ng/ml only approximately 14 will have a clinically significant prostate cancer detected, or 86 men underwent a biopsy with the associated risks for a benign disease. A prostate biopsy is not without risks, infection and hemorrhage being the main potentially serious side effects, with a 30 day complication rate of 3.7%, especially in older patients [10], thus avoiding unnecessary biopsies is a worthwhile aim if the number of clinically significant cancers detected is not prejudiced.

Attempts to try to improve the predictive value of serum PSA, using free percent PSA, age adjusted PSA, PSA density or velocity are still controversial [11], although PSA velocity has been incorporated into clinical guidelines. An alternative is the incorporation of known risk factors into predictive tools based on statistical models [12], of which some have been externally validated and compared [13].

An ideal biomarker should be able to differentiate between
benign and non-significant prostate cancer on the one hand and clinically significant prostate cancer needing treatment on the other should be cost effective and could be implemented in the setting of a general hospital practice [14]. The detection of primary malignant circulating prostate cells (mCPCs) could be one candidate as a biomarker for prostate cancer. Although the detection of circulating tumor cells is not new, Ashworth first described cells in the blood at autopsy that were similar to that of the primary breast tumor in 1869 [15], the technologies to detect the few circulating cells has only been developed in the last few decades.

In men with prostate cancer there is, at least, one subpopulation of cancer cells that disseminates first to the neuro-vascular structures and then on to the circulation [16]. These primary circulating prostate cells will be eliminated by host defenses in the majority of cases [17], but their presence could indicate the presence of clinically significant prostate cancer.

The CellSearch System® is semiautomatic and using immunoferreromagnetic enriches mononuclear cells from the blood using an EpCAM based system. Then these cells are analyzed using anti-cytokeratin and anti-CD45 and DAPI (4′, 6-diamidino-2-phenylindole) nuclear staining. A positive cell is one that is cytokeratin positive, pan-leucocyte CD45 negative and has a stained nucleus. It is approved by the FDA for use in men with metastatic prostate cancer, where it has been associated with reduced survival [18]. However studies on patients with localized cancer were unable to differentiate patients with cancer from controls [19, 20], and has been suggested that EpCAM may not be the ideal biomarker in men with non metastatic cancer or localized cancer [21]. There may be two reasons, firstly during the epithelial to mesenchyme transition there is down regulation of both EpCAM and cytokeratin expression in tumor cells, increasing cell plasticity and facilitating dissemination [22]. In breast cancer patients 34% of patients had EpCAM negative CTCs detected [23].

Secondly, there is a concept that only cancer cells disseminate to the blood, however in some benign conditions there could be trafficking of normal epithelial cells. These normal cells would be detected using EpCAM or cytokeratin based methods and contribute to false positive results. The need for unambiguous criteria to define the malignant nature of circulating tumors cells has therefore to be defined. The frequency of epithelial cells detected in the blood of healthy individuals or those with benign disease is low, representing 0.3% of subjects [24]. This may be an underestimate, using the CellSearch System®, Pantel et al. [25] found circulating epithelial cells in between 0% and 18% of patients with benign colonic diseases.

To address these problems, the use of standard differential gel centrifugation to obtain mononuclear cells has been used, and to detect malignant circulating prostate cells the combined use of anti-PSA and anti-P504S. Although P504S is not prostate specific its use has facilitated the identification of pre-malignant and malignant cells in prostate biopsy samples [26]. Normal or benign cells are P504S negative, while those arising from prostatic intra-epithelia dysplasia or cancer are positive [27]. The Chilean Prostate Tumor Early Cancer Test (ProTECT) study started in 2008, with a blood simple being taken immediately before prostate biopsy in men with suspicion of prostate cancer based on an elevated serum PSA or abnormal digital rectal examination (DRE).

The presence of malignant primary CPCs (Figure 1), defined as cells staining positive for PSA and P504S increases with age and serum PSA [28]. In this group of 1117 men, 559 underwent a prostate biopsy, 32.7% had a biopsy positive for cancer, and 37% were positive for malignant CPCs. There was a significant association between the presence or absence of CPCs and a positive or negative biopsies (Table 1), with a sensitivity of 88.5%, specificity of 88.0%, positive predictive value of 78.3% and negative predictive value of 94.5% [28].

**a) comparing with PSA derived parameters:**

When compared with PSA parameters, free percent PSA, PSA density and velocity the detection of primary malignant CPCs proved to be superior in terms of sensitivity, specificity, positive and negative predictive values [29]. Using the PSA parameters in the 113 men with a biopsy positive for prostate cancer, the cut off values used were unable to differentiate between cancers needing treatment and those that could be observed, whereas CPC detection could differentiate between the two (Table 2).
A PSA velocity of >0.75ng/ml/year has been incorporated as a screening marker in the NCCN guidelines [30]; this is because serum total PSA does not differentiate between high and low risk men [31], high grade disease may occur at low serum total PSA values. Thus the use of PSA velocity expands the definition of a positive PSA test and increases the likelihood of a referral for biopsy. In prospective screening studies PSA velocity does not appear to add value to PSA levels or improve accuracy [31, 32]. In this study of Chilean men, PSA velocity was not more accurate than free percent PSA or PSA density in terms of diagnostic yield.

b) in the elderly:

