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Identification of early stage and metastatic prostate cancer using electrochemical detection of beta-2-microglobulin in urine samples from patients

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To improve prostate cancer (PCa) diagnosis, it is imperative to identify novel biomarkers and establish effective screening techniques. Here, we introduce electrochemical biosensing of β -2-Microglobulin (β 2M) in urine as a potential diagnostic tool for PCa. The immunosensor is composed of a screen-printed graphene electrode coated with anti β 2M antibodies. The sensor is capable of detecting the protein directly in urine without any sample pretreatment within 45 min including sample incubation and a lower limit of detection of 204 µg/L. The sensor demonstrated a significant difference in the β 2M-creatinine ratio in urine between control and both local- and metastatic PCa (mPCa) (P = 0.0302 and P = 0.0078 respectively), and between local- and mPCa (P = 0.0302). This first example of electrochemical sensing of β 2M for the diagnosis of PCa may set the stage for an affordable, on-site screening technique for PCa.

Prostate cancer (PCa) is the second most common cancer in men¹. Worldwide, PCa accounts for 7.3% of total cancer cases and 3.8% of cancer-related deaths¹. The lack of more specific diagnostic and better prognostic biomarkers for significant PCa often leads to over-diagnosis and overtreatment². Thus, there is a critical need to develop better non-invasive diagnostic methods with new biomarkers that are more sensitive and specific for PCa. β -2-Microglobulin (β 2M) is a glycoprotein that is synthesized by all nucleated cells. It consists of 99 amino acids and forms a small subunit of major histocompatibility class I (MHC I)³. The noncovalent association of β2M with the α-chain of MHC class I molecules and no direct attachment to the cell membrane gives rise to free $\beta 2M$ in the body fluids due to intercellular release⁴. Free and soluble $\beta 2M$ in the blood is mainly linked to kidney diseases, although it is suspected to be linked to other diseases. The β 2M in body fluids such as blood and cerebrospinal fluids have been hypothesized to potentially play a role in the detection of various diseases, such as diabetes, heart disease, and myeloma, and more generally immunodeficiency in the innate and adaptive immune system^{3,5}. Furthermore, recent studies demonstrate that $\beta 2M$ is associated with the regulation of survival, proliferation, and apoptosis in cancer cells, and therefore targeting β2M signaling pathways provides a new strategy for cancer therapeutics⁴. Heretofore, methods used to detect β 2M include radioimmunoassay⁶, western blotting⁷, enzyme-linked immunosorbent assay (ELISA), and matrix-assisted laser desorption/ionization/ time-of-flight (MALDI-TOF)^{3,5,8}. However, most of the methods available in centralized laboratories, are timeconsuming, costly, and non-portable^{3,5}. Thus, there is a need for a cheap, fast, and simple detection technique that may be used for fast bedside screening. Electrochemical biosensors hold great promise in the biomedical area due to the direct conversion of the physical interaction with the sensor to electronic signal⁹⁻¹¹. The enhanced specificity, sensitivity, and cost-effectiveness make them ideal for rapid point-of-care detection of diseases at the bedside¹⁰. Earlier studies have demonstrated that detection of $\beta 2M$ with biosensors is possible^{3,5,12-14} (Table 2).

¹Department of Science and Environment, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark. ²Department of Biochemistry and Immunology, Lillebaelt Hospital, University Hospital of Southern Denmark, Vejle, Denmark. ³Department of Oncology, Lillebaelt Hospital, University Hospital of Southern Denmark, Vejle, Denmark. ⁴Department of Urology, Lillebaelt Hospital, University Hospital of Southern Denmark, Vejle, Denmark. ⁵Department of Regional Health Research, University of Southern Denmark, Odense, Denmark. [©]email: alzahraa@ruc.dk Rizwan et al.³ fabricated an electrochemiluminescence immunosensor based on gold nanoparticles-doped/carbon nano-onions chitosan nanocomposite modified cadmium selenide quantum (QDs-SPE/AuNPs/CNOs-CS) for detection of β 2M. This sensor demonstrated the potential to detect β 2M in serum and urine samples based on recovery studies³. Maity et al. demonstrated the quantification of β 2M in human tears. The sensor consists of aqueous gold nanoparticles coated with anti- β -2-microglobulin antibodies using a linker. The suspension of AuNPs shows a specific coloration when the interaction with light occurs⁵. Liu et al.¹² developed an electrical sandwich immunoassay based on different inorganic nanocrystal tracers.

