SmartBioSurface® Slides for Prostate Cancer Detection and Risk Stratification through CTC Enumeration and Biomarker Expression Profiling

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Introduction

Prostate cancer (PCa) is one of the most prevalent cancers in men and a significant cause of cancer-related deaths [1].

Gold standard procedure for Prostate Cancer diagnosis

- **Prostate-Specific Antigen (PSA) testing**
- **Digital rectal examination**
- **Tissue biopsy**

Limitation and negative aspects

- **Low specificity** (PSA testing is a not cancer-specific marker)
- **Invasive procedure** (side effects as bleeding, infection, hematuria or sepsis)
- **Limited Accuracy** (Biopsy sampling can results in undergrading or overgrading the cancers)

Blood-based liquid biopsy offers a non-invasive alternative for analyzing circulating tumor cells (**CTCs**) in bloodstream, providing **molecular insights** and **prognostic value.** [2].

Our study evaluates the use of **SmartBioSurface® (SBS) slides** [3-5] in patients undergoing biopsy for suspected PCa, focusing on its effectiveness in identifying clinically significant disease (Gleason Score ≥7 or Gleason Grade group ≥2 according to European Association of Urology Guidelines) [6].

Methods

We developed a **novel method** [4] to identify and characterize **CTCs** using **SBS slides** and **prostate-specific biomarker staining**.

CTCs and clusters were detected via immunofluorescence, targeting Prostate-Specific Membrane Antigen (PSMA), Prostate-Specific Antigen (PSA), Androgen Receptor (AR), epithelial (panCK/EpCAM), and hematopoietic (CD45/CD66b) biomarkers.

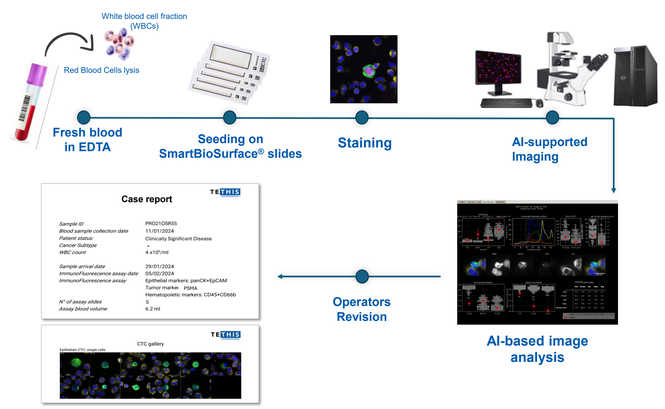

Figure 1. CTCs detection workflow using SBS slides: from blood to Case report
A blood sample is processed to isolate white blood cells (WBCs) that are seeded as a monolayer on SBS slides at a density of approximately 2.5 million WBCs/slide. After immunofluorescence staining, slides are scanned with an automated fluorescence microscope (BioView, Israel). CTCs are identified by a proprietary software according to their biomarker expression profiles. The gallery of target cells is then reviewed by two operators to confirm CTC identification. For each sample, a Case report is generated, detailing findings such as CTC count and biomarker expression profiles.

Results

The clinical trial (NCT04964271) included 60 men undergoing prostate biopsy: 15 with benign prostatic hyperplasia (BPH), 13 with low-risk disease (LR), and 24 with clinically significant PCa (csPCa), along with 8 healthy donors (HD) as controls.

TABLE 1	LR	csPCa
Number of patients	16	20
Clinical T Stage		
T1	15	7
T2	1	4
T3	0	9
ISUP Prostate Biopsy		
ISUP 1	15	0
ISUP 2-3	1	3
ISUP 4-5	0	17

TABLE 2	LR	csPCa
Number of patients	13	24
ISUP Final Pathology		
NA	7	2
ISUP 1	4	1
ISUP 2-3	2	7
ISUP 4-5	0	14
Pathological T Stage		
NA	7	2
pT1	1	0
pT2	4	5
pT3	1	17
Pathological N Stage		
NA	7	2
pNx	6	4
pN0	0	9
pN1	0	9

CTCs are here defined as cells lacking hematopoietic markers and expressing prostate-specific markers (PSMA, PSA, or AR) with or without epithelial biomarkers expression.

Using **SBS slides and specific immunostaining biomarkers** we identified the following CTC per ml (as mean ± SD).

- 1.41 ±1.56 CTC/ml in HD
- 3.18 ± 2.39 CTC/ml in BPH patients
- 2.53 ± 1.73 in LR patients
- 6.41 ± 5.11 in csPCa patients

Preliminary results indicate that **PSA, PSMA, and AR** are informative biomarkers for detecting CTCs (single cells and clusters). A statistically significant difference ($p<0.050$) was observed between the csPCa and LR groups, while no significant differences were observed between the LR and the BPH cohort ($p>0.6$).

Under the most stringent conditions, a **3.41 CTC/ml threshold** distinguished HD from csPCa, **achieving 100% specificity and 59% sensitivity.**

Table 1. Clinical characteristics of patients at trial enrollment.
Table 2. Clinical characteristics of patients following surgical reclassification.

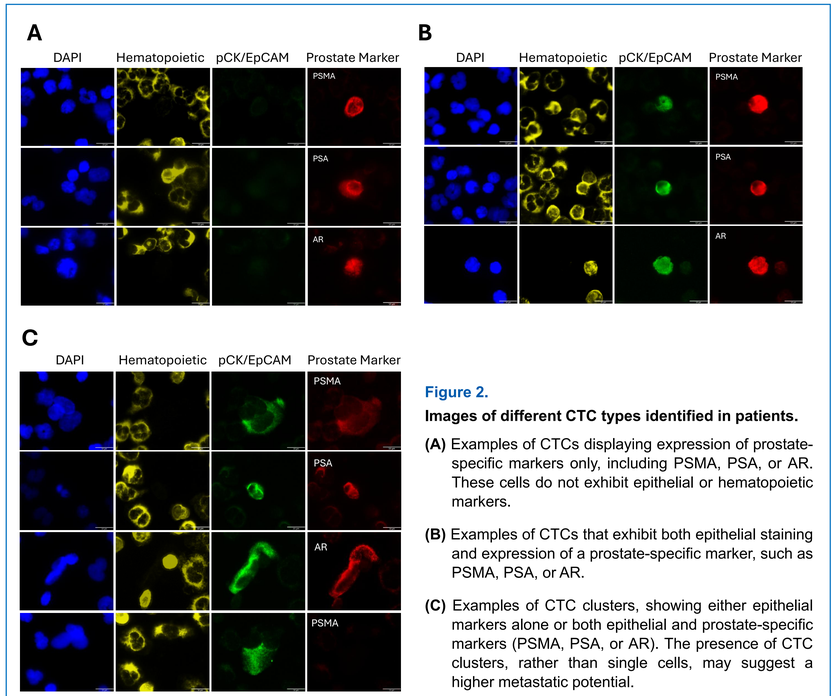

Figure 2.
Images of different CTC types identified in patients.

(A) Examples of CTCs displaying expression of prostate-specific markers only, including PSMA, PSA, or AR. These cells do not exhibit epithelial or hematopoietic markers.

(B) Examples of CTCs that exhibit both epithelial staining and expression of a prostate-specific marker, such as PSMA, PSA, or AR.

(C) Examples of CTC clusters, showing either epithelial markers alone or both epithelial and prostate-specific markers (PSMA, PSA, or AR). The presence of CTC clusters, rather than single cells, may suggest a higher metastatic potential.

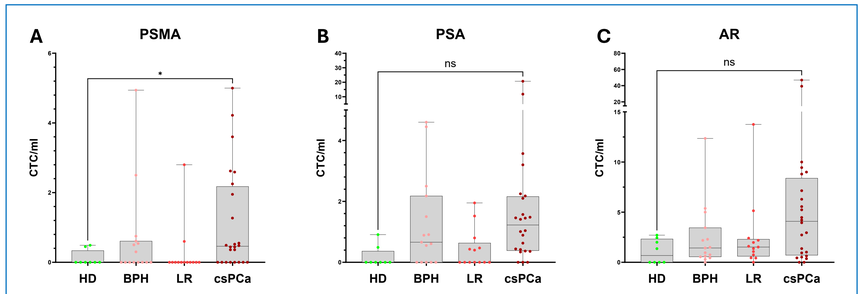

Figure 3. Analysis of CTCs by Individual Prostate-Specific Marker Staining. Scatter plots display the number of CTCs per ml (CTC/ml) across clinical classifications, for each of the three independent prostate-specific marker: **(A)** PSMA, **(B)** PSA, and **(C)** AR. Statistical analysis was performed using unpaired Brown-Forsythe and Welch ANOVA tests. * $p < 0.050$, $p > 0.050$ noted as non-significant (ns).