The median age at diagnosis of prostate cancer is 68 years, with 71.2% of prostate cancer specific deaths occurring in the over 75 years [33] and in the elderly is more aggressive [34]. Guidelines differ with respect to screening in the over 70s, the AUA recommends against screening, while EAU and NCCN guidelines indicate that radical treatment is appropriate in men with a life expectancy of over 10 years. As pathological and oncological outcomes in men over 70 years treated by radical prostatectomy are not significantly different than in younger men screening may be considered appropriate [35]. For individual patients population based life expectancy tables are not helpful. The International Society of Geriatric Oncology classified the elderly into four groups, according to co-morbidities, functional status and weight loss in the previous three months. Those in Group 1 with controlled co-morbidities, full Independence in daily living and good nutritional status have a median life expectancy of 14.2 years at the age of 75 years. In the elderly the specificity of serum PSA decreases as a result of benign hyperplasia and chronic prostatitis [36], to compensate for this age related serum total PSA levels were proposed.

In a study of fit elderly men, the same detection test using mCPC proved superior to age related PSA [37] (Table 3). Men older than 70 years had a higher frequency of cancer detection 41.5% versus 38.7% (p=0.04), a higher median serum PSA (p=0.05). Using a cutoff value of 6.5ng/ml for serum total PSA, 56/90 cancers would not have been detected in men >70 years, of which 48/56 (85.7%) complied with the criteria of Epstein for active treatment. In comparison only 2 clinically significant cancers would have been missed using mCPC detection, and 58.5% of biopsies would have been avoided in men mCPC negative.

c) the use of nomograms:

There are an increasing number of statistically based predictive tools to predict the results of an initial prostate biopsy [12]. The Montreal (Canada) predictive tool has been externally validated [39], and uses simple readily available markers, age, DRE, PSA and percent free PSA to give a percent risk calculation in an individual patient. Compared with two other validated models, it was shown to be superior to the European Randomized Study of Screening for Prostate Cancer derived Prostate Risk Indicator and the North American Prostate Cancer Prevention Trial derived Cancer Risk Calculator [13]. The Montreal tool does not require data on prostate volume, the prostatic ultrasound is not a routine test in Chilean patients, the volume is only assessed at the moment of ultrasound guided trans-rectal biopsy and therefore does not have a role in the decision to biopsy the patient, nor does it incorporate race or family history.

In a head to head comparison with the Montreal nomogram the use of primary CPCs was shown to be superior in predicting cancer detection at first biopsy [39]. The area under the curve in this study of Chilean men for the Montreal nomogram was similar to that reported in other studies [13] and was superior to serum total PSA alone. However it failed to improve on the use of free percent PSA in terms of clinically significant cancers that would be missed and the number of biopsies avoided using established

Table 2. Use of PSA parameters and mCPC detection to determine active treatment or active observation in 113 men diagnosed with prostate cancer (Ref: 29)

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Free PSA</th>
<th>PSA velocity</th>
<th>PSA density</th>
<th>CPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needs treatment</td>
<td>≤15%</td>
<td>&gt;15%</td>
<td>≥0.75</td>
<td>0.75 &lt;0.75</td>
</tr>
<tr>
<td>Needs observation</td>
<td>71</td>
<td>24</td>
<td>58</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>p= Chi squared</td>
<td>p=0.07</td>
<td>p=0.25</td>
<td>p=0.11</td>
</tr>
</tbody>
</table>

Table 3. Diagnostic yield for mCPC in men <70 years and men ≥ 70 years (Ref 37)

<table>
<thead>
<tr>
<th></th>
<th>Men &lt;70 yrs.</th>
<th>Men ≥ 70 yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value %</td>
<td>95% CI</td>
</tr>
<tr>
<td>specificity</td>
<td>86.4</td>
<td>81.6-90.3</td>
</tr>
<tr>
<td>sensitivity</td>
<td>88.1</td>
<td>81.3-93.0</td>
</tr>
<tr>
<td>PPV</td>
<td>76.6</td>
<td>69.1-83.1</td>
</tr>
<tr>
<td>NPV</td>
<td>93.4</td>
<td>89.6-96.2</td>
</tr>
<tr>
<td>LR (+)</td>
<td>6.46</td>
<td>4.74-8.80</td>
</tr>
<tr>
<td>LR (-)</td>
<td>0.14</td>
<td>0.09-0.22</td>
</tr>
</tbody>
</table>
cut off points [39].

In terms of an ideal biomarker the use of primary mCPC failed to detect small low grade small volume tumors [40] and for its high negative predictive value was able to decrease the number of potential prostate biopsies [28]. P504S negative CPCs were not found in men with a biopsy positive for cancer, there was a higher frequency of detection in men with chronic prostatitis than with benign hyperplasia, being present in 21/245 men with a benign biopsy [41]. Although specific for benign disease the sensitivity is low, the authors concluding that it is not a test for the detection of benign disease, however when present implies the absence of cancer [41].

Thus, it would appear from the published results that the sequential use of the detection of CPCs using anti-PSA and anti-P504S that the test fulfills the criteria of an ideal biomarker, that it detects most clinically significant prostate cancers, does not detect indolent cancer and has a high negative predictive value. This being true in older men where the high negative discriminating power was superior to age related PSA. The results of these studies suggest that men, independent of age, with an increased PSA but CPC negative could be considered at low risk of significant cancer and thus a biopsy might not be necessary. This is part of an ongoing study, with active follow-up of men CPC positive and negative and an initial biopsy negative for prostate cancer.

Conflicting interests

The authors have declared that no competing interests exist.

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