



Original Article

## Diagnostic value of a combined AMACR, P63, and ERG immunohistochemical panel in distinguishing benign from malignant prostate lesions

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Received: 11 December 2025

Accepted: 07 March 2026

Published: 01 May 2026

**DOI**

10.25259/IJHS\_385\_2025

**Quick Response Code:**



Supplementary material is available at:

[https://dx.doi.org/10.25259/IJHS\\_385\\_2025](https://dx.doi.org/10.25259/IJHS_385_2025)

### ABSTRACT

**Objectives:** Diagnosing prostate cancer is difficult because its histological traits often overlap with those of benign conditions. Alpha-methylacyl-CoA racemase (AMACR), tumor protein 63 (P63), and erythroblast transformation-specific-related gene (ERG) are commonly utilized immunohistochemical markers. The triple-marker panel showed a marginal incremental diagnostic benefit over AMACR alone and may improve diagnostic confidence in selected difficult cases.

**Methods:** The specimens were immuno-stained with antibodies targeting AMACR, P63, and ERG. Sensitivity and specificity for AMACR, P63, and ERG were determined. Hematoxylin and eosin staining is the reference standard as it is a rapid, cost-effective diagnostic tool offering excellent morphological detail, allowing accurate identification of architectural and cellular features for diagnosis, grading, and prognosis.

**Results:** Among the evaluated specimens, 128 (66%) showed benign prostatic hyperplasia, while 66 (34%) showed prostatic adenocarcinoma on histopathological examination (HPE). Perineural invasion occurred in 32 (48.5%) of malignant cases. AMACR immunohistochemical expression was found in 54 (82%) of malignant cases and 24 (18.7%) of benign cases. P63 expression was present in 122 (95.3%) benign lesions. ERG expression was observed in 23 (34.8%) of malignant cases. The diagnostic accuracy of AMACR, P63, and ERG was 98.3%, 89.7%, and 86%, respectively. The triple-marker panel (AMACR + P63 + ERG) achieved 97% sensitivity, 99.2% specificity, and 98.5% diagnostic accuracy, indicating an incremental diagnostic gain in prostate biopsy specimens.

**Conclusion:** The triple-marker panel yielded a diagnostic accuracy rate of 98.5%, despite ERG's comparatively lower sensitivity. The combined use of AMACR, P63, and ERG may be considered in selected diagnostically challenging cases.

**Keywords:** Alpha-methylacyl-CoA racemase (AMACR), Diagnostic accuracy, Erythroblast transformation-specific-related gene (ERG), Prostate adenocarcinoma, Tumor protein 63 (P63)

### INTRODUCTION

Prostate cancer (PC) has an age-adjusted incidence rate of 29.4/100,000 individuals.<sup>[1]</sup> PC rates are higher in North America, Europe, and Australia than in Asia and Africa.<sup>[2]</sup> Although it

**How to cite this article:** Sharma G, Sharma S, Kumar P, Bhargava R, Agrawal H. Diagnostic value of a combined AMACR, P63, and ERG immunohistochemical panel in distinguishing benign from malignant prostate lesions. *Int J Health Sci (Qassim)*. 2026;20:163-71. doi: 10.25259/IJHS\_385\_2025

predominantly affects older men, there has been a significant rise in cases among younger individuals, especially in urban areas. Enhanced awareness and improved access to health care contribute to higher rates of case detection.<sup>[3-5]</sup>

The differentiation of benign, malignant, or atypical prostate lesions continues to present significant diagnostic challenges, underscoring the necessity for improved detection methods. In recent years, immunohistochemical markers have emerged as critical adjuncts to conventional histopathology in the classification of prostate lesions.<sup>[6]</sup>

Alpha-methylacyl-CoA racemase (AMACR) stains the cytoplasm of malignant prostate cells. However, its expression can be heterogeneous and sometimes present in benign mimics, necessitating additional markers to prevent false-positive or negative results. Incorporating additional diagnostic markers is advisable for effective stratification between benign and malignant prostatic lesions. Tumor protein 63 (P63) stains basal cell nuclei found in normal prostate glands, but not in cancer cells.

