Research Article

Cancer Control
Volume 26: 1-7
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DOI: 10.1177/1073274819887697
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Prostate Cancer Diagnosis Using Urine Sediment Analysis-Based α-Methylacyl-CoA Racemase Score: A Single-Center Experience

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Abstract

To evaluate the diagnostic value of α-methylacyl-CoA racemase (AMACR) score in Han Chinese patients with prostate cancer (PCa) through urine sediment analysis. We collected 292 urine sediment samples after digital rectal examination. Levels of AMACR and prostate-specific antigen (PSA) messenger RNA (mRNAs) were evaluated by quantitative real time-polymerase chain reaction. The diagnostic value of AMACR score was assessed by receiver-operating characteristic analysis (ROC), Mann-Whitney test, logistic regression analysis and decision curve analysis. In all patients (n = 292), the area under the curve (AUC) for serum PSA, AMACR score, and a combinative model of these 2 parameters were 0.745 (95% confidence interval [CI]: 0.691-0.794), 0.753 (95% CI: 0.700-0.802), and 0.784 (95% CI: 0.732-0.830). No statistical difference was found between AMACR score and serum PSA ($P = .826$), while the combinative model was better than AMACR score ($Z = 5.222$, $P < .001$). Among patients with serum PSA level of 4 to 10 ng/mL (n = 121), the AMACR score was significantly higher in patients with PCa ($P = 0.0002$), while serum PSA showed no difference ($P = 0.3023$). Alpha-methylacyl-CoA racemase score (AUC = 0.712, 95% CI: 0.623-0.790) and a combinative model (AUC = 0.714, 95% CI: 0.626-0.793) showed a better diagnostic value than serum PSA (AUC = 0.559, 95% CI: 0.466-0.649), ($P = .048$, $P = .042$). Decision curve analysis showed a biopsy prediction model including AMACR score have a better net benefit when the threshold probability greater than 20%. The diagnostic model combing serum PSA and AMACR score has a better diagnostic value in patients with abnormal PSA level (including PSA level ranging from 4-10 ng/mL), and could reduce unnecessary prostate biopsy in clinical use.

Keywords

prostate cancer, α-methylacyl-CoA racemase, prostate-specific antigen, biomarkers, clinical diagnosis

Received April 3, 2019. Received revised August 8, 2019. Accepted for publication September 30, 2019.

Introduction

As with the highest morbidity and the second fatality rate of males in the United States and Europe, prostate cancer (PCa) remains a major health challenge worldwide.[1] Prostate cancer is the most common age-related cancer, which has become a substantial health burden in China with its rapidly aging population. Prostate cancer has the sixth highest morbidity and seventh highest mortality among cancers in China, which has been growing rapidly in recent years.[2] Early PCa screening has been advocated when curative radical surgery or local radiotherapy is possible.[3]

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Currently, the mainstay screening modalities for PCa are serum prostate-specific antigen (PSA) and digital rectal examination (DRE), positive results for which generally justifies patients to be recommended for transrectal ultrasonography (TRUSG)-guided prostate biopsy for further pathological diagnosis.

Prostate-specific antigen is particularly useful as an early diagnostic biomarker of PCa and it is the only marker that is clinical available for the diagnosis and prognosis prediction of PCa currently. It has been reported that PSA screening can decrease the mortality of PCa patients by 20%.[4,5] However, PSA represents the prostate tissue as a whole rather than being specific for PCa, since cancer cell is not the only cell type that produces PSA.[6] Some benign diseases like benign prostatic hyperplasia, or medical procedures such as TRUSG, can also lead to an elevated PSA level. Particularly, for patients with a PSA level arrangement grey zone (4-10 ng/mL), the detection rate of PCa by PSA is only 25%.[7] More importantly, the lack of specificity of PSA and the consequent high false negative rate could result in overdiagnosis and unnecessary treatment of PCa. The proportion of prostate biopsy in the United States each year after elevated PSA level was detected has been as high as 70-80%,[8] which is costly and invasive and may result in patient distress and potential side effects including urinary incontinence, erectile dysfunction, and patient anxiety.[9,10] Therefore, molecular markers with higher sensitivity and specificity are urgently needed to improve the diagnostic accuracy of PCa, and thus reduce the social and economic burden brought by the disease.

