Section: Pathology

Original Research Article

# HISTOMORPHOLOGICAL SPECTRUM OF PROSTATIC LESIONS AND THE DIAGNOSTIC UTILITY OF SERUM PSA, AMACR AND P63 IMMUNOHISTOCHEMISTRY: A CROSS-SECTIONAL STUDY FROM A TERTIARY CARE CENTRE

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***Received*** *: 01/08/2026*
***Received in revised form*** *: 26/08/2026*
***Accepted*** *: 07/09/2026*

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**DOI:** 10.70034/ijmedph.2026.3.813

**Source of Support:** Nil,
**Conflict of Interest:** None declared

**Int J Med Pub Health**
2026; 16 (3); 5280-5288

ABSTRACT

**Background:** Histopathological differentiation of benign prostatic lesions from acinar adenocarcinoma is often confounded by overlapping morphology and the limited specificity of serum PSA alone. This study characterized the histomorphological spectrum of prostatic lesions, correlated histopathological diagnoses with serum PSA levels, and evaluated the diagnostic performance of AMACR and p63 immunohistochemistry in a tertiary care setting.

**Materials and Methods:** A descriptive combined prospective and retrospective study was conducted on 96 prostatic specimens (71 benign, 25 malignant), primarily obtained via transurethral resection of the prostate (TURP) and core needle biopsy. Serum PSA levels were correlated with morphological diagnoses. Immunohistochemistry was performed using monoclonal antibodies directed against p63 (a basal cell marker) and AMACR (a carcinoma-associated metabolic marker). Diagnostic performance metrics, including sensitivity, specificity, positive predictive value, and negative predictive value, were statistically derived.

**Results:** The most frequent diagnoses were benign prostatic hyperplasia with chronic nonspecific prostatitis (36.6%) and conventional acinar adenocarcinoma (52.0% of malignancies). Median age was 70 years across both cohorts ($p=0.503$). Median PSA was significantly higher in malignant (56.7 ng/mL) versus benign (6.86 ng/mL) cases ($p<0.001$); however, 22.5% of benign cases exhibited PSA >10 ng/mL, all possessing concurrent prostatitis. For detecting malignancy, p63 negativity showed 100% sensitivity and 93.0% specificity, while AMACR positivity showed 84.0% sensitivity and 95.8% specificity. Combined interpretation (AMACR-positive and p63-negative) achieved 100% specificity and 100% positive predictive value.

**Conclusion:** While elevated PSA strongly suggests prostatic adenocarcinoma, but can also rise markedly in benign inflammation, necessitating definitive histopathological confirmation. Combined p63 and AMACR immunohistochemistry markedly improves diagnostic accuracy, offering a highly specific, biologically synergistic adjunct to routine morphology for distinguishing benign hyperplastic or inflammatory mimics from invasive carcinoma.

**Keywords:** Prostatic adenocarcinoma, BPH, PSA, AMACR, p63, Immunohistochemistry

## INTRODUCTION

Prostatic diseases represent a profound public health challenge and a major source of urological morbidity among ageing male populations globally.[1] The prostate gland, a fibromusculoglandular organ encircling the proximal urethra, is uniquely susceptible to a broad spectrum of age-related pathological alterations. These range from inflammatory conditions and benign hyperplastic proliferations to premalignant intraepithelial neoplasia and overtly invasive adenocarcinoma.[2] Benign prostatic hyperplasia (BPH) is essentially ubiquitous in the elderly demographic, predominantly arising within the transition zone and precipitating varying degrees of lower urinary tract symptoms, including hesitancy, frequency, and acute urinary retention. Concurrently, prostatic adenocarcinoma is recognized as one of the most frequently diagnosed non-cutaneous malignancies worldwide and remains a leading contributor to cancer-related mortality.[2] While the incidence of prostate cancer has historically peaked in Western countries, epidemiological data demonstrate a steadily rising incidence in developing nations, including India. This shifting landscape is largely attributed to expanding life expectancies, the adoption of sedentary lifestyle factors, and the increased availability of advanced diagnostic infrastructure in tertiary healthcare settings.[3]

Serum prostate-specific antigen (PSA), a glycoprotein enzyme belonging to the kallikrein-related peptidase family (KLK3), has revolutionized the clinical approach to prostatic disease over the past several decades.[4] Synthesized almost exclusively by the luminal secretory cells of the prostatic epithelium, PSA functions physiologically to liquefy seminal coagulum. Under normal conditions, the integrity of the prostatic epithelial barrier restricts the entry of PSA into the systemic circulation, maintaining low serum concentrations. However, any pathological disruption of this barrier –whether through neoplastic architectural destruction, hyperplastic glandular expansion, or inflammatory exudation– facilitates the leakage of PSA into the bloodstream.[5] Consequently, while elevated serum PSA levels are powerfully correlated with the presence and volume of prostatic adenocarcinoma, the biomarker suffers from a critical lack of cancer specificity. Marked elevations in serum PSA are frequently documented in benign entities, most notably acute and chronic prostatitis, robust glandular hyperplasia, and secondary to iatrogenic instrumentation.[6] This biochemical overlap dictates that PSA cannot function as a standalone diagnostic determinant; rather, it necessitates definitive histopathological corroboration to avert the catastrophic consequences of overdiagnosis and unwarranted oncological intervention.[7]

The histopathological evaluation of prostatic tissue remains the undisputed gold standard for diagnosis. However, surgical pathology practice, particularly when evaluating limited tissue from transrectal ultrasound-guided core needle biopsies or fragmented transurethral resection of the prostate (TURP) specimens, is fraught with morphological challenges.[8] The diagnostic hallmark of invasive acinar adenocarcinoma relies on identifying an infiltrative architectural growth pattern, the presence of distinct cytological atypia (such as prominent nucleoli and nuclear enlargement), and critically, the complete absence of the basal cell layer. In contemporary practice, pathologists frequently encounter minute foci of atypical small acinar proliferations (ASAP) measuring less than 1 mm, where these classical architectural and cytological criteria are equivocal or incomplete.[9] This diagnostic ambiguity is exacerbated by a multitude of benign morphological mimics, including atypical adenomatous hyperplasia (adenosis), partial atrophy, post-atrophic hyperplasia, and basal cell hyperplasia. Misinterpretation of these subtle lesions can lead to severe clinical ramifications, underscoring the necessity for objective ancillary diagnostic tools.[10]

Immunohistochemistry has fundamentally transformed the diagnostic evaluation of morphologically ambiguous prostatic lesions. The contemporary diagnostic algorithm relies on a synergistic approach: demonstrating the biological absence of the basal cell compartment alongside the positive expression of carcinoma-associated metabolic antigens.[11] The nuclear transcription factor p63, a homologue of the p53 tumour suppressor gene, plays an indispensable role in the development and maintenance of stratified epithelial structures. In the prostate gland, p63 (specifically the ΔNp63 isoform) is selectively and strongly expressed in the nuclei of the basal cells that line benign acini and ducts. Because the transition to invasive acinar adenocarcinoma involves the exclusive proliferation of transformed luminal cells and the complete dissolution of the basal cell layer, p63 serves as a highly reliable negative marker for malignancy. To provide positive confirmation of malignant transformation, alpha-methylacyl-CoA racemase (AMACR, or P504S) has emerged as an invaluable adjunctive biomarker. AMACR is an enzyme localized to peroxisomes and mitochondria that orchestrates the β-oxidation of branched-chain fatty acids.[12] Transcriptomic and proteomic profiling has demonstrated that AMACR is profoundly upregulated in prostatic adenocarcinoma cells relative to benign epithelium. Immunohistochemically, AMACR yields a characteristic granular cytoplasmic staining pattern, frequently displaying striking luminal accentuation within malignant acini. When interpreted in tandem, the simultaneous loss of p63 and the overexpression of AMACR provide a powerful, complementary

immunophenotype that maximizes diagnostic confidence in resolving atypical foci.[11,12]

Despite the established utility of these markers in Western cohorts, there remains a critical need to rigorously evaluate their performance characteristics within the specific demographic and histomorphological context of Indian tertiary care centres, where advanced presentation and a high baseline prevalence of infectious and inflammatory prostatic diseases are common.

Aim and Objectives: The aim of the present study was to comprehensively evaluate the histomorphological spectrum of benign and malignant prostatic lesions, to ascertain the correlation between final histopathological diagnoses and pre-procedural serum PSA levels, and to precisely quantify the diagnostic utility of p63 and AMACR immunohistochemistry in differentiating invasive carcinoma from its benign morphological mimickers.

## MATERIALS AND METHODS

**Study Design and Setting:** A descriptive, cross-sectional study incorporating both retrospective and prospective cohorts was conducted over an 18-month period at the Department of Pathology in a tertiary care teaching hospital.

**Study Population and Sampling:** The sample size was calculated based on previous published data which reported that approximately 90% of malignant prostatic lesions showed AMACR positivity, and 90% of benign lesions showed p63 positivity, at a 5% level of significance with 6% absolute precision. The study population was derived using a consecutive sampling methodology, culminating in a total of 96 male patients who had undergone therapeutic or diagnostic prostatic tissue excision. The cohort was categorized into two fundamental groups based on the final histopathological diagnosis: 71 cases comprised the benign prostatic lesions cohort (including hyperplastic, inflammatory, and atrophic conditions), and 25 cases comprised the malignant prostatic lesions cohort (predominantly invasive adenocarcinoma).

**Inclusion and Exclusion criteria**

Inclusion criteria were strictly defined as patients diagnosed with benign or malignant prostatic lesions via transurethral resection of the prostate (TURP), core needle biopsy, or prostatectomy, provided there was adequate, well-fixed tissue for both morphological and immunohistochemical evaluation, alongside documented preoperative serum PSA values. Exclusion criteria encompassed critically ill patients, specimens demonstrating severe crush artifact, autolysis, or inadequate tissue volume, patients with known secondary metastatic tumours involving the prostate gland, and cases lacking complete biochemical or clinical demographic data.

**Study Procedure**

**Histopathological Processing**

Tissue specimens fixed in 10% neutral buffered formalin were processed, embedded in paraffin wax, and sectioned at a thickness of 2-3 µm for routine Haematoxylin and Eosin (H&E) staining. Invasive prostatic adenocarcinomas were graded utilizing the classic Gleason scoring system, mapping to contemporary International Society of Urological Pathology (ISUP) Grade Groups where sufficient architectural data permitted.

For immunohistochemistry, 2-3 µm sections on positively charged slides were deparaffinized in xylene and rehydrated through a descending ethanol gradient. Heat-induced antigen retrieval was performed using Tris-EDTA buffer (pH 9.0) in a multi-epitope retrieval system for 45 minutes, followed by cooling. After circumscribing the tissue with a PAP pen, endogenous peroxidase activity was neutralized with a hydrogen peroxide-based quencher for 10 minutes. The sections were then incubated for 45 minutes with primary antibodies: p63 (4A4 mouse monoclonal) and AMACR/P504S (13H4 rabbit monoclonal). Signal detection utilized a Target Binder and an HRP-conjugated secondary system (10 minutes each), followed by visualization with DAB chromogen (4-7 minutes). Finally, the slides were counterstained with Harris haematoxylin, dehydrated, cleared, and permanently mounted using DPX resin.

**Interpretation of Immunohistochemistry**

The interpretation of the immunohistochemical stains was conducted in conjunction with the H&E morphological findings:

- **p63 Interpretation:** The stain was designated as positive when crisp, continuous, or distinctly patchy dark brown nuclear reactivity was observed confined to the basal cell layer of the prostatic acini. Complete absence of nuclear staining in the target atypical glands was recorded as negative.
- **AMACR Interpretation:** The stain was recorded as positive when moderate to strong, continuous, or near-continuous granular cytoplasmic reactivity, predominantly highlighting the apical/luminal aspect of the epithelial cells, was observed.

**Statistical Analysis:** data were compiled in Microsoft Excel and analyzed using SPSS Statistics for Windows, Version 17.0. Continuous variables (age, serum PSA) were summarized as medians with interquartile ranges (IQR) or means ± standard deviations (SD) and compared using the Mann-Whitney U or Wilcoxon rank-sum tests. Categorical variables were expressed as absolute frequencies and percentages, with group associations evaluated via the Chi-square or Fisher's exact tests. Diagnostic performance metrics (sensitivity, specificity, PPV, NPV) were calculated from standard 2×2 contingency tables. Statistical significance was defined as a two-tailed p-value $<0.05$.

**Ethical Considerations:** The study was conducted in strict adherence to the ethical guidelines delineated in the Declaration of Helsinki. Institutional Ethics Committee approval was secured from IEC of GRMC, Gwalior, prior to the initiation of data collection. Written informed consent was obtained from all prospectively enrolled patients, and retrospective data were completely anonymized to ensure patient confidentiality.

## RESULTS

**Table 1: Distribution of Histopathological Spectrum of Prostatic Lesions**

| Histopathological Diagnosis | Count (n) | Percentage of Group |
|---|---|---|
| Benign Lesions (Total n = 71) | | |
| Benign prostatic hyperplasia (BPH) | 16 | 22.5% |
| BPH with chronic nonspecific prostatitis | 26 | 36.6% |
| Adenomyomatous hyperplasia with chronic prostatitis | 18 | 25.4% |
| BPH with chronic nonspecific prostatitis and prostatic abscess | 3 | 4.2% |
| BPH with focal low-grade PIN | 2 | 2.8% |
| Adenomyomatous hyperplasia + chronic prostatitis + focal low-grade PIN | 1 | 1.4% |
| Adenomyomatous hyperplasia + chronic prostatitis + squamous metaplasia | 1 | 1.4% |
| BPH with chronic prostatitis and infected polyp | 1 | 1.4% |
| BPH with tubercular granuloma | 1 | 1.4% |
| Chronic nonspecific prostatitis | 1 | 1.4% |
| Mild glandular proliferation with hyperplastic lining epithelial cells | 1 | 1.4% |
| Malignant Lesions (Total n = 25) | | |
| Acinar adenocarcinoma of prostate | 13 | 52.0% |
| Adenocarcinoma of prostate | 8 | 32.0% |
| Acinar adenocarcinoma + chronic prostatitis | 2 | 8.0% |
| Ductal adenocarcinoma | 1 | 4.0% |
| Acinar adenocarcinoma + high-grade PIN | 1 | 4.0% |

Of the 96 prostatic specimens, 71 (74.0%) were benign and 25 (26.0%) were malignant. Benign prostatic hyperplasia with chronic nonspecific prostatitis was the most prevalent benign diagnosis (36.6%, 26 cases), followed by adenomyomatous hyperplasia with chronic prostatitis (25.4%, 18 cases), highlighting widespread concurrent inflammation. Focal low-grade prostatic intraepithelial neoplasia (LGPIN) was noted in 3 benign cases. Within the malignant cohort, conventional acinar adenocarcinoma (52.0%, 13 cases) and adenocarcinoma not otherwise specified (32.0%, 8 cases) predominated. Less common malignant entities included acinar adenocarcinoma with background chronic prostatitis (8.0%, 2 cases), ductal adenocarcinoma (4.0%, 1 case), and acinar adenocarcinoma with high-grade PIN (4.0%, 1 case).

**Table 2: Age-wise Distribution of Prostatic Lesions by Diagnosis**

| Age Group | Malignant n (%) | Benign n (%) | Total (N=96) | % Malignant within Age Group |
|---|---|---|---|---|
| <50 years | 0 (0.0%) | 2 (2.8%) | 2 | 0.0% |
| 50–59 years | 5 (20.0%) | 8 (11.3%) | 13 | 38.5% |
| 60–69 years | 7 (28.0%) | 25 (35.2%) | 32 | 21.9% |
| 70–79 years | 12 (48.0%) | 27 (38.0%) | 39 | 30.8% |
| ≥80 years | 1 (4.0%) | 9 (12.7%) | 10 | 10.0% |
| Total | 25 (100.0%) | 71 (100.0%) | 96 | |
| Age (Years) | Malignant (n=25) | Benign (n=71) | Overall (N=96) | p-value* |
| Median (IQR) | 70 (60, 74) | 70 (62, 74) | 70 (62, 74) | 0.503 |
| Range | 50-80 | 49-87 | 49-87 | |
| *Mann-Whitney U test | | | | |

Detailed age-wise stratification revealed that the highest absolute volume of malignant cases presented in the 70-79 years decile, comprising 12 of the 25 malignant diagnoses (48.0%). However, examining the proportional rate of malignancy within each specific age bracket illuminated that the 50-59 years group carried the highest relative burden of malignancy, with 38.5% (5 out of 13) of patients in this bracket receiving a malignant diagnosis. No malignant lesions were detected in the small subset of patients under 50 years of age.

The median age of the overall study population was 70 years, spanning a range from 49 to 87 years. When stratified by diagnostic category, the median age for both the malignant and benign cohorts was identical at 70 years. Statistical evaluation utilizing the Mann-Whitney U test confirmed that there was no significant divergence in age distribution between patients harbouring benign versus malignant disease (p=0.503).

**Table 3: Specimen Type Distribution and Serum PSA Levels by Histopathological Diagnosis**

| Specimen Type | Malignant n (%) | Benign n (%) | Total (N) | p-value* |
|---|---|---|---|---|
| TURP | 21 (84.0%) | 69 (97.2%) | 90 | 0.063 |
| Core Needle Biopsy | 4 (16.0%) | 2 (2.8%) | 6 | |
| Total | 25 (100.0%) | 71 (100.0%) | 96 | |
| *Chi-square test with Yates' continuity correction | | | | |
| Parameter | Malignant (n=25) | Benign (n=71) | | p-value* |
| Mean ± SD (ng/mL) | 64.95 ± 43.99 | 9.23 ± 12.08 | | <0.001 |
| Median (IQR) (ng/mL) | 56.7 (37.0, 65.7) | 6.86 (4.8, 9.55) | | |
| Range (ng/mL) | 14.4-180.6 | 0.77-100.0 | | |
| *Wilcoxon rank-sum test | | | | |

Most specimens were obtained via TURP (93.8%, 90/96). Core needle biopsies (6.2%, 6/96) yielded a higher proportion of malignancies (66.7%, 4/6) compared to TURP specimens (23.3%, 21/90), though this difference was not statistically significant (p=0.063).

Clinical impressions correlated well with histopathology: "Suspicious for Carcinoma" and "BPH" had positive predictive values of 92.9% and 88.9%, respectively. However, a generalized diagnosis of "Prostatomegaly" (n=45) was non-specific, encompassing 8 malignant and 37 benign cases.

The analysis of serum PSA levels revealed profound differences between the diagnostic cohorts. The malignant group exhibited a mean PSA of 64.95 ± 43.99 ng/mL (median: 56.7 ng/mL; IQR: 37.0-65.7), which was significantly higher than the benign group's mean PSA of 9.23 ± 12.08 ng/mL (median: 6.86 ng/mL; IQR: 4.8-9.55) (p<0.001).

**Table 4: Benign Cases Presenting with Elevated PSA (>10 ng/mL)**

| Histopathological Diagnosis | Count (n=16) | % of Elevated PSA Benign Cases |
|---|---|---|
| BPH with chronic nonspecific prostatitis | 10 | 62.5% |
| Adenomyomatous hyperplasia with chronic prostatitis | 4 | 25.0% |
| Adenomyomatous hyperplasia + chronic prostatitis + focal LGPIN | 1 | 6.2% |
| BPH + chronic prostatitis + infected polyp | 1 | 6.2% |
| Total | 16 | 100.0% |

Despite this marked statistical divergence, the absolute ranges demonstrated critical clinical overlap. Specifically, 22.5% (16 out of 71) of the benign cases presented with PSA values exceeding the widely recognized high-risk threshold of 10 ng/mL, reaching as high as 100 ng/mL in one instance. A meticulous histomorphological review of these 16 specific cases unequivocally identified background inflammation or active prostatitis in every single instance (100%). BPH with chronic nonspecific prostatitis accounted for the majority (62.5%) of these biochemically elevated false positives.

**Table 5: Expression of p63 and AMACR by Histopathological Diagnosis**

| Marker Status | Malignant (n=25) | Benign (n=71) | Total (N=96) | p-value |
|---|---|---|---|---|
| AMACR Expression | | | | |
| Positive | 21 (84.0%) | 3 (4.2%) | 24 | <0.001 |
| Negative | 4 (16.0%) | 68 (95.8%) | 72 | |
| p63 Expression | | | | |
| Positive | 0 (0.0%) | 66 (93.0%) | 66 | <0.001 |
| Negative | 25 (100.0%) | 5 (7.0%) | 30 | |

The application of p63 and AMACR immunohistochemistry yielded highly statistically significant reciprocal expression patterns (p<0.001 for both markers). AMACR exhibited robust cytoplasmic positivity in 84.0% (21/25) of the malignant cases. In sharp contrast, AMACR was negative in 95.8% (68/71) of the benign lesions, with only 3 cases demonstrating aberrant false positivity.

p63 established itself as an exceptionally reliable basal cell marker. It demonstrated intact nuclear positivity in 93.0% (66/71) of the benign cases. Crucially, p63 was entirely absent (100% negative) in all 25 cases of prostatic adenocarcinoma, directly mirroring the biological dissolution of the basal cell compartment during invasive transformation.

**Table 6: AMACR Expression Stratified by Gleason Score (ISUP Grade Group)**

| Grade Group | Gleason Score | AMACR Positive n (%) | AMACR Negative n (%) | Total (n=25) |
|---|---|---|---|---|
| Group 1 | 3+3=6 | 3 (75.0%) | 1 (25.0%) | 4 |
| Group 2 | 3+4=7 | 5 (83.3%) | 1 (16.7%) | 6 |
| Group 3 | 4+3=7 | 3 (75.0%) | 1 (25.0%) | 4 |
| Group 4 | 4+4=8, 5+3=8 | 5 (100.0%) | 0 (0.0%) | 5 |
| Group 5 | 4+5=9, 5+4=9 | 5 (83.3%) | 1 (16.7%) | 6 |
| Total | | 21 (84.0%) | 4 (16.0%) | 25 |

Subgroup analysis within the malignant cohort evaluated the relationship between AMACR expression and the degree of histological dedifferentiation, as categorized by the Gleason Score (ISUP Grade Group). The data indicated that AMACR positivity was broadly sustained across all tiers of malignancy. There was no evidence of significant biomarker attenuation in higher-grade disease; expression rates ranged from 75.0% in Grade Groups 1 and 3, to a peak of 100.0% in Grade Group 4.

Analysis of marker expression relative to specimen type revealed statistical significance for both p63 (p=0.011) and AMACR (p=0.033). Specifically, p63 positivity was predominantly observed in TURP specimens (65/90 cases), corresponding to the high volume of benign transition zone hyperplasia typically resected via this modality. Conversely, AMACR positivity was proportionally higher in core biopsies, aligning with their clinical function as a targeted diagnostic tool for peripheral zone malignancies.

**Table 7: Diagnostic Performance of IHC Markers for the Detection of Malignancy**

| IHC Criterion | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| AMACR (Positive = Malignant) | 84.0% | 95.8% | 87.5% | 94.4% |
| p63 (Negative = Malignant) | 100.0% | 93.0% | 83.3% | 100.0% |
| Combined (AMACR+ & p63-) | 84.0% | 100.0% | 100.0% | 94.7% |

The mathematical evaluation of the immunohistochemical markers as diagnostic tests for malignancy underscored their clinical utility. Utilizing the complete absence of p63 as the sole criterion for malignancy generated an absolute sensitivity of 100%, establishing it as a flawless rule-out assay within this dataset. AMACR positivity alone provided excellent specificity (95.8%) and a PPV of 87.5%.

The biological synergy of the markers was most evident in their combined application. Defining a positive malignant immunophenotype strictly as the simultaneous presence of AMACR positivity and p63 negativity resulted in a specificity of 100% and a PPV of 100%. This combined threshold eliminated all false-positive diagnoses, confirming its role as a highly stringent confirmatory test.

## DISCUSSION

**Principal Findings and Histopathological Demographics:** The present study confirms the indispensable role of integrating histomorphological evaluation, biochemical correlation, and advanced immunohistochemistry in the diagnostic pathology of prostatic diseases. The demographic and histopathological distribution observed in this cohort accurately reflects the realities of urological pathology in a tertiary care setting. Benign prostatic hyperplasia, deeply intertwined with chronic inflammatory processes, constituted the vast majority of the surgical workload. Acinar adenocarcinoma emerged as the exclusive epithelial malignancy, corroborating extensive epidemiological data outlining its dominance in the ageing male population.[1,2]

Age analysis revealed a median of 70 years across both benign and malignant cohorts, confirming that chronological age, while a fundamental risk factor, provides negligible discriminatory power between neoplastic and hyperplastic aetiologies. The detection of the highest relative malignant burden within the 50-59 years demographic (38.5%) serves as a critical clinical caveat. It suggests that relatively younger men subjected to tissue sampling in a tertiary referral center likely present with highly suspicious clinical profiles or alarming PSA velocities that warrant aggressive investigation.[13] Similar observations were noted by Fatima et al., who documented that while benign hyperplasia remains the most common finding overall, adenocarcinoma constitutes a significant subset requiring meticulous differentiation, particularly in symptomatic men.[14]

**The Confounding Role of Inflammation in PSA Biochemistry:** The evaluation of serum PSA levels in this cohort exposes the profound clinical limitations of isolated biochemical screening. While the median PSA of the malignant group was unequivocally and significantly higher than that of the benign group, the ranges overlapped dangerously. The critical finding that 22.5% of the definitively benign cohort exhibited marked PSA elevations exceeding 10 ng/mL underscores a persistent diagnostic dilemma. Detailed histological review of these specific cases uniformly identified active or chronic prostatitis.

The biological mechanism governing this phenomenon is well documented: inflammation drives the breakdown of the glandular-stromal barrier, disrupting the structural integrity of the basement membrane and tight junctions. This pathological permeability allows intraluminal kallikrein-related peptidase 3 (PSA) to extravagate directly into the vascular and lymphatic microcirculation, generating alarming systemic spikes independent of malignant transformation.[4,5] Similar findings have been reported by Pariyar et al., who emphasized that integrating clinical findings with histopathology is vital because PSA elevation in isolation frequently leads to the misclassification of prostatitis as suspected carcinoma.[15] These data emphatically reiterate that an elevated PSA, regardless of its magnitude, mandates rigorous histopathological confirmation, as inflammatory mimics routinely masquerade as biochemical malignancies.[16]

**Diagnostic Utility and Biology of p63:** The diagnosis of acinar adenocarcinoma essentially requires the verification that the basal cell layer has been biologically extinguished. Identifying basal cells on routine H&E sections is frequently compromised by tangential sectioning, crush artifact, or florid inflammatory infiltrates.[8] In this context, p63 proved to be an exceptionally robust diagnostic adjunct. The complete absence of p63 nuclear staining in 100% of the malignant cases confirms its biological fidelity. The ΔNp63 isoform acts as a master transcriptional regulator essential for the maintenance of basal stem cells in stratified epithelia; the transition to invasive acinar adenocarcinoma represents a complete shift toward luminal differentiation, resulting in the absolute loss of this basal compartment.[17]

Conversely, p63 demonstrated a high sensitivity for benignity, retaining nuclear positivity in 93.0% of benign lesions. The 7.0% false-negative rate in the benign cohort likely reflects known diagnostic pitfalls: severe atrophy where basal cells are flattened and discontinuous, tissue processing artifacts, or severe acute inflammation obscuring antigenic sites. These findings align seamlessly with the reports of Nisar et al., who validated p63 as a highly reliable negative marker for invasive carcinoma in a South Asian cohort, providing a crucial safety mechanism against the overdiagnosis of crowded benign mimickers such as adenosis and partial atrophy.[18]

**Diagnostic Utility and Biology of AMACR (P504S):** As a positive marker of malignant transformation, AMACR demonstrated a strong diagnostic performance, yielding 84.0% sensitivity and 95.8% specificity. The robust, granular cytoplasmic staining pattern, frequently displaying marked luminal accentuation, is biochemically driven by the profound upregulation of peroxisomal β-oxidation of branched-chain fatty acids–a metabolic shift characteristic of prostate cancer cell survival and proliferation.[12]

Crucially, the present study demonstrates that AMACR expression is sustained across all Gleason scores (ISUP Grade Groups 1-5). This indicates that the metabolic derangements driving AMACR overexpression are early, fundamental oncogenic events that are maintained even as the tumour architecture progressively dedifferentiates.[19] Similar conclusions were drawn by Alinezhad et al., who confirmed that AMACR transcripts and proteins are significantly upregulated in carcinoma relative to benign tissues, functioning as reliable indicators of malignant transformation regardless of tumour grade.[19]

However, reliance on AMACR as a solitary marker is cautioned by two observations in this study. First, 16.0% of the true adenocarcinomas failed to express AMACR. This known phenomenon is often associated with specific morphological subtypes, such as the foamy gland, pseudohyperplastic, and atrophic variants of adenocarcinoma, or inherent intratumoural heterogeneity.[20,21] Second, false-positive staining occurred in 4.2% of benign cases. Upregulation of AMACR is a recognized pitfall in high-grade prostatic intraepithelial neoplasia (HGPIN), nephrogenic adenomas, and occasionally in floridly inflamed regenerative glands.[22,23] Therefore, interpreting AMACR without concurrent morphological assessment and a basal cell marker is diagnostically hazardous.[24]

**The Synergistic Superiority of the Combined Panel:** The intellectual centerpiece of this diagnostic evaluation is the unparalleled performance of the combined dual-marker panel. By demanding a reciprocal immunophenotype – specifically, the simultaneous demonstration of p63 loss and AMACR overexpression– the assay achieved a flawless 100% specificity and 100% positive predictive value. The biological synergy of this approach effectively neutralizes the isolated weaknesses of each marker. The risk of misinterpreting discontinuous p63 staining in benign atrophy is mitigated by the absence of AMACR, while the risk of overinterpreting AMACR positivity in HGPIN is checked by the retention of the p63-positive basal layer.[25]

These findings strongly corroborate the extensive literature endorsing the dual-marker approach. Rathod et al. demonstrated that combining AMACR with a basal cell marker significantly reduces ambiguity in atypical small acinar proliferations, effectively eliminating false-positive diagnoses.[25] Similarly, Hasan et al. and Biswas and Talukdar reported that the dual application of these markers yields sensitivity and specificity metrics superior to either assay performed in isolation.[26,27] The current dataset emphatically supports the routine integration of this combined panel into surgical pathology practice to resolve challenging prostatic biopsies.[28]

Strengths and Limitations: This study's major strength is its multi-parametric approach, correlating clinical features, PSA levels, H&E morphology, and dual-marker immunohistochemistry within a real-world tertiary cohort. Including diverse inflammatory lesions rather than only suspicious biopsies authenticates routine diagnostic challenges, highlighting prostatitis as a confounding factor in PSA screening.[29] Limitations include the modest malignant sample size (n=25), which restricts deep subgroup analyses of ISUP Grade Groups. The predominance of TURP specimens (93.8%) biases the cohort toward transition zone pathologies like BPH, underrepresenting early-stage peripheral zone adenocarcinomas typically diagnosed via core biopsies.[30,31] Additionally, the cross-sectional design lacked longitudinal follow-up, precluding prognostic assessments of biomarker expression regarding recurrence or survival.[22] Finally, the IHC panel was limited to p63 and AMACR; integrating additional markers like ERG or HMWCK could further refine recalcitrant diagnoses.[23,24,32]

**Clinical and Pathological Implications:** The clinical implications of these findings are significant

for both urologists and pathologists. Clinicians must exercise caution when interpreting elevated PSA levels, recognizing that profound biochemical spikes frequently emanate from florid chronic prostatitis.[16] The decision to proceed with aggressive oncological intervention must never rely on biochemistry alone. For the surgical pathologist, the data validate the routine deployment of the p63 and AMACR dual-stain in cases of atypical small acinar proliferation or when crushing/cautery artifact obscures nuclear detail. This synergistic approach drastically minimizes interobserver variability, prevents catastrophic diagnostic errors, and provides objective evidence to support definitive patient management.[14]

## CONCLUSION

The accurate diagnostic classification of prostatic lesions necessitates a rigorous synthesis of clinical parameters, biochemistry, and precise tissue pathology. While serum PSA demonstrates a highly significant quantitative correlation with prostatic adenocarcinoma, its diagnostic specificity is severely compromised by coexistent inflammatory conditions; chronic prostatitis frequently simulates the biochemical profile of invasive malignancy. Consequently, definitive diagnosis relies irrevocably on histomorphological examination. The present study establishes that the targeted application of p63 and AMACR immunohistochemistry provides an exceptionally robust diagnostic adjunct to routine morphology. The absolute absence of p63 expression is highly sensitive for the malignant loss of the basal cell layer, while AMACR upregulation serves as a specific metabolic marker for invasive carcinoma across all histological grades. The combined reciprocal immunophenotype –defined by p63 negativity and AMACR positivity– yields unparalleled specificity and positive predictive value. This biological synergy effectively resolves diagnostic ambiguity in challenging prostatic specimens, safeguarding against the perilous misclassification of benign prostatic mimics and ensuring the delivery of accurate, evidence-based oncological care.

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