The erythroblast transformation-specific-related gene (ERG) transcription factor oncogenic properties have been identified in various malignancies, notably Ewing's sarcoma, leukemia, and PC.<sup>[7]</sup> Within the field of PC, ERG gene rearrangements – most notably those involving *TMPRSS2* – are commonly observed across various stages of the disease. These genetic alterations lead to androgen receptor-mediated upregulation of ERG expression in carcinoma cells. Research by investigating ERG expression through both immunohistochemistry and fluorescent *in situ* hybridization (FISH) has demonstrated that immunohistochemical detection of ERG in prostate adenocarcinoma strongly correlates with ERG rearrangements identified by FISH.<sup>[8]</sup>

This study analyzed AMACR, P63, and ERG expression in benign and malignant prostate lesions. The primary objective was to assess the diagnostic accuracy of each marker individually and to examine the potential advantages of utilizing a combination of markers concurrently.

## MATERIALS & METHODS

An observational analytical study was conducted at a tertiary care teaching hospital in the northern region of the subcontinent from December 2023 to April 2025. The study received approval from the ethics committee. Written informed consent was obtained from all patients as per the tenets of the Declaration of Helsinki. The manuscript adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines when presenting findings on diagnostic accuracy [Supplementary Material].

A total of 202 samples from the Urology department were examined by HPE, followed by immunostaining for AMACR,

P63, and ERG. Prostate tissue was collected through ultrasound-guided transrectal biopsy, transurethral resection of the prostate, or radical prostatectomy in 12 (5.9%) cases.

## Histopathology

The tissue was preserved using formalin fixation to maintain structural integrity, followed by dehydration, clearing, and infiltration before embedding in paraffin wax. Sections measuring 3–5  $\mu\text{m}$  in thickness were prepared with a microtome, mounted onto glass slides, and subsequently stained with hematoxylin and eosin (H&E). Two independent pathologists examined the tissue morphology, cellular characteristics, and staining patterns to diagnose the presence and type of prostate pathology, including malignancies. The malignant cases were analyzed for Gleason grading and perineural invasion. Each case received a grade group based on WHO's latest recommendations.<sup>[9]</sup> The Gleason grade compression was conducted based on the following criteria: Grade group 1 included Gleason scores of 6 or less, grade group 2 included Gleason 3 + 4 = 7, and grade group 3 included Gleason 4 + 3 = 7. Grade group 4 included Gleason 8, and grade group 5 included Gleason scores of 9 and 10. Another pathologist re-examined the H&E-stained slides to perform a repeat Gleason scoring.<sup>[10]</sup> Patients were excluded if they had previous PC treatment, non-acinar PC variants, or insufficient biopsy material.

## Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks were sectioned (typically 4–5  $\mu\text{m}$  thick) and mounted on slides. Slides were deparaffinized (removal of paraffin) and rehydrated through a series of alcohol solutions to prepare them for staining. Immunostaining was conducted using an automated Dako Omnis immuno-stainer. An anti-AMACR (13H4) Rabbit immunoglobulin G (IgG) monoclonal antibody (#IR060: Agilent, USA) and anti-ERG (EP111) Rabbit IgG monoclonal antibody (#IR659: Agilent, USA) were utilized. For P63 immunostaining, sections were deparaffinized, rehydrated, and microwaved in citrate or ethylenediaminetetraacetic acid buffer as per the manufacturer's instructions. After 30 min of cooling at room temperature, a 1:50 dilution of P63 4A4 mouse monoclonal antibody (AS Bioscience, Telangana, India) was applied.

The immunoreactivity of the antibodies was evaluated using a semiquantitative scoring method that considered both the proportion of positively stained tumor cells and the intensity of staining. The expression levels of each protein were determined by calculating a total immunoreactive score, derived from the product of the proportion and intensity scores. For AMACR, the staining patterns were categorized as follows: 0 for non-circumferential staining, 1+ for focal apical

granular staining, 2+ for diffuse weak cytoplasmic staining, and 3+ for strong cytoplasmic staining. Optimal P63 staining shows moderate to strong, distinct nuclear staining in the basal cells of prostate glands. For ERG, 0 indicated negative staining, 1+ represented weak staining, 2+ signified strong staining but lighter than endothelial cells, and 3+ matched the intensity of staining of endothelial cells. The staining proportion score estimates the fraction of positively stained tumor cells: 0 (none), 1 (1–10%), 2 (11–50%), and 3 (>51%).

#### Criteria for defining positivity of the triple-marker panel

The statistical method for defining triple-marker panel positivity when comparing HPE with AMACR, P63, ERG, and their triple-marker panel was the “any two out of three positive” rule. According to this approach, a case was considered positive if at least two out of the three markers (AMACR, P63, and ERG) tested positive. The second method used was receiver operating characteristic (ROC) curve analysis, which assessed the combined diagnostic effectiveness and accuracy of these markers and identified the best threshold for positivity.