Alpha-methyl CoA-racemase (AMACR) is a peroxisomal and mitochondrial enzyme, which contributes to catalyze β-oxidation of branched fatty acids and catabolism of bile acid metabolites.[11] The AMACR gene, encoding 382 amino acids, is located on chromosome 5p13. Previous studies have reported that AMACR is overexpressed at both protein and mRNA levels in cancerous prostatic tissue,[12] with specificity and sensitivity of 97% and 100%, respectively, suggesting AMACR to be an excellent immunohistological biomarker for Pca.[13] As an important source of prostatic secretions and tumor shedding cells, urine sample is an essential tool in researches on noninvasive diagnostic markers of PCa. A study has demonstrated that AMACR mRNA can be detected in the cellular component of patients' urine collected postprostate biopsy or massage.[14] However, the current application of urine AMACR is mostly an assistive diagnostic marker to other existing PCa-related indicators such as PCA3,[15] or one of the multiple diagnostic indicators.[16] Meanwhile, studies on urine AMACR mRNA were mostly conducted in European and American populations,[17] with relatively small sample size and limited representativeness. Our purpose is to investigate the distribution of AMACR score in urinary sediment cells of Chinese patients who underwent prostate biopsy, to explore the diagnostic role of AMACR in PCa patients with intermittent or "grey-zone" level of PSA, and to establish a more efficient noninvasive diagnostic model for PCa.

Materials and Methods

This study was approved by the Ethics Committee of Changhai Hospital, Naval Medical University (Second Military Medical University; NO. CHEC2013-115). To conduct a retrospective analysis, we collected 292 urine samples of patients with elevated serum PSA who visited the urology clinic from March 2011 to April 2017 (Supplementary Table S1). Informed consents were obtained from all patients. All patients underwent ultrasound-guided prostate biopsy with 6 to 12 needles, and the biopsy samples and the pathological diagnosis were confirmed by 2 senior pathologists. The study cohort consisted of both patients with positive needle biopsy (PCa, n = 138) and non-PCa patients (n = 154).

Sample Collection and Preparation

First morning urine was collected after prostate massage before prostate examination in all patients. The massage maneuver: Press from both sides of the prostate to the central line for 3 times, followed by massaging the central line from top to bottom for 3 times. The initial urine after micturition was collected (about 50 mL) and stored at 4°C, which was then centrifuged at ×4000 rpm for 15 minutes at 4°C within 3 hours. After removing the supernatant, the urine sediment was washed again with cold PBS (×1) and centrifuge at ×5000 rpm for 15 minutes at 4°C. Discard the supernatant, homogenize the sediment in 1.5 mL centrifuge tubes for RNA extraction or further use. Extract the total RNA of urine sediment with the manufacturer's instructions (HiPure Total RNA Mini Kit, Magen, China). The RNA concentration and purity were measured using Nanodrop 2000 (Thermo Scientific, US) and those with an RNA concentration lower than 5 ng/μL were excluded. Amplify the complementary DNA with TransPlex Complete Whole Transcriptome Amplification Kit (WTA2, SIGMA, China) according to the manufacturer's instructions.

Quantitative RT-PCR Analysis

Quantitative real time-polymerase chain reaction (RT-PCR) was performed to detect the expressions of AMACR and PSA mRNAs in urinary sediment using SYBR Green Realtime PCR Master Mix (QPK-201, Toyobo, Japan) with Applied Biosystem QuantStudio 3 Real-Time PCR System (Thermo Fisher, US) according to the manufacturer's recommended cycling conditions. Quantitative real time-polymerase chain reaction primers were designed as follow: AMACR forward primer 5′-CTGGTGGTGGCCTTATGTGT-3′, AMACR reverse primer 5′-CCAAGTCCTTTGATCAGCAGC-3′; PSA forward primer 5′-GTGACGTGGATTGGTGCTG-3′, PSA reverse primer 5′-GAAGCTGTGGCTGACCTGAA-3′. All experiments were performed in triplicate wells. The data were analyzed using QuantStudio Design & Analysis Software (Thermo Fisher, US). The expression of AMACR was represented by the AMACR score (AMACR score = AMACR mRNA/PSA mRNA × 1000 = Ct[PSA]−Ct[AMACR] × 1000].

Statistical Analysis

Statistical analyses were performed using SPSS Software version 21.0 (IBM, US). To analyze the difference between patients

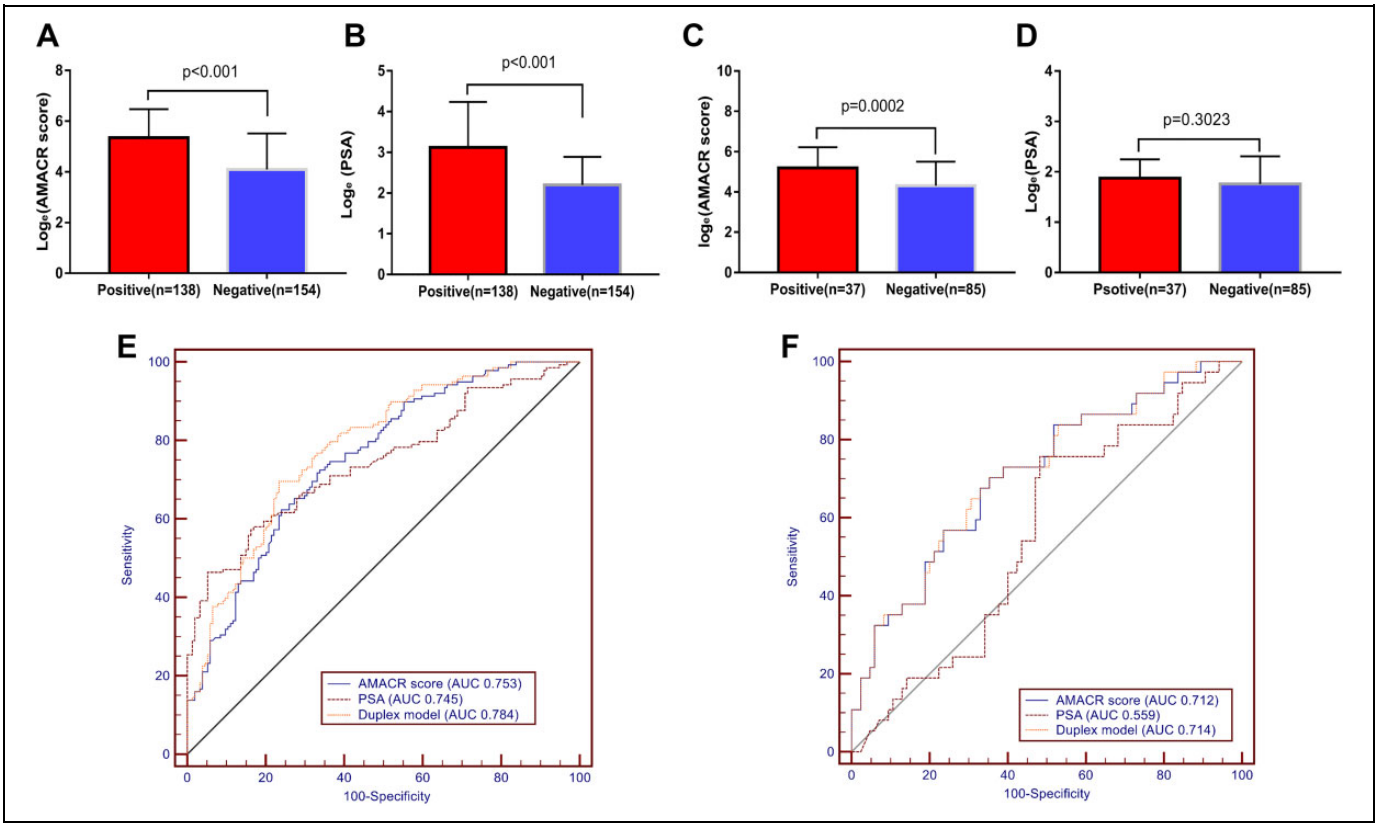

Figure 1. Comparison of urinary sediment AMACR scores (A) and serum PSA (B) between patients with positive (n = 138) and negative (n = 154) biopsy in all patients. Comparison of urinary sediment AMACR scores (C) and serum PSA (D) between patients with positive (n = 37) and negative (n = 85) biopsy in "grey-zone PSA level" patients. Receiver operating characteristic-AUC analysis of AMACR score, serum PSA and duplex model in all prostate biopsy patients (E) and in the diagnosis of "PSA grey zone" biopsy patients (F). AMACR indicates α-methylacyl-CoA racemase; AUC, area under the curve; PSA, prostate-specific antigen.

with positive and negative biopsy results, we performed the Mann-Whitney *U* test. We assessed the relationships between AMACR score and clinical variables by the Spearman rank correlation test. The regression model of AMACR score and serum PSA was established using logistic regression analysis. We also constructed the receiver-operating characteristic (ROC) curves among different groups of patients, and the predictive power of AMACR score, serum PSA and the combination of the 2 was evaluated by the ROC curve and area under the curve (AUC). The AUCs of different diagnostic models were compared by Pairwise comparison. The patient's benefit threshold was assessed by the decision curve. All *P* values were 2-sided, and $P < .05$ was considered statistically significant.

Results

The Predictive Value of AMACR Score for Prostatic Biopsy Result

Among all 292 patients undergoing TRUS-guided prostate biopsy, 138 had positive and 154 had negative results with a positive rate of 47.3%. The patients had a mean age of 66 ± 7.29 years old and the mean prostate volume of them was 62.55 mL (interquartile range [IQR]: 34.31-75.12). The mean serum PSA level, fPSA level, and fPSA/PSA ratio of the patients were 26.65 ng/mL (IQR 7.31-21.93), 3.22 ng/mL (IQR 0.97-3.04), and 0.21 (IQR 0.11-0.21), respectively. Digital rectal examination revealed positive results in 76 (26%) cases.

The AMACR scores in all patients were not normally distributed, we used Mann-Whitney test to evaluate the diagnostic values of AMACR score and serum PSA level. Both parameters were found to be elevated in patients with PCa (Figure 1A, $P < .001$, Figure 1B, $P < .001$). Spearman test showed that the AMACR score was correlated with age ($P = .014$), serum PSA level ($P < .001$), fPSA/tPSA ratio ($P = .047$), DRE ($P = .021$) and PSA density ($P < .001$). However, serum fPSA level, prostate volume, and Gleason score were unrelated to the AMACR score (Supplementary Table S2).

We used logistic regression analysis to identify the risk factors for PCa diagnosis. However, except for AMACR score and serum PSA, other indexes showed no statistical significance in the logistic regression analysis (age, $P = .185$, fPSA/tPSA ratio, $P = .180$, DRE, $P = .454$, PSA density, $P = .35$). Therefore, a regression model of serum PSA and AMACR score was established accordingly. Among all patients, the odds ratios (ORs) of serum PSA and AMACR score were 15.78 and 5.16, which discriminated patients with

PCa from patients who had a negative biopsy result. Receiver-operating characteristic analysis (**Figure 1E**) was then used to evaluate the AUCs for serum PSA, AMACR score, and the combinative model of these 2 parameters (logit(P) = −1.99316 +0.06779 × PSA + 0.00385 × AMACR score) for the discrimination between PCa and non-PCa samples. The AUCs for serum PSA, AMACR score, and the combinative model were 0.745 (95% confidence interval [CI]: 0.691-0.794), 0.753 (95% CI: 0.700-0.802), and 0.784 (95% CI: 0.732-0.830), respectively. No statistical difference ($Z = 0.22$, $P = 0.826$) was found in the comparison of diagnostic value between AMACR score and serum PSA. Nevertheless, the combinative model showed a significantly better diagnostic value compared to AMACR score alone ($Z = 5.222$, $P < .001$), which indicates that the combination of AMACR score and serum PSA can enhance the accuracy of prediction of prostate biopsy results in all patients.

The Predictive Role of AMACR Score in Patients With PSA Level of 4 to 10 ng/mL

One hundred twenty-two patients who underwent TRUS guided prostatic biopsy had PSA levels of 4 to 10 ng/mL (grey zone). Among which, 37 (47.26%) were confirmed to have positive PCa and 85 patients were negative. The mean age of these patients was 64 ± 8.19 years and the mean prostate volume was 57.35 mL (IQR 33.12-72.10). Mean serum PSA levels, fPSA levels, and fPSA/PSA ratio in these patients were 6.57 ng/mL (IQR 5.35-8.16), 1.39 ng/mL (IQR 0.71-1.56), and 0.18 ng/mL (0.12-0.22), respectively. Meanwhile, DRE was proved to be positive in 30 (24.59%) cases.

The AMACR score was significantly higher in PCa group compared to non-PCa group (Figure 1C, $P = 0.0002$), while no difference was observed in serum PSA level between both groups (Figure 1D, $P = 0.3023$). Spearman test showed that AMACR score was not associated with patient age, serum PSA and fPSA levels, fPSA/tPSA ratio, DRE, PSA density, prostate volume and Gleason score in patients with PSA level of 4 to 10 ng/mL (Supplementary Table S3). The ORs of serum PSA level and AMACR score were 3.34 and 4.33, respectively. Area under the curve-ROC analysis was then conducted for serum PSA, AMACR score and the combinative model of these 2 values (logit(P) = −2.39525 + 0.12487 × PSA + 0.00402 × AMACR score) to discriminate between PCa and non-PCa samples (Figure 1F). The AUCs for serum PSA, AMACR score, and the combinative model were 0.559 (95% CI: 0.466-0.649), 0.712 (95% CI: 0.623-0.790), and 0.714 (95% CI: 0.626-0.793), respectively. Alpha methylacyl-CoA racemase score showed a significantly better diagnostic value ($Z = 1.972$, $P = .048$) than serum PSA alone in patients with PSA level among 4 to 10 ng/mL, and the combinative model had a better predictive capability than serum PSA level alone ($Z = 2.029$, $P = .042$).

Evaluating the Diagnostic Performance of Established Models With Decision Curve Analysis

Decision curve analysis was conducted for biopsy prediction in base model (PSA, age, prostate volume, fPSA/PSA, and DRE) and the optimized model combining the base model and the AMACR score showing the optimized model had a better performance when the threshold probability was greater than 20% (Figure 2). Furthermore, after analyzing for different threshold to reduce unnecessary prostate biopsies, the combinative model was found to have a better net benefit when the threshold probability was greater than 20% in both the whole cohort and patients with grey-zone PSA level (Table 1), and the combinative model could avoid 18 unnecessary prostate biopsies, without missing one case of PCa, while the basic model could avoid 12 unnecessary prostate biopsies at the predicted probability threshold value of 15% in the whole cohort (Table 2). However, no significant difference in this respect was found between these 2 models in patients with grey-zone PSA level.

Discussion

New molecular biomarkers are particularly needed in clinical practice to distinguish PCa from benign disease. Due to the limitations of PSA, the diagnostic rate of PCa in patients, especially a specific group of patients, that is, those with PSA level of 1 to 4 ng/mL, is between 16% and 39%,[5,18] which results in unnecessary prostate biopsies in a great number of patients with benign diseases which increases patients' social and economic burdens. Since prostatic secretion products and shedding tumor cells are present in the urine, the discovery of potential urine molecular biomarkers for PCa is of vital importance as a non-invasive diagnostic approach for PCa. Several urine PCa biomarkers have been reported, such as fusion gene TMPRSS2: ETS, prostate cancer antigen 3 (PCA3), glutathione S-transferase P1, vascular endothelial growth factor (VEGF), matrix metalloproteinases-9, and annexin A3, among which PCA3 is the most widely used in current practice. As a long noncoding RNA, PCA3 is located on chromosome 9 (9q21-22) which has optimal diagnostic potential because it only expresses in PCa tissues. Previous studies[19] have shown that detecting patients' urine PCA3 using RT-PCR can help avoid unnecessary biopsies. Additionally, a study[20] showed that the combination of PCA3 and TMPRSS2: ERG or PCA3 and PSA[21] can improve the detecting rate of PCa. Notably, there are studies[22,23] claiming that the diagnostic efficacy of PCA3 could be significantly different in different populations. Therefore, PCA3 is not necessarily the most suitable for the Chinese population, which warrants the need to identify molecular markers that are more suitable for the Chinese population.

Alpha-methylacyl-CoA racemase is highly expressed in PCa tissues, which has been demonstrated with good diagnostic value[7,24] with a sensitivity between 82% and 100% and a specificity between 79% and 100%. However, most of the studies about AMACR were conducted on the basis of tumor tissue samples, and the relationship between AMACR expression and

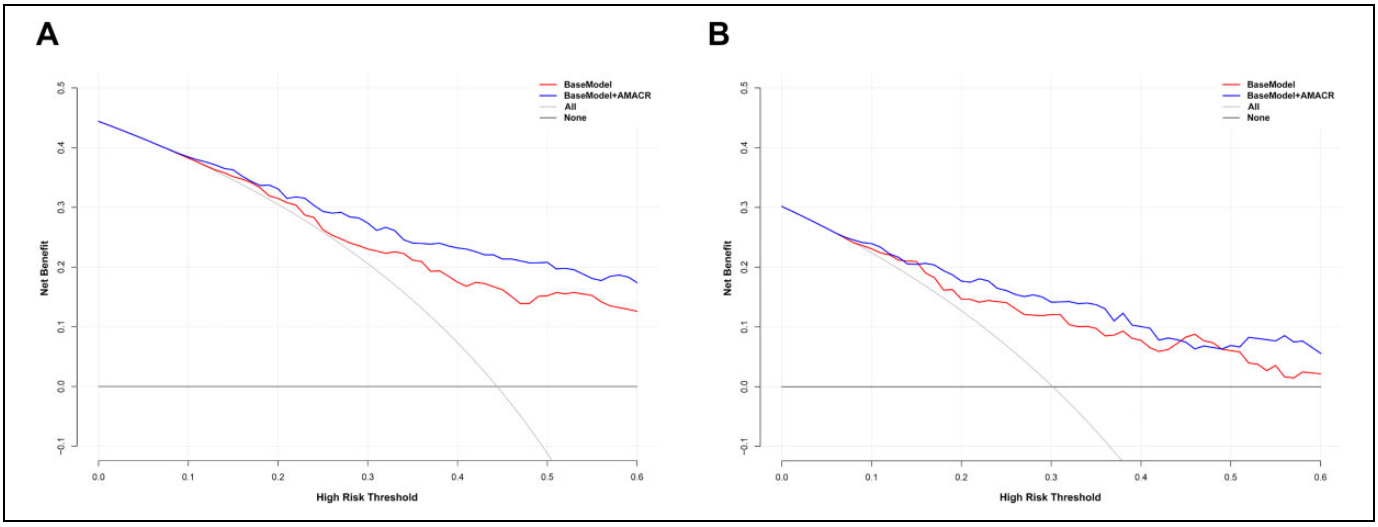

Figure 2. Decision curve analysis for biopsy prediction in the whole cohort (A) and patients with grey zone PSA level by the base model (PSA, age, prostate volume, fPSA/PSA and DRE). The yellow line represents the combinative model (base model plus AMACR score); the green line represents the base model. The horizontal line and the x-axis represent no patient underwent biopsy, while the solid blue line represents the assumption of all patients will have PCa (biopsy all). AMACR indicates α-methylacyl-CoA racemase; DRE, digital rectal examination; PSA, prostate-specific antigen.

Table 1. Comparation of Net Benefit for the Base Model and Combinative Model With AMACR Score Between all Biopsy Patients (Treat all) for Different Threshold Probabilities.

	Threshold Probability (%)	10	15	20	25	30	35	40	45	50
Net benefit for all patients	Base model	38.27	35.157	31.50	26.267	23.03	21.17	17.47	16.22	15.20
	Base model +AMACR	38.44	36.28	33.10	29.33	27.37	24.03	23.20	21.35	20.80
	Treat all	38.22	34.59	30.50	25.87	20.57	14.46	7.33	−1.09	−11.20
Net benefit for grey zone patients	Base model	23.08	20.94	14.66	14.08	12.07	9.74	7.76	8.31	6.03
	Base model +AMACR	23.95	20.49	17.67	16.09	14.16	13.73	10.06	7.45	6.90
	Treat all	22.41	17.85	12.72	6.90	0.25	−7.43	−16.38	−26.96	−39.66

Abbreviation: AMACR, α-methylacyl-CoA racemase.

Table 2. Number of Prostate Cancer Missed and Unnecessary Biopsies Reduction Using Base Model and Base Model Plus AMACR Score With Probability Threshold Value in the Range of 15% to 40% for all Patients.

Probability Cut-Off (%)	Model	PCa Missed, No (%)	Unnecessary Biopsies Spared, No (%)
15	Base model	0	12 (4.1)
	Base model + AMACR	0	18 (6.2)
20	Base model	2 (0.7)	21 (7.2)
	Base model + AMACR	5 (1.7)	42 (14.4)
25	Base model	7 (2.4)	30 (10.3)
	Base model + AMACR	11 (3.8)	65 (22.3)
30	Base model	19 (6.5)	56 (19.2)
	Base model + AMACR	17 (5.8)	80 (27.4)
35	Base model	27 (9.2)	82 (28.1)
	Base model + AMACR	26 (8.9)	100 (34.2)
40	Base model	35 (11.9)	95 (32.5)
	Base model + AMACR	33 (11.3)	106 (36.3)

Abbreviation: AMACR, α-methylacyl-CoA racemase.

PCa cannot be studied precisely due to the subjective limitation of histochemical staining score.[12] Rogers et al[25] found that patients with negative prostate biopsy result could be diagnosed by elevated urine AMACR protein level; while Sroka et al[26] reported that the expression of AMACR protein in urine could not distinguish benign prostate diseases from PCa. In the present study, we used AMACR score firstly proposed by Zielie et al[17] to evaluate its diagnostic efficacy for PCa in Chinese population.

In this study, we used 198 samples in the preliminary experiment and other 94 samples for internal validation. In the total 292 urine samples, we found that both AMACR score and serum PSA level could be used as diagnostic markers for PCa ($P < .001$). However, ROC analysis indicated that there was no statistical difference ($Z = 0.22$, $P = .826$) between AMACR score and serum PSA, indicating that serum PSA is still of great value in the diagnosis of PCa in Chinese people, and single usage of AMACR score has no superiority over serum PSA in PCa diagnosis.

Kanyong et al[13]stated that the application of AMACR was limited by its low specificity on PCa diagnosis when used alone. Meanwhile, an appropriate serum PSA level to be included in the diagnostic criteria for the Chinese population is to be determined.[27] Consistently, we investigated a diagnostic model combining AMACR score and serum PSA level in the Chinese population, this model was superior to the single use of serum PSA or AMACR score. Similarly, Ouyang et al[28] has measured the transcription levels of PCA3, AMACR, and PSA in urine sediments in 92 patients indicating combing AMACR and PCA3 could reach a more superior diagnostic efficiency with a sensitivity of 81% and specificity of 84%. Jamaspishvili et al[15] also reported that the combined use of 4 markers (TRPM8, MSMB, AMACR, and PCA3) could complement the limitations of single markers. Therefore, the combination of multiple indicators would be a better solution to improve the diagnostic efficiency for PCa.

Previous studies have reported AMACR was the only biomarker that could play a diagnostic role for PCa in patients with serum PSA levels among 3 to 15 ng/mL, with an AUC of 0.645.[9] In this study, data on 122 patients with grey zone PSA levels (4-10 ng/mL) revealed that patients with positive biopsy results had significantly higher AMCAR scores ($P = 0.0002$), while serum PSA showed no differences for positive biopsy result, which is consistent to conclusions in previous studies. Furthermore, ROC analysis indicated that both AMACR score alone (AUC = 0.712, $P = .048$) and the combinative model of serum PSA and AMACR score (AUC = 0.714, $P = .042$) had better predictivity in grey-zone PSA patients compared to serum PSA alone. Therefore, this study suggests a limited diagnostic value of serum PSA in PSA grey zone patients while AMACR score alone or in combination with serum PSA can be more effective in the diagnosis of PCa. On the basis of the above evidence, we thus recommend AMACR score to be adopted as a clinical diagnostic marker for PCa, patients with gray-zone PSA levels.

In this study, we first demonstrated the novel model proposed in this study provided better prediction in the whole patient cohort to avoid unnecessary prostate biopsies than the basic model (18 vs 12). These results indicate that the combined use of AMACR score and basic model in all patients can reduce unnecessary biopsy without increasing the chance of missed diagnosis thereby reducing potential injuries to patients and lowering their financial burden. However, in patients with gray-zone PSA level, the optimized model was not significantly more superior than the basic model, which may due to the limited sample size in this study.

There are several limitations in this study: Firstly, the study is limited by its single-centered data with a small number of patients; secondly, there is a lack of comparison of urine AMACR with either tissue and blood samples, or other biomarkers already reported elsewhere. Previous studies have shown that combination of multiple clinical markers can improve the diagnostic efficiency, more diagnostic indicators, such as PCA3, should be included in further studies to improve the diagnostic efficiency of this model. Therefore, the urine AMACR score needs to be verified by future studies with multicenter nature and large-scale clinical samples.

Authors' Note

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of Changhai Hospital, Naval Medical University (Second Military Medical University). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (No. 81430058); Shanghai Key Laboratory of Cell Engineering (14DZ2272300).

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Supplemental Material

Supplemental material for this article is available online.

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