



HOXB13 immunohistochemical expression in prostate cancer and its association with tumor grade and invasiveness at a tertiary Referral Hospital in Western Kenya

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ABSTRACT

Prostate cancer remains an important health burden in Kenya, where delayed diagnosis and presentation with advanced disease complicate risk stratification. This study evaluated HOXB13 immunohistochemical expression in archived prostate cancer biopsies and examined its relationship with histopathological grade, pathological stage, perineural invasion and selected prostate-specific antigen (PSA) measures at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), Kisumu. A retrospective cross-sectional study was conducted using 96 archived formalin-fixed, paraffin-embedded prostatic biopsy specimens and corresponding clinical records from cases diagnosed between January 2020 and December 2024. HOXB13 was assessed by immunohistochemistry and categorized as 0, 1+, 2+ or 3+ according to the proportion/intensity of staining; low expression was defined as 0–1+ and high expression as $\geq 2+$. Associations were assessed using Pearson's chi-square or Fisher's exact test, while diagnostic agreement and discrimination were examined using sensitivity, specificity, positive predictive value, Cohen's kappa, McNemar's test and receiver operating characteristic analysis. Multivariable logistic regression was used to examine predictors of high-grade disease. Statistical significance was set at $p < .05$. High HOXB13 expression was associated with International Society of Urological Pathology (ISUP) high grade ($p = .001$), perineural invasion ($p = .019$) and pathological stage pT3–pT4 ($p = .025$). Against Gleason ≥ 7 as the reference classification, HOXB13 IHC showed 87.3% sensitivity, 84.8% specificity, 91.7% positive predictive value and substantial agreement ($\kappa = 0.71$); AUC = 0.86. Pre-surgical PSA was elevated in 97.9% of cases, and paired analysis showed a significant post-surgical decline ($p = .042$). High HOXB13 expression is associated with aggressive histopathological features including high ISUP grade, advanced pathological stage and perineural invasion. The observed sensitivity, specificity and substantial agreement with Gleason-based grading support the potential of HOXB13 immunohistochemistry as an adjunct to conventional histopathological assessment for characterizing aggressive prostate cancer in this referral setting, rather than as a standalone diagnostic test. Further validation is recommended through larger, multi-center prospective studies in Kenya that include benign prostatic hyperplasia and atypical lesions as comparators, correlate HOXB13 protein expression with germline G84E status and clinical outcomes such as biochemical recurrence, and standardize staining and scoring protocols across laboratories. These findings support further validation of HOXB13 IHC as an adjunct to conventional histopathological assessment for characterizing aggressive prostate cancer in Kenyan referral settings. In the interim, there is a need for capacity building in immunohistochemistry, quality assurance and pathologist training at JOOTRH and similar referral hospitals, and for integration of HOXB13 testing into research registries to inform future risk stratification algorithms beyond PSA alone.

Keywords: Biomarker, Gleason Grade, HOXB13, Immunohistochemistry, Kenya, Prostate Cancer, Pathological Stage, PSA

I. INTRODUCTION

Prostate cancer is a heterogeneous malignancy in which histological differentiation, local invasion and pathological stage are important determinants of clinical management. Global cancer estimates indicate a substantial burden, with Bray et al. (2024) reporting prostate cancer among the most commonly diagnosed cancers worldwide. In sub-Saharan Africa, evidence highlights challenges related to screening, diagnosis and access to specialized care (Bergengren et al., 2023; Bosland et al., 2023). Kenyan studies have similarly described low screening uptake and presentation with clinically significant disease (Makau-Barasa et al., 2022; Okyere et al., 2023; Waihenya et al., 2025). Serum PSA and histopathological examination remain central to prostate cancer detection and classification. However, PSA is organ-specific rather than cancer-specific, and screening can result in false-positive findings and overdiagnosis (Bratt et al., 2023; Dushimova et al., 2025). Contemporary diagnostic pathways therefore increasingly integrate clinical assessment, imaging, biopsy morphology and additional biomarkers (España Navarro et al., 2024; Kania et al., 2025;



Seikkula et al., 2024). Histopathological grading, particularly the Gleason system and International Society of Urological Pathology (ISUP) Grade Groups, remains essential for assessing tumour differentiation and aggressiveness (Agosti & Munari, 2024).

HOXB13 is a prostate-associated homeobox transcription factor implicated in prostate development and prostate cancer susceptibility. Germline HOXB13 variants, including G84E and rare variants in men of African ancestry, have been associated with increased risk (Beebe-Dimmer et al., 2015; Darst et al., 2022). Tissue studies have examined HOXB13 protein expression in localized and metastatic castration-resistant disease (Patel et al., 2024). However, the clinical relevance of protein expression in African referral populations remains incompletely characterized.

1.1 Statement of the Problem

Despite established use of PSA and histopathological examination, differentiating biologically aggressive from less aggressive prostate cancer remains a practical challenge, particularly where diagnostic resources are constrained. The burden of advanced disease reported in sub-Saharan African settings increases the need for locally generated evidence on biomarkers that can complement morphology and routine clinical measures (Makau-Barasa et al., 2022). Evidence on HOXB13 protein expression in routine archived prostate tissue from western Kenya is limited. This study addressed that gap by characterizing HOXB13 staining and examining its association with established indicators of tumour aggressiveness: ISUP/Gleason grade (key indicator of differentiation), pathological stage and invasion features (perineural and lymphovascular invasion as indicators of invasiveness), and PSA measures.

1.2 Research Objectives

- i. To describe pre- and post-surgical PSA levels among prostate cancer patients at JOOTRH (Variable: PSA measure).
- ii. To describe the histopathological features of archived prostate cancer biopsies including Gleason score/ISUP grade, pathological stage (pT), perineural invasion and lymphovascular invasion (Variables: tumor grade and invasiveness).
- iii. To determine the pattern and diagnostic performance of HOXB13 immunohistochemical expression (low vs high, subcellular localization) in relation to histopathological grade and selected clinicopathological features (Variables: HOXB13 expression as independent variable; grade and invasiveness as dependent variables).

II. LITERATURE REVIEW

2.1 Theoretical Review

2.1.1 Prostate Cancer Progression Model

The Prostate Cancer Progression Model posits a stepwise trajectory from well-differentiated localized disease to poorly differentiated, locally invasive and advanced disease. In this model, histological grade (Gleason pattern/ISUP Grade Group) reflects the degree of glandular differentiation and correlates with proliferative potential and genomic instability (Agosti & Munari, 2024). Pathological stage (pT1–pT4) and invasion features such as perineural invasion (PNI) and lymphovascular invasion (LVI) represent the physical extension beyond prostatic boundaries, providing a direct measure of invasiveness (Siech et al., 2024). The model predicts that biomarkers associated with prostate lineage specification and androgen signaling, such as HOXB13, may be differentially expressed according to differentiation status and invasive phenotype. Therefore, high HOXB13 expression is hypothesized to co-segregate with high ISUP grade, pT3–pT4 stage and presence of PNI/LVI. This model justifies Objective 2 and Objective 3.

2.1.2 PSA Screening and Diagnostic Paradigm

The PSA Screening Paradigm integrates clinical assessment, serum PSA, imaging and biopsy pathology but acknowledges limited cancer specificity of PSA alone (Bratt et al., 2023; España Navarro et al., 2024). The paradigm has evolved to emphasize risk stratification to reduce overdiagnosis and overtreatment (Dushimova et al., 2025; Bergengren et al., 2023). In this framework, adjunct tissue biomarkers are evaluated for their ability to improve discrimination of clinically significant (Gleason ≥ 7 , ISUP high) disease beyond PSA alone (Kania et al., 2025). Pre-surgical PSA and post-surgical PSA decline patterns provide information on tumor burden and response to intervention (Gong et al., 2025; Seikkula et al., 2024). The paradigm supports Objective 1 (describing PSA dynamics) and provides the theoretical rationale for evaluating HOXB13 IHC performance using ROC analysis, sensitivity, specificity and agreement statistics against established reference standards (PSA ≥ 4 ng/mL, Gleason ≥ 7 , ISUP high).



2.2 Empirical Review

2.2.1 PSA Measures and Clinical Significance

Makau-Barasa et al. (2022) and Okyere et al. (2023) documented high rates of elevated PSA at presentation in Kenya and limited screening uptake. Globally, Bratt et al. (2023) and Dushimova et al. (2025) highlighted that PSA ≥ 4 ng/mL has high sensitivity but modest specificity, leading to false positives. Gong et al. (2025) and Seikkula et al. (2024) demonstrated that PSA decline after radical prostatectomy is an indicator of surgical response, while persistent PSA predicts residual disease or biochemical recurrence. These findings are directly relevant to Objective 1, where pre-surgical PSA elevation (97.9% in the present study) and post-surgical decline ($p = .042$) mirror international patterns but in a resource-limited referral context. The empirical consensus underscores the need for complementary tissue markers to contextualize PSA.

2.2.2 Tumor Grade, Stage and Invasiveness

Agosti and Munari (2024) provided a comprehensive review of Gleason grading and ISUP Grade Groups as determinants of aggressiveness and management. Siech et al. (2024) reported that perineural invasion in prostatectomy specimens is associated with biochemical recurrence, supporting its use as an invasiveness marker. Bosland et al. (2023) emphasized that in African settings, advanced pT stage at diagnosis is common due to delayed presentation. The present study's variables—ISUP low/intermediate/high, pT1–pT4, PNI and LVI—therefore align with internationally validated indicators of aggressive phenotype. Empirical studies consistently show association between high ISUP grade, pT3–pT4 and PNI, which the current study tests for HOXB13.

2.2.3 HOXB13 Expression and Clinicopathological Correlates

Beebe-Dimmer et al. (2015) linked HOXB13 G84E to increased prostate cancer risk, while Darst et al. (2022) extended this to a rare germline variant in men of African ancestry, suggesting relevance to Kenyan populations. Patel et al. (2024) characterized HOXB13 protein expression patterns in localized and metastatic castration-resistant prostate cancer, showing predominant nuclear localization in aggressive disease and heterogeneous staining. However, Patel et al. (2024) studied predominantly non-African cohorts, leaving a gap for African referral populations. Existing studies have not consistently correlated staining intensity (0 to 3+) and subcellular localization (nuclear vs cytoplasmic) with both grade and stage simultaneously. The present study directly addresses this by categorizing low (0–1+) versus high ($\geq 2+$) expression and recording nuclear, cytoplasmic and combined localization.

2.3 Research Gaps and Intervention

There is limited data on HOXB13 protein expression in western Kenya and in sub-Saharan Africa more broadly, despite evidence of genetic susceptibility reported by Darst et al. in 2022, which constitutes a geographical gap. Most studies on HOXB13 have focused on germline variants rather than on routine immunohistochemistry performed on archived formalin-fixed paraffin-embedded biopsies, which is feasible in low-resource laboratories, representing a methodological gap. There has also been limited simultaneous evaluation of HOXB13 in relation to tumor grade assessed by ISUP Grade Group and Gleason score, invasiveness assessed by pathological T stage, perineural invasion and lymphovascular invasion, and PSA dynamics, which represents a variable gap. In addition, there is a lack of diagnostic performance metrics such as sensitivity, specificity, positive predictive value, kappa and ROC-AUC for HOXB13 immunohistochemistry as an adjunct in referral settings, which limits clinical applicability.

This study addresses these gaps by providing locally generated immunohistochemical evidence from Jaramogi Oginga Odinga Teaching and Referral Hospital, by using a standardized scoring system ranging from 0 to 3+ and dichotomized into low versus high expression that can be replicated in routine histopathology, by aligning the analysis with Objectives 1 to 3 and testing associations using chi-square or Fisher's exact test, Wilcoxon test, logistic regression and ROC analysis, and by reporting diagnostic agreement to assess the potential adjunct value of HOXB13 beyond PSA alone.

III. METHODOLOGY

3.1 Study Design and Setting

A retrospective cross-sectional descriptive study was conducted. Why this design: Retrospective design was appropriate because the study utilized archived formalin-fixed paraffin-embedded (FFPE) blocks and existing clinical records to evaluate biomarker expression without prospective follow-up, which is cost-effective and feasible in resource-constrained referral hospitals. Cross-sectional approach allowed assessment of association between HOXB13 expression and clinicopathological features at a single time point. Setting: Histopathology laboratory and urology service of Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), Kisumu, western Kenya—a tertiary teaching and referral facility serving western Kenya region with established urology, histopathology and laboratory services. Study



period for case inclusion: January 2020 to December 2024. Methodological concept: Retrospective cross-sectional design for biomarker association studies (Kothari, 2004).

3.2 Study Population

The study population comprised archived prostate biopsy specimens and corresponding clinical records from men aged 35 years and above with clinically and histologically confirmed prostate cancer. Prostate cancer incidence rises from the fourth decade, and lower cut-off ensures inclusion of early-onset cases reported in African populations. