RESEARCH ARTICLE

Does the Presence of Primary Circulating Prostate Cells Imply the Presence of Aggressive Prostate Cancer with Early Biochemical Failure: a Comparison with the Walz Nomogram

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Abstract

**Background:** To determine the utility of primary circulating prostate cells (CPC) for predicting early biochemical failure after radical prostatectomy for prostate cancer and compare the results with the Walz nomogram. **Materials and Methods:** A single centre prospective study of men with prostate cancer treated with radical prostatectomy was conducted between 2004 and 2014. Clinical-pathological details were registered, along with total serum PSA pre-surgery, Gleason score, extracapsular extension, positive surgical margins, infiltration of lymph nodes, seminal vesicles and pathological stage. Primary circulating prostate cells were obtained using differential gel centrifugation and detected using standard immunocytochemistry with anti-PSA. Biochemical failure was defined as a PSA >0.2ng/ml, predictive values were calculated using the Walz nomogram and CPC detection. **Results:** A total of 285 men participated, of whom 103/285 (36.1%) suffered biochemical failure; 32/103 (31.1%) within two years of radical prostatectomy. Men with higher Gleason scores, higher pathological stage, infiltration of the surgical margin or prostate capsule and infiltration of seminal vesicles were more likely to undergo biochemical failure. There was a significant increase in the frequency of biochemical failure with increasing number of CPCs detected (p<0.0004 Chi squared for trend) and increasing percent prediction for the Walz nomogram (p<0.0001 Chi squared for trends). The positive predictive value of primary CPC detection, even using a cutoff point of ≥ 4 cells/sample was very low. **Conclusions:** The detection of primary CPCs in men as a prognostic factor pre-treatment fails to identify those at high risk of biochemical failure within two years of curative therapy. This is in keeping with their biological significance, that the majority of them will be eliminated by the primary therapy and thus have no influence on the subsequent clinical history of the patient.

Keywords: Prostate cancer - biochemical failure - circulating prostate cells - Walz nomogram.
as they circulate in the blood and lymph systems (Fidler
11, 1970). These cells have been defined as primary
circulating prostate cells (CPCs). While secondary CPCs,
those detected after primary treatment have been reported
to increase the risk of biochemical failure (Murray et al.,
2013; Murray et al 2015) an ideal biomarker would be
able to assess the risk of early failure before primary
treatment was decided.

We present the finding of the presence of primary
CPCs in men undergoing radical prostatectomy for
prostate cancer, their association with the risk of early
biochemical failure and compare their presence with the
Walz nomogram in predicting this biochemical failure

Materials and Methods

Between 2008 and 2014 patients who underwent
open retropubic radical prostatectomy for prostate cancer
were enrolled in the study. Pre-treatment serum total PSA
was measured before digital rectal examination using the
Siemens Advia CentaurXR® assay. Pathological study
of the surgical piece was performed by dedicated
genitourinary pathologists according to the Gleason
system. Pathological stage was defined according to the
Partin criteria, organ confined, extra capsular extension,
seminal vesicle invasion and lymph node invasion (14).
A positive surgical margin was defined as cancer cells
in contact with the inked surface of the specimen. The
registered data were entered in the on-line nomogram
(www.nomogram.org) and the risk of biochemical failure
in the first two years after surgery registered.

Detection of primary circulating prostate cells:
immediately pre-surgery an 8mL venous blood sample
was taken and collected in a tube containing EDTA
(Beckinson-Vacutainer). Three months was chosen for
the sampling, to give time for prostate cells disseminated
during surgery would be cleared from the circulation.
Samples were maintained at 4º C and processed within
48 hours. CPC detection was independently evaluated
with the evaluators being blinded to the clinical details.

Collection of CPCs: Mononuclear cells were obtained
by differential centrifugation using Histopaque 1.077
(Sigma-Aldrich), washed, and resuspended in an 100 µL
aliquot of autologous plasma. 25 µL aliquots were used
to make slides (silanized, DAKO, USA), were dried in
an alkaline phosphatase-anti alkaline phosphatase based
system (LSAB2, DAKO, USA), with new fuchsin as the
chromogen. Positive samples underwent a second process
in the first two years after surgery registered.