#### Statistics

Statistical analysis was performed using IBM Statistical Package for the Social Sciences Statistics version 30 (IBM Inc.). Continuous variables were reported as means with standard deviation. Frequencies and percentages were calculated for all categorical variables. The independent-sample *t*-test was used to determine if a difference exists between the means of two independent groups on a continuous dependent variable. Chi-square tests were used for proportions. The sensitivity and specificity of ERG and AMACR were determined using H&E staining as the reference standard. H&E staining is widely recognized as a rapid, cost-effective diagnostic method that provides superior morphological detail, enabling accurate evaluation of architectural and cellular characteristics for diagnosis, grading, and prognosis of malignant lesions. The binary method was applied using a 2 × 2 contingency table (confusion matrix). This analysis was complemented by the generation of an ROC curve, with the area under the curve (AUC) calculated accordingly. Sensitivity and specificity were further validated through coordinate points on the ROC curve. The apex of the ROC curve, marked by arrows, reflects the sensitivity for each parameter, while specificity is denoted by the corresponding coordinate point. The results obtained from both the binary method and ROC curve analysis were consistent.

A ROC curve analysis was performed for AMACR P63, ERG, and the triple-marker panel. AUC with 95% confidence intervals informed in a single numerical value about the overall diagnostic accuracy of the test. All tests

were conducted using two-sided analysis, and  $p < 0.05$  was regarded as significant.

## RESULTS

Out of 202 urology samples, 194 (96%) were assessed successfully, while 8 (4%) were excluded because they lacked enough biopsy material. Among the evaluated samples, 128 (66%) had benign prostatic hyperplasia (BPH) and 66 (34%) had prostatic adenocarcinoma on HPE. Figure 1 illustrates participant flow. The average age of patients diagnosed with BPH was  $57.6 \pm 5.6$  years (ranging from 50 to 68 years), while the average age of patients diagnosed with prostatic adenocarcinoma was  $69.5 \pm 6.6$  years (ranging from 58 to 78 years). The difference in mean ages was statistically significant (independent *t*-test,  $p < 0.001$ ). The mean prostate-specific antigen (PSA) level in patients with BPH was  $7.8 \pm 6.6$  ng/mL, while in patients with prostatic adenocarcinoma, it was  $28.4 \pm 18$  ng/mL (independent *t*-test,  $p < 0.001$ ). Among the malignant cases, 6 (9%) cases were classified as Gleason's grade 1, 5 (7.6%) cases as grade 2, 17 (25.7%) cases as grade 3, 20 (30.3%) cases as grade 4 [Figure 2], and 18 (27.3%) cases as grade 5, respectively. Perineural invasion was found in 32 (48.5%) of the malignant cases.

Table 1 presents the clinical and pathological characteristics of AMACR expression in the study participants. AMACR immunohistochemical expression [Figure 3] was found in 54 (82%) of malignant cases and 24 (18.7%) of benign cases, showing varying levels of expression. AMACR expressions are significantly correlated with the type of prostatic lesion ( $p < 0.001$ ), patient age ( $p < 0.001$ ), carcinoma grade groups ( $p < 0.001$ ), PSA ( $p < 0.001$ ), and perineural invasion ( $p < 0.001$ ). A higher expression of AMACR correlated with higher PSA levels (Pearson's correlation coefficient,  $r = 0.634$ ).

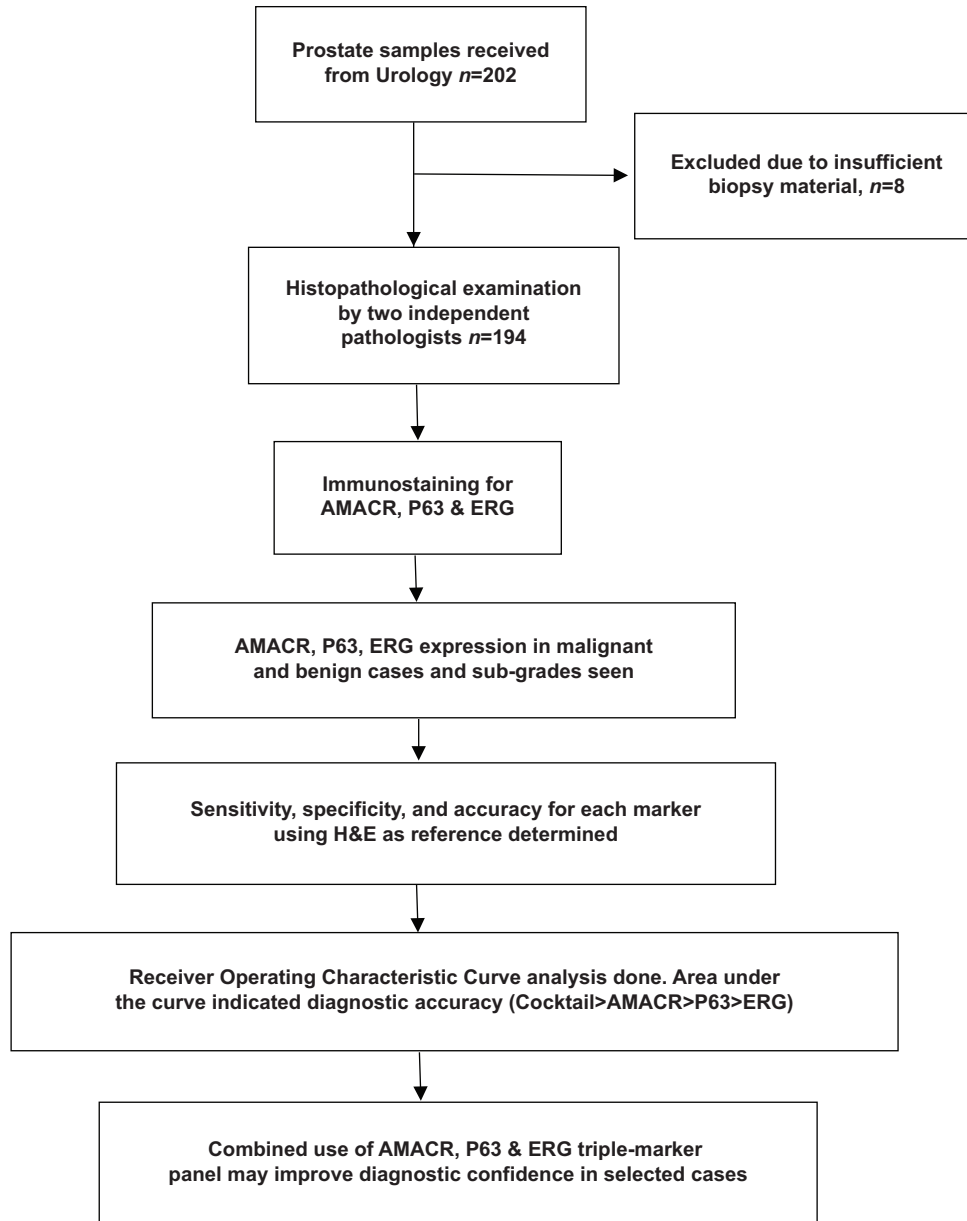
P63 expression was found in 118 (92.2%) benign cases and 10 (15.1%) malignant cases [Figure 4]. ERG expression was absent in benign cases but detected in 23 (34.8%) of malignant cases [Figure 5], demonstrating a statistically significant difference between the two groups.

In malignant cases, ERG expression showed a significant correlation with age ( $p < 0.001$ ), Gleason's grade ( $p < 0.001$ ), and perineural invasion ( $p = 0.010$ ) as detailed in Table 2.

Table 2 presents the clinical and pathological characteristics of ERG expression in the study participants. Our study identified a statistically significant correlation between AMACR and ERG expression, as determined by the Chi-square test ( $p = 0.004$ ).

#### Sensitivity, specificity, and diagnostic accuracy

Sensitivity was calculated as the proportion of true positives relative to the combined total of true positives and false



**Figure 1:** Flow diagram illustrating participants' flow. AMACR: Alpha-methylacyl-CoA racemase, ERG: Erythroblast transformation-specific-related gene, H&E: Hematoxylin & eosin.

negatives. Specificity represents the proportion of true negatives compared to the sum of true negatives and false positives. Diagnostic accuracy was determined by dividing the sum of true positives and true negatives by the total number of cases.