Here we present an immunosensor composed of screen-printed graphene electrodes coated with anti- β -2-microglobulin (anti- β 2M) antibodies using a linker, 1-pyrene butyric acid N-hydroxysuccinimide ester (PBASE), a concept shown in Fig. 1. The introduction of PBASE, antibodies, and bovine serum albumin (BSA) onto the sensor surface result in a reduction in current due to the obstruction of electron transport towards the electrode surface. As a result, when β 2M protein binds to the electrode, the expected signal output is diminished in comparison to a sample without protein binding. Our measurements show a significant difference in the levels of β 2M between 5 patients referred with a suspicion of PCa, but prostate biopsies showed no evidence for cancer (controls) and 10 PCa patients (5 with local PCa and 5 with metastatic PCa). To the best of our knowledge there have been no reports on electrochemical immunosensors for use in point-of-care detection of β 2M in PCa patients and our method may be a breakthrough in the field of diagnostic tools for PCa.

Material and methods

Study population and sample collection. The research project received approval from the Southern Denmark Regional Committee on Health Research Ethics (Reference No.: S-20210085) and utilized urine samples from the PerPros Biobank (Reference No.: 18/11174), Department of Urology at Vejle Hospital. All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects prior to sample collection. A total number of 15 male individuals were included, 5 patients referred with a suspicion of PCa, whose biopsies were without any evidence for PCa (controls), and 10 PCa patients (five diagnosed with local PCa and five have de novo metastatic PCa). Urine samples from all PCa patients were collected before initiation of androgen deprivation therapy (ADT) and were obtained at any time during the day with no manipulation. The urine samples were collected from patients with normal renal function. All samples were stored at - 80 °C. Table 1 gives baseline clinical information's about the patients included in this study.

Electrochemical detection of β2M. β 2M was quantified using screen-printed graphene sensors from PreDiagnose (Karlslunde, Denmark) featuring a three-electrode design, which comprised a graphene working electrode (WE), a counter electrode (CE), and a graphene reference electrode (RE). The data acquisition was performed using PS-trace 5.8 software (PalmSens BV, Houten, The Netherlands) and the potentiostat Palmsense 4 device (PalmSens BV, Houten, The Netherlands).

Functionalization of graphene sensors. To enable binding between the anti- β 2M antibody and the graphene-based sensor, a surface modification step is employed, which allows for the attachment of the anti- β 2M antibody to the graphene surface via a specific linker. Graphene screen-printed sensors were modified according to the protocol developed by Mojsoska et al.¹⁵. Initially, the Pyrenebutyric acid N-hydroxysuccinimide ester (PBASE, #63520) from Lumiprobe (Hannover, Germany) was dissolved in a 1:5 volume/volume ratio of dimethylformamide (68-12-2, Sigma-Aldrich) and methanol (67-56-1, Sigma-Aldrich). The PBASE solution was then diluted with pure methanol to attain a concentration of 2.5 mM.



Figure 1. Concept of urinary β -2-microglobulin detection using an electrochemical immunosensor composed of graphene working electrode with anti- β -2-microglobulin functionalization. (i) A urine sample is applied on an (ii) electrochemical scree-printed sensor. (iii) The working electrode of the sensor is functionalized with β -2-microglobulin-specific antibodies.

Patient	Age (Years)	Baseline PSA	Earlier cancer	Gleason score	T-stage	Control	Local PCa	mPCa
1	67	6.3	No			x		
2	76	3.9	No			x		
3	70	3.9	No			x		
4	63	3.5	No			x		
5	70	4.8	No			x		
6	64	3.7	No	6 (3+3)	N/A		x	
7	68	4.3	No	6 (3+3)	N/A		x	
8	67	8.3	No	6 (3+3)	T1		x	
9	78	8.8	No	6 (3+3)	T1		x	
10	56	3.6	No	6 (3+3)	N/A		x	
11	91	45	No	9 (4+5)	T2			x
12	79	-	No	9 (4+5)	T3			x
13	70	-	No	7 (4+3)	T3			x
14	91	135	No	9 (4+5)	T3			x
15	66	38	No	7 (4+3)	T2			x

 Table 1. Demographic characteristics of the patients based on age, PSA, earlier cancer, (Gleason score (GS), and T-stage.