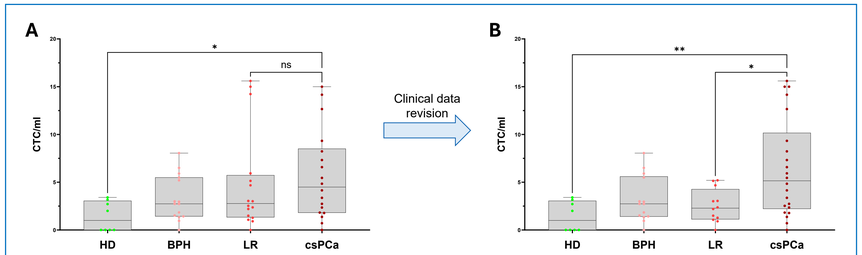

Figure 4. Cumulative analysis of CTCs. CTCs from each prostate biomarker staining were combined to provide an aggregated count of CTC/ml per case. **(A)** Scatter plot displaying the number of CTC/ml based on the initial clinical classification derived from biopsy results at enrollment. **(B)** Scatter plot following reclassification using post-surgical clinical data. This reclassification enhances the distinction in CTC levels between patient categories, leading to an increase in the statistical significance of differences observed across clinical stages. Statistical analysis was performed using unpaired Brown-Forsythe and Welch ANOVA tests. *$p < 0.050$, **$p < 0.010$,

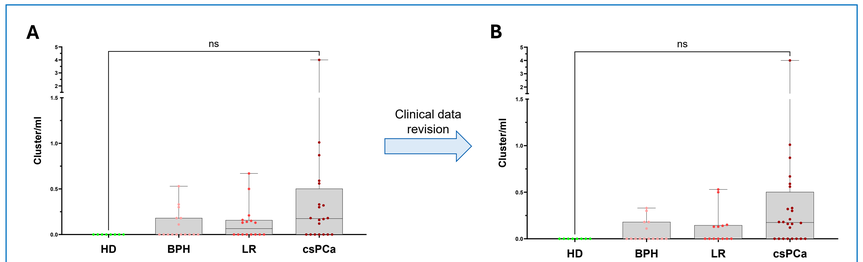

Figure 5. Cumulative Analysis of CTC Clusters. Several cluster (defined as a group of two or more cells ($n \geq 2$) that express either epithelial markers, prostate-specific markers, or both) have been identified in patient samples.
(A) Scatter plot of cluster concentration (Cluster/ml) across clinical classifications based on biopsy results taken at enrollment; **(B)** Scatter plot following reclassification using post-surgical clinical data. Statistical analyses were conducted using unpaired Brown-Forsythe and Welch ANOVA tests. * $p > 0.050$ are indicated as non-significant (ns).

Conclusions

This pilot study successfully established a **novel protocol** for CTC and cluster detection using **SmartBioSurface® slides** and prostate-specific biomarker staining.

Moreover, we demonstrated :

- PSMA, PSA, and AR as **informative** biomarkers for CTC identification;
- a potential correlation between the total number of CTCs and risk classification, enabling the **stratification** of patients with clinically significant PCa from those with low-risk cancer.

These findings support the potential for **larger clinical studies** to evaluate whether CTC **enumeration and characterization** could provide **more precise diagnostics** than standard methods for accurately detecting tumors and assessing disease severity.

CONTACT INFORMATION
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[1] Siegel, R.L.; Miller, K.D.; Jemal, A., 2020, Cancer statistics. CA *Cancer J. Clin.*, 70, 7–30.
[2] Matuszczak M, Schalken JA, Salagierski M. 2021 Prostate Cancer Liquid Biopsy Biomarkers' Clinical Utility in Diagnosis and Prognosis. *Cancers*, 13(13):3373
[3] Carbone R. et al., 2006, Biocompatibility of cluster-assembled nanostructured TiO2 with primary and cancer cells, *Biomaterials*, 27(17):3221-9
[4] Krol I., Schwab F.D., Carbone R., Ritter M. et al., 2021, Detection of clustered circulating tumour cells in early breast cancer, *Br J Cancer* , 125(1):23-27
[5] Mastromarino M.G., Parini S. et al., 2023, Liquid Biopsy Detecting Circulating Tumor Cells in Patients with Non-Small Cell Lung Cancer: Preliminary Results of a Pilot Study, *Biomedicines*, 11(1):153
[6] https://uroweb.org/guidelines/prostate-cancer