Immunocytochemistry: primary CPCs were detected
using a monoclonal antibody directed against PSA, clone
28A4 (Novocastro Laboratory, UK), and identified using an
alkaline phosphatase-anti alkaline phosphatase based
system (LSAB2, DAKO, USA), with new fuchsin as the
chromogen. Positive samples underwent a second process
with anti-PS504S clone 13H4 (DAKO, USA) and were
identified using a peroxidase based system(LSAB2,DAKO,
USA) with DAB (3,3 diaminobenzidine tetrahydrochloride)
as the chromogen. A primary CPC was defined according
to the criteria of ISHAGE (International Society of
Hemotherapy and Genetic Engineering) (Borgen et al.,
1999) and the expression of P504S defined according to
the Consensus of the American Association of Pathologists
(Ruben et al., 2001). A primary CPC was defined as a
cell that expressed PSA and P504S, a leucocyte did not
express PSA but may or may not express P504S. A test
was considered positive for primary CPCs when at least
1 cell/8mL of blood was detected, the number of CPCs
detected/8ml blood simple was registered.

Statistical analysis

Descriptive statistics were used for demographic
variables, expressed as mean and standard deviation
in the case of continuous variables with a normal
distribution. In case of an asymmetrical distribution the
median and interquartile range (IQR) values were used.
Noncontiguous variables were presented as frequencies.
The Shapiro-Wilk test was used to determine a normal
distribution. The Student T-Test was used to compare
continuous variables with a normal distribution, the Mann-
Whitney test for ordinate and continuous variables with a
nonnormal distribution, and the Chi-squared test for the
differences in

frequency. Statistical significance was defined as a p-
value less than 0.05, all tests were two-sided. Area
under the curve analysis was performed using the online
programe Vassarcal.

The CPC test was considered positive if ≥1 cell was
detected per sample and negative if no cell was detected,
we used a cutoff value of 20% probability of biochemical
failure within two years for the Walz nomogram, based
on the sensibility to detect 50% of patients who would
undergo biochemical failure.

Ethical Considerations

The study was approved by the hospital ethics
committee and in complete agreement with the Declaration
of Helsinki. All patients provided written informed
consent.

Results

A total of 285 men participated in the study, of whom
103/285 (36.1%) suffered biochemical failure; 32/103
(31.1%) within two years of radical prostatectomy. The
clinical pathological details of men with and without
biochemical failure are shown in Table 1. The median
follow up of the study population was 5 years (IQR 2-9
years). 225/285 (79%) of men were primary CPC positive.

Association of biochemical failure with clínico-
pathological features: a) Gleason score: men with higher
Gleason scores were more likely to suffer biochemical
failure (p<0.001) (Table 2), b) pathological stage: men
with higher pathological stage were more likely to
suffer biochemical failure (p<0.001). c) according
to margen status: 47/62 (75.8.4%) of men with the surgical
margin positive for tumor suffered biochemical failure
in comparison with 56/223 (25.1%) of those with margins
negative for tumor (p<0.0001; RR 3.02 (95% CI 2.31-
3.94); OR 9.34 (95% CI 4.85-17.99). d) according to
extra-capsular extension: 76/133 (57.1%) of men with

extra capsular extension by tumor underwent biochemical failure in comparison with 27/152 (17.8%) of those without extra-capsular extension (p=0.0001; RR 3.47 (95% CI 2.36-5.12); OR 6.77 (95% CI 3.91-11.73). e) seminal vesicle infiltration: 17/103 (16.5%) of men with biochemical failure had seminal vesicle infiltraion versus 3/182(1.6%) without biochemical failure (Fisher exact test p=0.0004). f) lymph node infiltration: 1/103 (0.9%) of men with biochemical failure had lymph node infiltration versus 1/182 (0.5%) without biochemical failure ) (Fisher exact test p=0.99).