The working electrode was coated with a 50 μ L layer of the 2.5 mM PBASE solution and incubated for 2 h at room temperature (RT) within a sealed petri dish to prevent evaporation. Subsequently, the sensors underwent washing with pure methanol followed by deionized water and drying with nitrogen. The linker-modified graphene sensors were incubated overnight at RT with 10 μ L of anti- β 2M antibody (#GTX42680, GeneTex) diluted 80× in Phosphate-buffer solution (PBS). Following the washing and drying phase, the sensors underwent incubation with a 50 μ L PBST solution (comprised of PBS and 0.05% Tween-20) containing 1% BSA at room temperature, to hinder non-specific binding. The sensors underwent washing with deionized water and rinsing in PBST (at 37 °C) on a magnetic stirrer at 500 rpm, followed by drying with a gentle flow of nitrogen. Subsequently, the electrochemical signal of the functionalized graphene sensor was measured.

Characterization of carbon electrochemical sensors. Characterization of the graphene electrode at each stage of functionalization was accomplished by cyclic voltammetry (CV). All electrochemical measurements were carried out using a 2.5 mM ferriferrocyanide solution in PBS and measured against a graphene reference electrode. The CVs were performed from -1.1 to 1.0 V with scan rates between 0.01 and 0.20 V/s.

Detection of β2M. β2M in the samples was measured after incubating the functionalized sensors with 10 μ L of the sample for 45 min at 37 °C. The sensors underwent washing with deionized water and rinsing in PBST (at 37 °C) on a magnetic stirrer at 500 rpm, followed by drying with nitrogen. The electrochemical measurement of β2M was performed using Square Wave Voltammetry (SWV). SWV was conducted with the following settings: amplitude: 0.25 V; frequency 15.0 Hz and the current ranges from 1 nA to 1 mA, $E_{begin} - 0.75$ V, and E_{end} 0.8 V. A new sensor was used for each measurement.

Stability of β2M. To examine the β2M degradation pattern, a stability examination was carried out. Urine samples containing 300 μ mol/L of β2M were stored at two temperatures, – 80 °C and 4 °C. On day 0 and day 10, triplicate electrochemical measurements were performed. The signals obtained from the stability experiment were then normalized based on the measurement taken on day 0.

Recovery test. The sensor was used for the determination of $\beta 2M$ in urine samples to evaluate the efficiency of the proposed method. The urine samples from three different individuals were measured in triplicates before and after spiking with a known quantity of 300 µg/L $\beta 2M$. The obtained peak heights were converted to concentrations using the standard curve in Fig. 4b.

Data analysis. The signal induced by β 2M binding to the sensor surface was obtained by subtracting the background signal from the analyte signal in PS. Trace 5.8. After subtraction of the background, the peak height was found. The average peak height for each patient was presented. The limit of detection (LOD) was calculated as 3 times the standard deviation of the lowest concentration signal divided by the slope of the calibration curve. Unpaired T-tests with Welch's test were performed using Graphpad.

Results

Characterization of functionalization steps. To assess each functionalization step, CV measurements of 2.5 mM ferriferrocyanide buffer were performed as seen in Fig. 2A–D. The introduction of PBASE, antibodies, and BSA on the surface leads to a decrease in the current due to the blocking of electron transport towards the



Figure 2. Characterization of functionalization steps and sensor reliability. (**A**) Cyclic voltammograms of (**A**) a bare electrode with no sensor modification, (**B**) an electrode with immobilized linker molecules, (**C**) an electrode with immobilized antibodies, (**D**) an electrode with bovine serum albumin (for blocking non-specific binding) shows the decrease in the current due to the blocking of electron transport towards the electrode surface. (**E**) Cyclic voltammograms at increasing scan rates using the fully functionalized sensor. (**F**) The Randle–Sevcik plot of the peak currents extracted from the cyclic voltammograms versus the square root of the scan rate, deeming the sensor compliant with analytical detection of concentration perturbations. All measurements are conducted in ferriferrocyanide.

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electrode surface. The performance and reversibility of the graphene sensor after completed functionalization were evaluated by CVs of 2.5 mM ferriferrocyanide at different scan rates (Fig. 2E). The results showed symmetrical voltammograms and the half-wave potential for a reversible system (each voltammogram) was calculated according to the standard equation $(E_0 = (E_{pa} + E_{pc})/2)$, yielding a value of -0.15 ± 0.0245 V vs. the reference electrode. The Randle–Sevcik plot indicated that the sensor is suitable for analytical detection, as the peak current of anodic and cathodic signals are proportional to the square root of the scan rates (Fig. 2F).