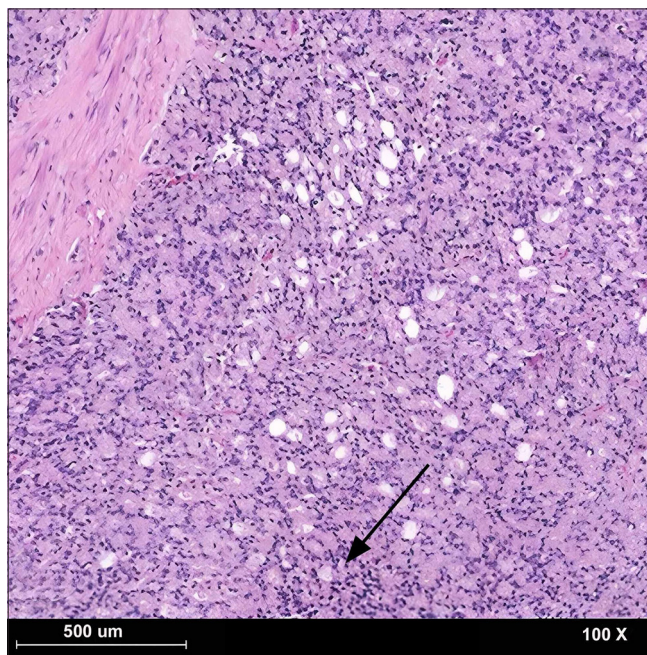
The sensitivity, specificity, and diagnostic accuracy of AMACR were 84.8%, 98.4%, and 98.3%, respectively. The sensitivity, specificity, and diagnostic accuracy of P63 were 92.2%, 84.6%, and 89.7%, respectively. The sensitivity, specificity, and diagnostic accuracy of ERG were 59.1%, 95.3%, and 86%, respectively.

### ROC curve analysis

The ROC curve analysis demonstrated that the AUC for AMACR, P63, ERG, and the triple-marker panel (AMACR + P63 + ERG) was 0.916, 0.885, 0.817, and 0.981, respectively [Figure 6]. The diagnostic accuracy in distinguishing between benign and malignant prostate lesions improved to 98.5% when the three markers were used in a triple-marker panel. Table 3 shows the ROC curve's coordinate points for estimating sensitivity and specificity.

## DISCUSSION

This study examined the immunohistochemical expression of AMACR, P63, and ERG in 66 prostatic adenocarcinoma cases. A control group included 128 patients with prostatic



**Figure 2:** Hematoxylin and eosin-stained photomicrograph ( $\times 100$ ) illustrating prostate adenocarcinoma (Gleason grade 4) with abundant malignant cells (arrow).

hyperplasia. The goal was to assess how well these individual markers, and their combination, could diagnose prostatic carcinoma at a tertiary care teaching hospital in Northern India.

Immunohistochemistry helps distinguish low-grade PC from benign conditions and identifies the origin of poorly differentiated carcinomas in metastatic samples. Recently, biomarkers like AMACR have improved diagnostic accuracy due to their high sensitivity and specificity, aiding difficult PC cases worldwide.

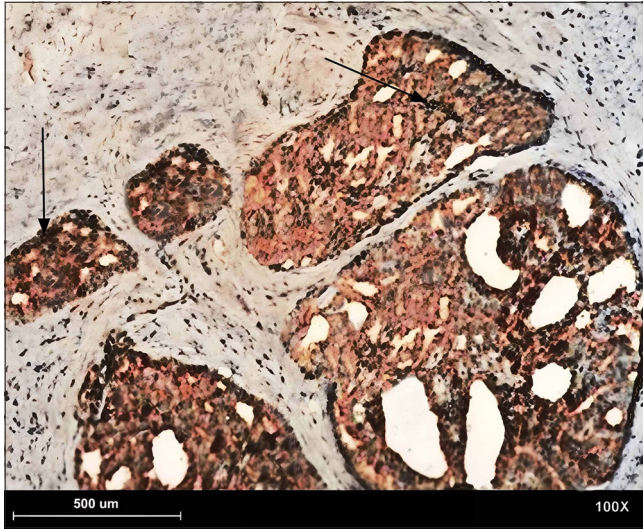
AMACR was the first marker to enhance carcinoma diagnosis beyond basal cell immunohistochemistry, after being identified as commonly overexpressed in PC.<sup>[11]</sup> Some diagnoses may require additional markers due to limitations of basal cell markers and AMACR. Basal cell markers like P63 can be inconsistent in benign glands, requiring multiple sections for diagnostic accuracy.<sup>[12]</sup> AMACR, while sensitive to malignant lesions, shows varied expression in PC and can appear weak to moderate in benign lesions.<sup>[13]</sup> ERG gene fusion is commonly observed in PC patients. Immunohistochemistry using anti-ERG antibody shows a strong correlation with ERG rearrangement as identified by FISH.<sup>[14]</sup>

In our study, AMACR expression was significantly higher in prostatic adenocarcinoma cases, indicating its strong potential as a diagnostic marker for distinguishing malignant from benign prostate lesions in clinical practice.<sup>[15]</sup> The positive (low and intermediate) AMACR expression observed

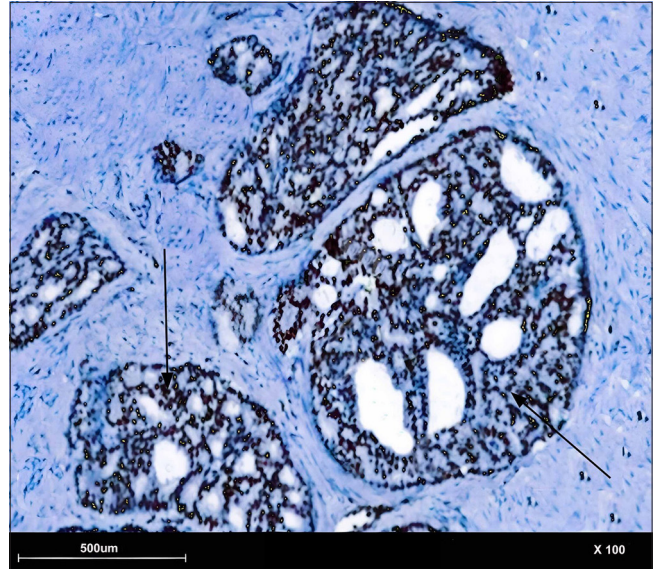
**Table 1:** Clinicopathological features and AMACR expression.

*Parameter	No expression	Low expression	Intermediate expression	High expression	**p-value
Age (years)					
<65 (n=134)	95 (48.9)	6 (3.1)	27 (13.9)	6 (3.1)	<0.001
>65 (n=60)	16 (8.2)	14 (7.2)	16 (8.2)	14 (7.2)	
Lesion type					
Benign (n=128)	104 (81.2)	4 (3.1)	20 (15.6)	0	<0.001
Malignant (n=66)	7 (10.6)	16 (24.2)	23(34.8)	20 (30.3)	
Grade (n=66)					
Grade 1 (n=6)	5 (7.6)	1 (1.5)	0	0	<0.001
Grade 2 (n=5)	1 (1.5)	4 (6)	0	0	
Grade 3 (n=17)	1 (1.5)	9 (13.6)	6 (9)	1 (1.5)	
Grade 4 (n=20)	0	0	9 (13.6)	11 (16.7)	
Grade 5 (n=18)	0	2 (3)	8 (12.1)	8 (12.1)	
Perineural invasion					
Present (n=36)	2 (1.3)	10 (5.1)	4 (2)	20 (10.3)	<0.001
Absent (n=30)	5 (2.6)	6 (3)	19 (9.8)	0	

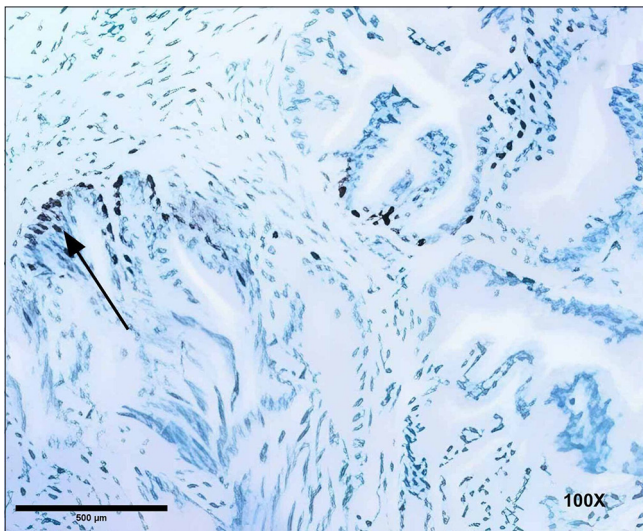
\*Expressed as frequency and percentage column-wise n (%), \*\* Chi-square, p-value<0.05. AMACR: Alpha-methylacyl-CoA racemase



**Figure 3:** Photomicrograph (×100) depicting strong cytoplasmic alpha-methyl acyl CoA racemase (AMACR) staining (arrow) in prostatic adenocarcinoma (Gleason grade 4).



**Figure 5:** Photomicrograph (×100) demonstrating strong nuclear erythroblast transformation-specific-related gene (ERG) staining (arrow) in a case of prostatic adenocarcinoma (Gleason grade 4).

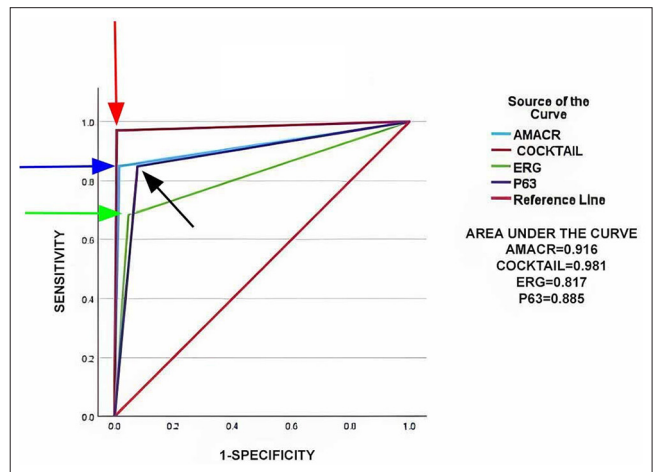


**Figure 4:** Photomicrograph (×100) depicting a cluster of basal cells with nuclear staining (arrow) for P63 in benign prostatic tissue.

in our study aligns with those reported in the literature, where the rate of AMACR positivity in benign prostatic lesions has been documented to range from 5.3 to 36.2.<sup>[16-18]</sup>

The AMACR positivity rate observed in our adenocarcinoma cases is consistent with published studies, which report rates ranging from 87.7 to 100% in prostatic carcinoma. Slight variations may be due to sample size, anti-AMACR antibody clone used, and the cut-off values for interpreting AMACR staining.<sup>[19-21]</sup>

The strong AMACR expression in adenocarcinoma cases in the present study aligns with Atta Ihab Shafek<sup>[17]</sup> who



**Figure 6:** Receiver operating characteristic curve with area under the curve for alpha-methyl acyl CoA racemase (AMACR), P63, erythroblast transformation-specific related gene (ERG), and their triple-marker panel. The red, blue, green and black arrows are refers to the highest point of the ROC curve.

reported strong expression in 21 (36.2%) of patients. Kars *et al.*, observed strong expression in 8 (61.5%), while Hasan *et al.*,<sup>[15]</sup> documented strong expression in 17 (68%) adenocarcinoma cases, respectively.<sup>[22]</sup>

Numerous studies have reported inconsistent AMACR expression in prostatic adenocarcinoma, reflected by differences in staining intensity and the proportion of positive cells. This variability poses a challenge to the reliable identification of small carcinoma foci in prostatic core biopsies using AMACR.

**Table 2:** ERG expression and clinicopathological features.

*Parameter	No expression	Low expression	Intermediate expression	High expression	p-value
Age (years)					
<65 (n=134)	127 (65.5)	3 (1.5)	1 (0.5)	3 (1.5)	<0.001
>65 (n=60)	44 (22.7)	4 (2)	3 (1.5)	9 (4.6)	
Lesion type					
Benign (n=128)	128 (100)	0	0	0	<0.001
Malignant (n=66)	43 (65.1)	7 (10.6)	4 (6)	12 (18.2)	
Grade (n=66)					
Grade 1 (n=6)	2 (3)	0 (1.5)	0	4 (6)	<0.001
Grade 2 (n=5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3)	
Grade 3 (n=17)	8 (12.1)	2 (3)	2 (3)	5 (7.6)	
Grade 4 (n=20)	17 (25.7)	2 (3)	1 (1.5)	0	
Grade 5 (n=18)	15 (22.7)	3 (4.5)	0	0	
Perineural invasion					
Present (n=36)	27 (13.9)	1 (0.5)	4 (2)	4 (2)	0.010
Absent (n=30)	16 (8.2)	6 (3)	0	8 (4.1)	

\*Expressed as frequency and percentage n (%), p-value<0.05, ERG: Erythroblast transformation-specific-related gene

**Table 3:** Coordinates of the ROC curve.

Variable	*Positive if greater than or equal to	Sensitivity	1-specificity
AMACR	0	1.000	1.000
	1.5	0.848	0.016
	3	0	0
P63	0	1	1
	1.5	0.846	0.078
	3	0	0
ERG	0	1.000	1.000
	1.5	0.591	0.047
	3	0	0
AMACR+ P63+ERG triple-marker panel	0	1.000	1.000
	1.5	0.970	0.008
	3	0	0

\*The test result variables: AMACR, P63, ERG, AMACR + P63 + ERG has at least one tie between the positive actual state group and the negative actual state group. The smallest cutoff value is the minimum observed test value, min 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values. ROC: Receiver operating characteristic, AMACR: Alpha-methylacyl-CoA racemase, ERG: Erythroblast transformation-specific-related gene, P63: Tumor protein 63.

Our study found a statistically significant link between AMACR expression and Gleason grade. Grade 4 cases had the highest expression rate, 20 (30.3%), with high expression (0 negative or weak). Grade 1 cases had the lowest expression

rate. Research on AMACR expression and PC grade shows varying results. Some studies indicate higher AMACR levels in high-grade cases, others in low-grade cases, while some studies find no correlation.<sup>[18,20,23,24]</sup>

Our findings indicate that the expression in PC may have prognostic significance, as it is notably higher in advanced cases, suggesting its potential as a therapeutic target. Nevertheless, from a diagnostic perspective, the limited expression of AMACR in lower-grade cases – which are often confused with benign counterparts – presents a challenge in using AMACR to distinguish between benign and malignant prostatic lesions.

In our study, ERG expressions were absent in benign cases, aligning with existing literature. Among adenocarcinoma cases, 34.9% showed varying expression levels. Previous studies reported higher ERG expression rates, between 35.2 and 70%.<sup>[25,26]</sup> A study by Nie *et al.*, found 16.7% positive ERG expression in prostatic adenocarcinoma cases. Our study revealed a significant difference in ERG expression between hyperplasia and adenocarcinoma cases.<sup>[27]</sup>

Our study observed a significant inverse correlation between ERG expression and Gleason grade, with higher grades showing reduced ERG levels. While some studies agree, others report the opposite relationship.<sup>[28,29]</sup> ERG expression diminishes as tumor grade, PSA levels, and tumor volume increase. This observation indicates that ERG expression might be an early event in the development of prostatic cancer. A notable correlation was observed between ERG and AMACR expression in our studied cases. The research conducted by Gouda and Eloiseily also found a significant

relationship between ERG and AMACR expression in prostatic lesions.

### Contextual commentary

The anticipated cost of triple-marker panel analysis within the subcontinent ranges from 100 to 150 USD, subject to variation based on whether the facility is privately or government operated.

### Limitations

We acknowledge several limitations in this study. First, the research was conducted at a single tertiary care teaching hospital in northern India, which may limit the generalizability of the results to broader populations or different healthcare settings. Although prostate biopsies were analyzed by experienced uropathologists, interobserver variability cannot be completely excluded nor can the choice to perform IHC, which was primarily based on the pathologists' discretion. All pathologies, however, were confirmed by an independent second pathologist. Regrettably, there is currently no long-term follow-up data or additional treatments/pathologies available for patients who underwent active surveillance following changes in biopsy results due to IHC performance. Finally, molecular tests to assess cancer aggressiveness and identify somatic or germline mutations were not performed.

### CONCLUSION

In our study, ERG showed a specificity of 95.3%, but its sensitivity in differentiating benign from malignant prostate lesions was lower than that of AMACR (59.1% compared to 84.8%). When the three markers were used in a triple-marker panel, the sensitivity significantly increased to 95.5%. In conclusion, the triple-marker panel may improve diagnostic confidence or provide marginal incremental diagnostic benefit.

AMACR was more accurate for diagnosis than ERG, showing a rate of 98.3% compared to ERG's 86%. When all markers were combined, diagnostic accuracy remained solid at 98.5%, even though ERG's sensitivity was lower (an incremental diagnostic gain). The findings support improved diagnostic discrimination in biopsy specimens; they do not directly establish prognostic benefit or mandate routine universal implementation. Emphasizing incremental diagnostic gain rather than broader clinical impact will improve balance and scientific restraint.

**Author contributions:** RB, GS, PK: Conceptualization; RB, GS, SS, PK: Methodology; PK, SS, GS: Data collection; RB: Statistical analysis; GS, RB, SS: Drafting; RB, GS: Review and editing; PK, SS: Supervision.

**Ethical approval:** The research/study was approved by the Institutional Review Board at Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, Ref No. MDC/JNMCIEC/233, dated February 21, 2025.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms.

**Financial support and sponsorship:** Nil

**Conflicts of interest:** There are no conflicts of interest.

**Availability of data and material:** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Use of artificial intelligence (AI)-assisted technology for manuscript preparation:** The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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