RESEARCH ARTICLE

Does the Presence of Primary Circulating Prostate Cells Imply the Presence of Agressive 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 nomagram and CPC detection. Results: A total of 285 men participated, of whom 103/285 (36.1%) suffered biochemcial 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.

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Introduction

After radical prostatectomy for prostate cancer approximately 25-30% of patients will eventually experience a biochemical failure, defined as a total serum PSA of >0.2ng/ml (Hull et al., 2002; Porter et al., 2006). Two thirds of these biochemical failures will occur in the first two years and implies a biologically more aggressive disease and poorer prognosis (Dillioglugil et al., 1997). This may be due to the presence of micrometastatic disease not detected by conventional testing or from locally advanced disease (Dillioglugil et al., 1997; Pound et al., 1999; Freedland et al., 2005). Early biochemical failure is associated with a higher rate of metastatic progression and increased cancer specific mortality (Pound et al., 1999; Freedland et al., 2006). Walz et al. (2009) produced a nomogram based on the total serum PSA at surgery, the presence or absence of extracapsular penetration, seminal vesicle and lymph node infiltration, positive or negative surgical margins and pathological Gleason score from the surgical piece. This externally validated nomogram, although it tends to over-estimate the risk of biochemical failure, identifies a group of men with a high risk of early biochemical failure. These individuals could be considered for adjuvant treatments, although some data have shown benefits to early therapy (MRC Working Party, 1997; Messing et al., 2006) this still is controversial.

Early in prostate cancer there are at least one subpopluation of cancer that disseminate firstly to the neurovascular structures and then into the circulation (Moreno et al., 1992). The majority of these cells are eliminated by host defences or destroyed by shear forces

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as they circulate in the blood and lymph systems (Fidler IJ, 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 failue (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 stud. 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 genitournary 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 air for 24 hours and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate buffered saline (PBS) pH 7.4 for five mintues and finally washed three times in PBS pH 7.4 .

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-P504S clone 13H4 (DAKO, USA) and were identified with 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 Vassarcalc.

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 biochemcial failure; 32/103 (31.1%) within two years of radical prostatectomy. The clinical pathological details of men with and without biochemcial 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

Table 1. Demographic Features of the Study Population

	With biochemical failure	Without biochemical failure	
Nº Patients	103	182	
Age mean (SD)	66.3 ± 8.9 years	65.6 ± 8.8 years	p=0.56
PSA at diagnosis ng/ml (IQR)	5.93 (4.76-9.30)	5.57 (4.64-7.81)	P<0.01

Table 2. Biochemical Failure According to Gleason Score

			Percent deviation		Standard residual	
	BF (+)	BF (-)	BF (+)	BF (-)	BF (+)	BF (-)
G ≤ 6	35	149	-47.4%	+26.8%	-3.86	+2.91
G7	43	29	+65.3%	-36.9%	+3.33	-2.50
G ≥ 8	25	4	+138.5%	-78.4%	+4.48	-3.37

Table 3. Biochemical Failure According to Pathological Stage

			Percent deviation		Standard residual	
	BF (+)	BF (-)	BF (+)	BF (-)	BF (+)	BF (-)
pT1	7	91	-78.8%	+44.6%	-6.69	+3.53
pT2	46	76	+3.2%	-1.8%	+0.21	-0.16
pT3	50	15	+112.8%	-63.9%	+5.47	-4.11

Table 4. Distribution of Biochemical Failure According to Nomogram and CPC Tests

Nomogram	BF (+)	BF (-)	CPC	BF (+)	BF (-)
0-5%	1	140	0 cells/8 ml	0	60
6-10%	4	47	1-2 cells/8ml	4	71
11-20%	7	23	3-4 cells/8ml	4	38
21-50%	8	25	5-8 cells/8ml	9	41
>50%	11	19	>8 cells/8ml	14	44

Table 5. Predictive Values of the two Tests (95% CI)

		sensitivity	specificity	PPV	NPV
nomogram	≥ 6%	97 (82-99)	55 (49-61)	21 (15-29)	99 (96-99)
	≥10%	81 (63-92)	73 (68-79)	28 (19-38)	97 (93-99)
	≥20%	61 (42-78)	83 (77-87)	30 (20-43)	95 (91-97)
CPC	≥1 cell/sample	97 (81-99)	23(18-29)	13 (9-19)	98 (90-99)
	≥2 cells/sample	87 (69-96)	52 (45-58)	18 (12-25)	94 (92-99)
	≥4 cells/sample	74 (55-87)	67 (60-72)	21 (14-30)	95 (91-98)

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 occuring 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 discrimative 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 nomagrams intergrates these variables to predict patient outcome on an individual basis, rather than catorgorizing 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 erradicated 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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