NOVEL MULTI SOURCE BIOMARKER PANEL IN SEMEN IMPROVES PROSTATE CANCER DIAGNOSIS OVER BLOOD PSA ALONE: A PRELIMINARY STUDY

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BACKGROUND

The current standard of care for prostate cancer (PC) screening (blood Prostate Specific Antigen, PSA) is unreliable. This has led to an annual 6.5% decline in PC screening rates, while new cases and deaths continue to increase[1]. According to the USPSTF, 55% of biopsies taken from those with elevated PSAs were negative, reaffirming the need for better, more thorough screening. Additionally, unnecessary biopsies cause bleeding, infection, urinary retention, and additional costs to the healthcare system[1]. It is more worrisome that biopsies cannot detect PC if systematic or targeted core sampling misses the tumor tissue. The unreliability of PSA and biopsy warrant an earlier, more accurate and non-invasive screening test for prostate cancer. More than 30% of seminal fluid is produced in the prostate, making semen a comprehensive and novel biomarker sample source. Our semen sample analysis contains a panel of heterogeneous biomarkers from multiple sources including protein, extracellular vesicles, and DNA methylation, as opposed to a single biomarker. Alpha-Methylacyl-CoA Racemase (AMACR) protein, measured in 96% (23 out of 24) of semen samples with biopsy confirmed PC, has proven a promising biomarker[2]. DNA methylation profiles of CAV1, EVX1, and FGF1 exhibit significant changes in methylation between normal and tumor-associated tissue groups[3,4]. Microparticles'(MP), submicron extracellular vesicles (100-1000nm), surface markers PSMA, STEAP1, and GHSR1a can differentiate PC prognosis[5]. Together, this comprehensive panel of biomarkers can detect prostate cancer earlier and more accurately than PSA alone.

METHODS

Semen samples were collected in a multi-center biorepository clinical trial of men aged 50 or older. Fourteen (14) sites participated across the United States. A single sample was collected from each study participant and assigned to four cohorts as defined in **Table 1**. Semen samples were processed and AMACR protein level was quantified using an ELISA assay. Microparticle quantity and surface marker profiles were characterized using antibody-stained flow cytometry. DNA methylation profile was characterized by methyl-specific PCR assay (**Figure 1**). Data normalization, univariate and multivariate LDA analysis was conducted to determine sensitivity and specificity of PC diagnosis between PSA alone and the Gregor panel. A subset of samples was analyzed to include only those without processing issues (45% of samples) and those with a biopsy confirmed 'Cancer' or 'No Cancer' status (excluding cohort 1), shown in **Figure 2**.

RESULTS

Cohort	N	PSA (ng/ml)	Biopsy Result	Gleason Score	Risk
1	64	< 2.0	NA	NA	Normal Sample
2	51	≥2.0	-	NA	BPH Elevated PSA
3	40	≥2.0	+	6 to 7(3+4)	Low
4	6	≥2.0	+	7(4+3) to 10	High

Table 1. *Distribution of Cohorts*

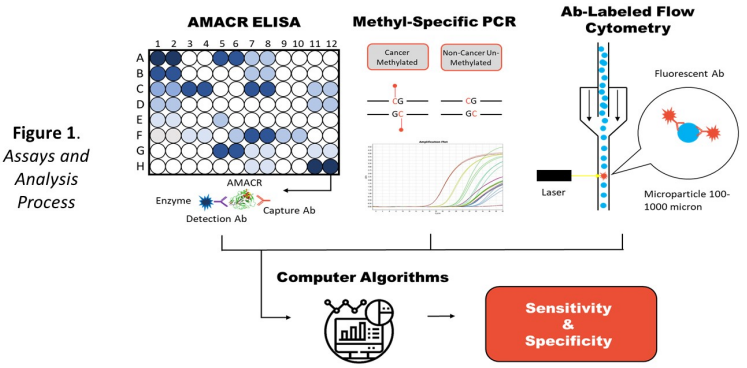

Figure 1. *Assays and Analysis Process*

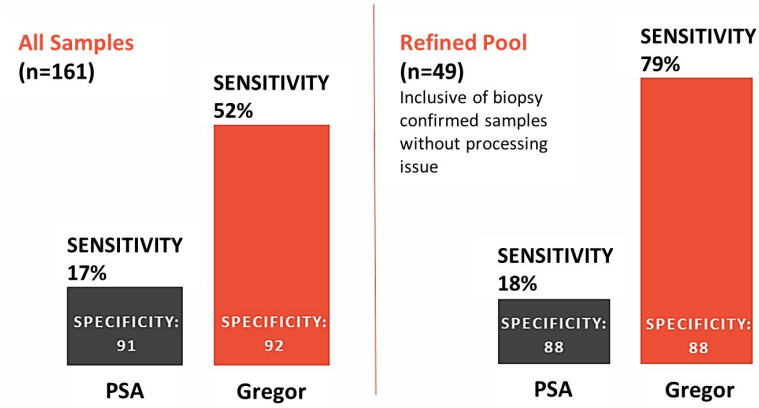

Figure 2. *Sensitivity of Biomarker Panel vs PSA-only*

CONCLUSIONS

This study demonstrates Gregor's diverse panel of biomarkers encompassing DNA methylation, protein, and MPs greatly improves prostate cancer detection sensitivity over PSA alone or any single marker alone, 18% compared to 79%. However, the improved sensitivity was manifested strongest in samples without any processing issues and those with biopsy confirmation. Improvements in sample processing procedures in parallel with further optimization of biomarker detection are currently underway and will aim to significantly increase cohesive diagnostic accuracy as a more effective early PC screening tool. This powerful multifaceted biomarker panel using seminal fluid as a superior source enables biomarker surveillance of the entire prostate and could increase PC screening adherence and ultimately lead to a decrease in new cases and death from prostate cancer.

Reference: 1. Carter et al., 2011 The Journal of Urology; 2. Jarrard et al., 2010 Cancer Research; 3. Bhusari et al., 2013 Neoplasia; 4. Jarrard et al., 2012 Patent; 5. Brar et al., Master's Thesis.