Matrix effect on electrochemical signal. If the β 2M protein is bound to the electrode, the anticipated signal output is suppressed compared to a sample with no protein binding (Fig. 3A,B). The effect of the urine matrix on the signal was characterized by measuring the signal with and without 300 µg/L β 2M spiking in unmodified urine, in 1:10 (urine: PBS), and in pure PBS (Fig. 3C). We found that the difference in electrochemical signal between urine with and without 300 µg/L β 2M was approximately 57% while the difference in signal





Figure 3. Matrix effect on an electrochemical signal using functionalized electrodes. (**A**) The absence of β 2M in the sample will result in a high peak height, (**B**) binding of β 2M to the electrode will result in an insulating layer which results in a lower peak height. (**C**) Peak heights of urine, 1:10 (urine: PBS), and pure PBS with and without 300 µg/L β 2M.

was 34% and 37% for 1:10 (urine dilution) and pure PBS, respectively. This suggested that no dilution of urine samples was needed.

Direct quantification of \beta 2M in urine. The modified graphene electrodes were used to detect $\beta 2M$ in urine at SWV scans from -0.75 to 1.0 V and the $\beta 2M$ peak appeared at 0.1 V (Fig. 4a). Square wave voltammograms of spiked urine samples show decreasing peak heights with increasing $\beta 2M$ concentration, which could indicate a saturation of the available binding sites. Another possible explanation could be that the effectiveness of antibodies to form immune complexes is impaired due to high levels of either antibody or antigen. Figure 4A shows that pure PBS and Betalactamase (control-protein) in PBS resulted in the highest peak heights due to no $\beta 2M$ present in the samples. The peak width of a peak in a voltammogram usually corresponds to the kinetics of the redox process. Changes in the peak width may happen when the surface area changes due to e.g., incubation with analytes of different matrices. Our data consistently show that the peak width than the measure-



Figure 4. Standard curve for $\beta 2M$ measurements. (A) Square wave voltammograms of increasing $\beta 2M$ concentration in urine using modified graphene sensors. (B) A standard curve with an inverse correlation between added $\beta 2M$ concentration and peak height measured in urine. The green dot in the graph is the outlier.

ments in PBS. The difference in signal between blank urine and pure PBS could be due to that urine naturally contains around 300 µg/L β 2M. Despite various urine controls from different healthy men, the observed signal levels were consistent and as expected. Therefore, the data suggest that no interference is observable. The increasing concentrations of added β 2M (0–600 µg/L) were measured in otherwise identical urine samples and followed a linear correlation with a slope of – 0.0162 µA/µg/L and a regression coefficient of 0.9876 (Fig. 4b). An outlier, represented by a green dot in the graph, was identified and could be attributed to a scratched sensor. It was subsequently removed and treated as a separate measurement. The LOD was calculated to 204 µg/L in urine as 3 times the standard deviation of the lowest concentration signal divided by the slope of the calibration curve. The graphene-modified electrodes can detect β 2M below the normal concentration range in urine which is reported to be 230–300 µg/L¹⁶.

Stability of β2M detection. To ensure that β 2M is stable despite the storage of samples under different temperatures, a stability test was performed in urine from one control patient in triplicates, by measuring the signal of a constant concentration at fixed temperatures for a period of 10 days. As patients' urine samples are regularly stored at 4 °C and – 80 °C, the stability of the electrochemical signal of β 2M detection was investigated after storage at these temperatures. Figure 5 shows the recovered concentration as a function of time for samples with 300 µmol/L of β 2M. After 10 days no significant degradation was observed.

Recovery of β2M in spiked urine samples. The functionalized graphene sensor was successfully applied for the detection of β 2M levels in human urine samples in recovery investigations (Table 2). The urine samples from three different individuals were measured before and after spiking with a known quantity of 300 µg/L. The obtained peak heights were converted to concentrations using the standard curve in Fig. 4b. All samples contained between 239 and 275 µg/L β 2M which is within the naturally occurring levels in healthy individuals (230–300 µg/L). The average recovery rate was $102 \pm 7\%$.



Figure 5. β 2M stability test: β 2M in urine was stored under different temperature conditions for a period of 10 days. The boxes represent the initial concentration of β 2M, which was 300 µmol/L, at Day 0, as well as the recovered concentration at Day 10 for storage conditions at 4 °C and – 80 °C. The test demonstrated that β 2M was stable under all conditions during the test period.

Sample	[β2M] before spiking (µg/L)	[β2M] after spiking (µg/L)	Recovery (%)
1	239	542	102
2	264	544	93
3	275	605	110

Table 2. Recovery of β 2M in spiked urine samples. Samples were spiked with 300 μ g/L β 2M.

Difference in \beta2M between controls and PCa patients. The modified graphene electrodes were finally applied to investigate the difference in β 2M levels between controls and patients with PCa. The β 2M to creatinine ratio was measured for all patients included in the study and compared between patient groups (Fig. 6). The data showed significant differences between the control group and both the local PCa (*P*=0.03) and the metastatic PCa (*P*=0.008). Also, a significant difference between local PCa and mPCa was observed (*P*=0.03). A significant difference for the measured β 2M levels (not corrected for creatinine) was observed



Figure 6. β 2M levels in the urine samples for the control group, local PCa, and metastatic PCa. The biosensor demonstrated that there is a significant increase in levels of β 2M in urine samples from PCa patients compared to those from the control group, **P* ≤ 0.05, ***P* ≤ 0.01.

between the controls and PCa group (P=0.009) (Supplementary Fig. 1). Even when we take into account the 7% uncertainty calculated from the recovery experiment, there is still a statistically significant difference between the control and patient groups. In one of the control samples β 2M was measured to be197 µg/L, which is under the LOD.

Other biosensors for β 2M quantification. Table 3 gives an overview of the previously reported biosensors for β 2M detection in comparison to this work. The comparison is based on the sensing materials, detection methods, limits of detection, media, and recovery.

Rizwan et al. fabricated an electrochemiluminescence (ECL) sensor and demonstrated very good sensitivity towards $\beta 2M$ in buffer solution³. Maity et al. demonstrated quantification of $\beta 2M$ by an immunosensor, however, the recovery percentages of $\beta 2M$ in urine with this sensor were very varying⁵. The mentioned sensors are the most sensitive towards $\beta 2M$ in buffer solutions, but they are based on plasmon resonance and ECL. Only one of the listed studies in Table 2 has developed an electrochemical immunosensor for the detection of $\beta 2M^{12}$. Liu et al. reported an immunoassay for measurements of multiple proteins based on inorganic nanocrystal tracers¹². In this immunoassay, the antigens are captured by magnetic beads conjugated with antibodies. The antigens are then detected by reactions with nanocrystal-labeled secondary antibodies and finally dissolution of the nanocrystals occurs, and electrochemical detection is possible¹². Liu et al. ¹² have not presented measurements in real samples. The current work demonstrated electrochemical measurements of $\beta 2M$ directly in patient urine samples with LOD at the lower end of the physiologically relevant concentration range and without any pretreatment of the patient sample.

Discussion

Increased levels of $\beta 2M$ have been reported for different cancer types^{20–23}. Bataille et al.²³ showed a significant positive correlation between $\beta 2M$ and myeloma stage. In the PCa population, Zhang et al.²⁴ demonstrated that serum $\beta 2M$ was significantly increased in PCa patients compared to benign prostatic hyperplasia (BPH) or normal controls. Furthermore, urinary $\beta 2M$ was reported to be elevated in 74% of advanced PCa patients compared to healthy subjects using radioimmunoassay^{20,21}. Additionally, elevated urinary $\beta 2M$ levels in PCa patients with bone metastasis were significantly associated with shorter overall survival²¹. These results suggest a role of $\beta 2M$ in both the development and progression of PCa.

The current diagnostic approaches for PCa have limited potential for distinguishing between indolent and aggressive disease²⁵. Due to the risk of overtreatment, more specific diagnostic and prognostic biomarkers are needed²⁶. In this respect, β 2M may have a potential role as an alternative biomarker for PCa. However, there no currently no low-cost and high sensitivity immunosensor available for use in the point-of-care detection of β 2M in urine, so this work can be seen as a potential breakthrough in the field of β 2M detection of.

When investigating biomarkers in urine, levels of markers are generally normalized to creatinine measurements²¹. Treatment by ADT results in rapid loss of muscle mass far exceeding that of normal aging, which results in reduced creatinine levels compared with normal subjects^{21,27}. Therefore, the normalization of β 2M levels with creatinine causes an elevation in the β 2M to creatinine ratio in patients that have been treated with hormonal therapy^{21,27}. The sampling of the patients included in this study was initiated before treatment by ADT. β 2M levels cannot be normalized to urine protein levels because evaluation in urine β 2M causes a comparable increase in urine protein levels²¹. In addition, β 2M is cleared by the kidneys, and its level also reflects renal function²⁸.

For validation of the observations made in this study, we wanted to perform ELISA. However, we were not able to quantify $\beta 2M$ in urine samples with direct or sandwich ELISA. Some of the common shortages of conventional ELISA are limited multiplexing options, the necessity for centralized laboratory equipment, and the relatively high sample volumes required²⁹. Also, consistency in handling ELISA comes with practice. Another limitation of using ELISA for the detection of $\beta 2M$ is the concentration range of the commercially available ELISA kits. The normal urinary levels of $\beta 2M$ are reported to be 230–300 µg/L¹⁶. No ELISA kit was found that could detect $\beta 2M$ in this concentration range. In our study, we chose not to dilute the samples due to the challenges

Material of sensor	Detection method	LOD in media	Media	Recovery in real samples	References
QDs-SPE/AuNPs@CNOs-CS	ECL	1 fg/mL	Tris-buffer	92–96%	3
Antigen-Antibody-DTSP-AuNP	Optical	100 fg/mL	PBS	67–136%	5
Colloidal nanocrystal tags	SWV	0.0001 fg/mL	PBS	-	12
Series S carboxymethylated	Surface plasmon resonance	$1.3 \times 10^{-5} \text{ fg/mL}$	500–1000-fold diluted serum in HBS-EP +	-	17
Quartz crystals microbalance	Piezoelectric	3×10^{-6} fg/mL	PBS	90-110%	18
Grating coupler	HeNe-laser	5×10 ⁹ μg/mL	PBS	-	19
Antigen-antibody-Gr	SWV PBS	$2.4 \times 10^8 \text{ fg/mL}$ $5.5 \times 10^7 \text{ fg/mL}$	Urine PBS	93–110%	This work

Table 3. Comparison of performance of this work with previously reported β 2M sensors. *ECL* electrochemiluminescence, *SWV* squarewave voltammetry, *LOD* limit of detection, *GCE* glassy carbon electrode, *Gr* graphene, *DTSP* n-Hydroxysuccinimide ester, *HBS-EP* + 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% (v/v) P20.

associated with achieving an excessively high dilution factor. Instead, we relied on internal electrode performance assessment to ensure the accuracy and reliability of our results. This is a proof-of-concept study, it is important to stress that further experiments are necessary to refine the proposed immunosensor in terms of practical and clinical applicability. To comprehensively evaluate the sensor performance, a larger validation study is required in the future as well as an investigation of the long-term stability, storage conditions, and shelf life of the sensors.

With the new electrochemical method, we have investigated the difference in urinary $\beta 2M$ levels between controls and patients diagnosed with PCa. Several studies have demonstrated that electrochemical detection of $\beta 2M$ is possible, but to the best of our knowledge, this study is the first to present electrochemical measurements of $\beta 2M$ in urine samples from PCa patients.

Considering the effectiveness and low cost of the electrochemical biosensor combined with the obtained data, usage of the biosensor and $\beta 2M$ as supplementary diagnostic tools potentially may improve the diagnostic accuracy in PCa. However, the observed significant differences between the control group and PCa patients need further validation in larger cohort studies.

Conclusion

This work established proof-of-concept for an electrochemical biosensor for fast and direct detection of $\beta 2M$ in urine. Cyclic voltammetry was used to characterize the electrode functionalization. Using square wave voltammetry, it was possible to detect $\beta 2M$ in the concentration range 204–600 µg/L, covering the physiologically relevant concentrations, in about 45 min. Using this device, we demonstrated a significant difference in urinary $\beta 2M$ levels between controls and PCa patients. A larger sample size is required for the validation of such observation.

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Author contributions

All contributors fulfill the authorship requirements. The study was designed by F.A.A. and H.N., with F.A.A. and H.N. also handling data management and statistical analysis. The first draft of the manuscript was created by H.N., and all authors contributed to editing. The resources for the study were provided by F.A.A., J.S.M., and P.J.S.O. The final manuscript was approved by all authors.

Competing interests

The authors declare no competing interests.

Additional information

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