Men with higher Gleason scores, higher pathological stage, infiltration of the surgical margin or prostate capsule and infiltration of seminal vesicles were more likely to undergo biochemical failure.

**Early Biochemical Failure**

32/103 (31.1%) of men underwent biochemical failure within two years of surgery, the predictive values of the Walz nomogram and primary CPC detection were compared, men with biochemical failure occurring after two years were classified as not having biochemical failure at the two year evaluation. Table 4 shows the distribution of patients according to CPC number/8ml blood sample and % prediction of biochemical failure.

Areas under the curve for both tests were for the nomogram 0.795 and for CPC detection 0.717 (p=0.29), there was no significant difference in the discriminative power between the two tests. There was a significant increase in the frequency of biochemical failure with increasing number of CPCs detected (p<0.0004 Chi squared for trends) and increasing percent prediction for the Walz nomogram (p<0.0001 Chi squared for trends).

The predictive values of sensitivity, specificity, positive predictive and negative predictive values were determined for each test for different values (Table 5).

The positive predictive value of primary CPC detection, even using a cutoff point of ≥ 4 cells/sample is very low, it must be remembered that the CPC test was designed to give a positive/negative result.

**Discussion**

In the study population the standard risk factors for predicting disease progression were significantly higher in the early biochemical failure group, confirming that the study population is a representative group of those who will experience progression after radical prostatectomy.

Early recurrence after radical prostatectomy is associated with a poor prognosis with early progression both in terms of metastasis and cancer specific mortality (Pound et al., 1999; Freedland et al., 2005). Therefore
identifying these patients at high risk of early relapse may have clinical applications, these patients being considered for adjuvant therapies. It must be emphasised that the use of adjuvant therapy is controversial, with conflicting results reported in the literature (Mitchell et al., 2008). Clinical decisions should be based on an understanding of the various parameters which play a role in defining the risk of disease progression. The use of nomograms intergrades these variables to predict patient outcome on an individual basis, rather than categorizing the patient as part of a risk group.

Studies analyzing the role of CPCs in localized disease are few (Helo et al., 2009; Khurana et al., 2013), recently Meyer et al. (2016) using the CellSearch assay failed to find an association between the presence of CPCs prior to treatment and early biochemical failure. Using this technique only 11% of patients had CPCs detected, using a cut-off value of ≥1 cell/sample to define a positive test. Loh et al. (2014) and Aragon et al. (2015) similarly found a low incidence of patients positive for CPCs, 14% and 8% respectively. This emphasizes the importance of the method used to detect CPCs, differing from the CellSearch assay, which uses an EpCAM based detection system we used a PSA based detection system, which identified primary CPCs in 79% of men with prostate cancer.

The positive predictive value of primary CPC detection was very low, much lower than that of the predictive value of secondary CPC detection and early biochemical failure (Murray et al., 2015a). This is because the two types of CPCs represent two different clinical entities. Secondary CPCs, detected after primary treatment, represent minimal residual disease, that is to say, they arise from a microfocus of micrometastatic disease not eradicated by the primary treatment. They do not indicate if this microscopic disease is local, in the prostatic bed or surrounding tissue or systemic such as in bone marrow. However, this minimal residual disease may in time progress causing biochemical failure. Primary CPCs are different, they arise from the primary tumor, not all these cells will survive or implant in distant tissues. Their elimination after primary treatment is associated with a lower rate of biochemical failure (Murray et al., 2014).

Therefore, primary CPCs have a different clinical application, their presence implies the presence of prostate cancer, their detection is reported to be clinically useful as a sequential test to detect prostate cancer, being absent in benign disease and low grade small volumen tumors (Murray et al., 2014a). However as a biomarker to predict prognosis they perform poorly as compared to other risk classification systems or nomograms.

In conclusion, the detection of primary CPCs in men as a prognostic factor pre-treatment fails to identify those at high risk of biochemical failure within two years of curative therapy. This is in keeping with their biological significance, that the majority of them will be eliminated by the primary therapy and thus have no influence on the subsequent clinical history of the patient.

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DETAILS?


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