Prostate-specific antigen level and detection of circulating tumour cells in castration-resistant prostate cancer

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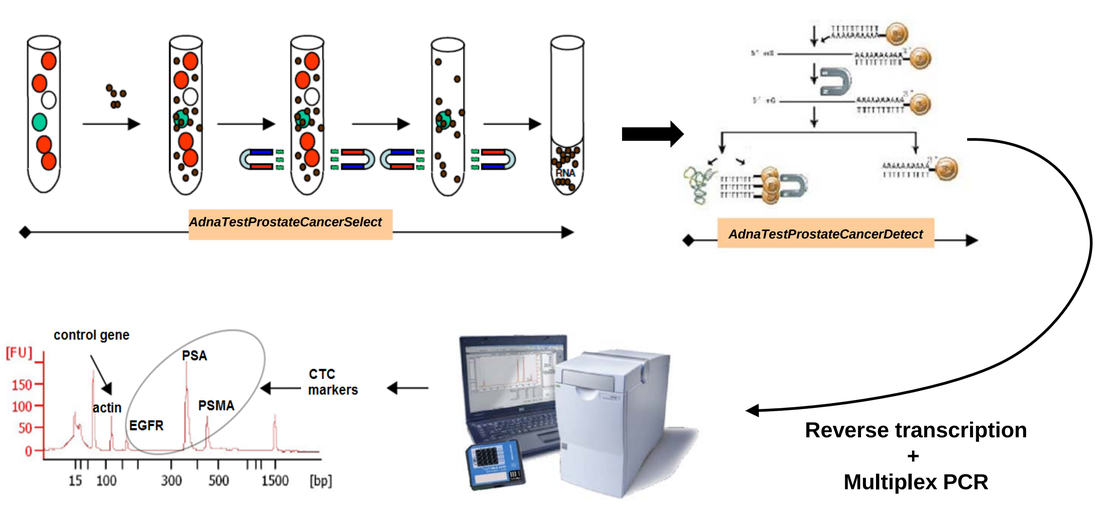
Aim : To define an association of the serum prostate-specific antigen (sPSA) level and expression of tumour-associated antigens in circulating tumour cells (CTC) in peripheral blood of patients with castration-resistant prostate cancer (CRPC).

Material and methods: Peripheral blood from patients with metastatic CRPC was taken prior to docetaxel therapy (DTx) and after the fourth cycle of chemotherapy. Detection of CTC was done by using a method of immunomagnetic separation and quantification of tumour-associated antigens (Fig. 1). The CTC count was evaluated verballly (positive vs. negative) together with a report of the absolute values (ng/µl). We have recorded the levels of sPSA and the fragments of respective antigens before and in the course of DTx and the values were compared. We have also evaluated the correlation between the change of sPSA and expression of CTC antigens during DTx. The correlation of the parameters was determined by using the Spearman correlation coefficient.

Fig.1 Immunomagnetic separation (IMS) of circulating tumour cells (CTC)



Peripheral blood (7,5ml) is taken to EDTA tubes and is used for CTC isolation (ProstateCancerSelect®, Adnagen). The IMS method is performed in two steps. In the first, the monoclonal antibodies conjugated with magnetic particles Dynabeads™ bind against antigens EpCAM and HER-2 on the surface of tumour cells. These cells are then separated from peripheral blood in the magnetic field and lysed subsequently.

In the second step, the lysed fraction is used for RNA isolation by using magnetic particles with oligo(dT)24 nucleotides (Adnatest ProstateCancerDetect®) and cDNA is synthetized from the isolated RNA. Part of the cDNA is used for multiplex-PCR reaction testing by PrimerMix ProstateDetect for control actin gene and tumour-associated genes PSMA, PSA and EGFR. Analysis of PCR products is done on Bioanalyzer 2100 (Agilent).

Test is positive, if at least one fragment of transcript is detected. The fragment of the control gene actin must be present in all samples, negative controls must be negative and no fragments greater than 500bp may be detected.

Graph 1. Overview of the tumour-associated CTC fragments before and after docetaxel therapy

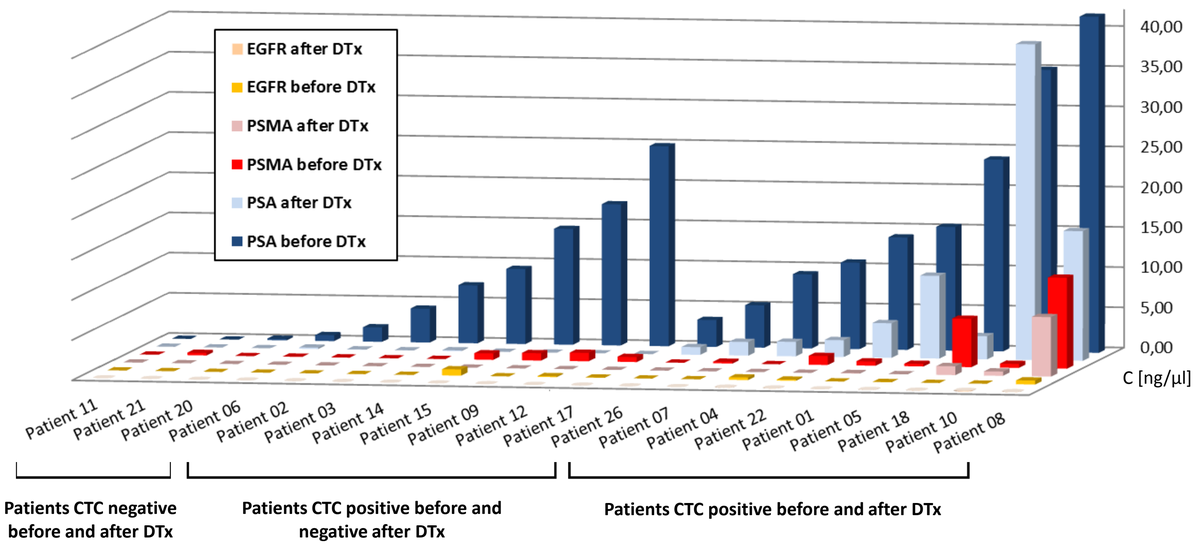


Fig 3. Examples of the capillary electrophoresis on the chip (Agilent Bioanalyzer 2100).
A - positive patient (No. 08), B- negative patient (No. 11), C - immunofluorescence cell staining

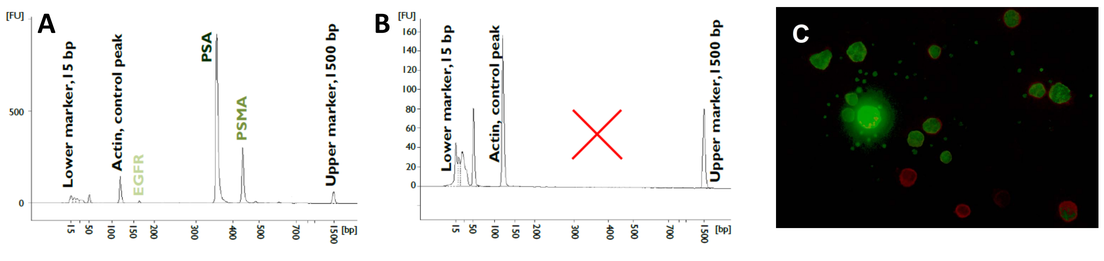


Table 2. Correlation of tumour-associated transcripts levels with various parameters

Parameter	Tumour-associated transcript (Spearman Correlation Coefficients)			Tumour-associated transcript (p-values)		
	PSA	PSMA	EGFR	PSA	PSMA	EGFR
sPSA 1	0.57783	0.47274	0.28452	0.0020	0.0147	0.1589
sPSA 2	0.72945	0.56501	0.57905	0.0013	0.0226	0.0188
Δ sPSA 1 vs. sPSA 2	0.32353	0.05189	0.57854	0.2216	0.8486	0.0189
Δ in verbal evaluation	0.14434	0.42363	0.35583	0.1020	0.8174	0.1610

sPSA1 - serum PSA level before initiation of chemotherapy, sPSA2 - serum PSA levels during chemotherapy, Δ sPSA 1 vs. sPSA 2 - change in serum PSA level during chemotherapy, Δ in verbal evaluation - change in verbal assessment of circulating tumour cells in a sample (positive or negative)

Note : Spearman's rank correlation coefficient assesses the relationship between two variables. If the coefficient is higher than 0, it is a positive correlation (higher values of one variable imply higher values of the second variable) and vice versa. The correlation coefficient takes values from -1 to +1, the closer is the value to -1 or +1, the stronger is the correlation.

Legend : high correlation | moderate correlation | significant correlation

Table 1. Study group characteristics (patients with both samples taken)

Patient's No.	11	21	20	06	02	03	14	15	09	12	17	26	07	04	22	01	05	18	10	08
Age (years)	71	69	76	76	75	78	69	82	72	67	76	65	77	73	64	70	80	78	68	66
Date of diagnosis	FEB-2006	APR-2004	SEP-2006	DEC-2009	AUG-2008	MAR-2006	JAN-2011	OCT-2003	APR-2006	NOV-2011	APR-2006	JAN-2012	SEP-2010	AUG-2008	SEP-2010	JUL-2005	SEP-2010	AUG-2002	JUL-2009	APR-1994
Biopsy GS	9	6	7	8	9	7	9	7	8	10	7	6	9	6	9	7	7	8	8	x
Radical treatment	RP	RT	none	none	RT	RT	none	none	RP	none	none	none	none	none	none	RP	none	none	none	RP
TNM classification	pT3bN1	T2-3bNxM0	T3NxM0	T4N1M1b	T3	T2cN0M0	T3-4NxM1b	T3-4NxMx	pT3bN0M0	T3-4NxM1b	T2bNxM0	T3NxM1b	T3bNxM1b	T3NxM1b	T4N1M1b	pT3bN0M0	T2NxM1b	T1cNcMx	T3NxM0	pT2NxMx
iPSA	12,3	137,0	53,5	782,0	108,9	11,0	18,9	41,3	8,8	79,2	96,9	125,2	150,0	74,2	232,0	14,8	117,0	42,7	66,0	13,5
sPSA 1	18,6	62,6	50,3	223,4	26,0	138,5	136,7	46,9	36,2	442,3	731,5	56,8	74,0	96,8	216,8	108,8	78,7	86,2	559,5	160,2
sPSA 2	12,0	5,4	3,8	110,7	21,5	20,4	30,7	36,0	4,2	35,2	136,4	49,3	92,7	46,3	58,4	221,3	167,2	57,6	891,2	69,5
Chemotherapy	D3W	D3W	D3W	D3W	D3W	D3W	D3W	C3W	D3W	D1W	C3W	D3W	D1W	D1W	D3W	D3W	D3W	C3W	D3W	D3W
CTC before CHT	negative	negative	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive	positive
CTC after CHT	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative	positive	positive	positive	positive	positive	positive	positive	positive	positive

GS - Gleason score, TNM - Tumour, Node, Metastasis classification, RT - radiotherapy, RP - radical prostatectomy, iPSA - prostate specific antigen at diagnosis, sPSA1 - serum PSA level before initiation of chemotherapy , sPSA2 - serum PSA level during chemotherapy, CTC - circulating tumour cells, CHT - chemotherapy, D3W - docetaxel every 3 weeks, D1W - docetaxel weekly, C3W - cabazitaxel every 3 weeks NOTE : patients on D1W regimen were sampled after the 12th cycle

Results : A total of 26 patients were included in the analysis with both samples taken in 20 of them. Median age was 72 years (54-82), mean sPSA level before and after DTx was 197.6 and 107.6 ng/ml, respectively. Two and 11 patients were considered CTC negative before and after DTx, respectively. Before DTx, positive detection of fragments of antigens for PSA, PSMA and EGFR was confirmed in 23, 16 and 2 patients, respectively, and during DTx in 9, 3 and 1 case, respectively. The sPSA level before DTx was associated with the level of fragments for PSA (p=0.0020) and PSMA (p=0.0147). During DTx the association was seen in all antigens. However neither a change in sPSA level nor a change in positive vs. negative CTC statement have correlated with a change of any of the tested antigens.

Conclusion : The sPSA level has the most accurate correlation with the level of gene fragment for PSA in CTC. A favourable change in CTC quantity will occur in more than a half of patients during DTx, however the change in CTC detection does not correlate with the change of the sPSA level.

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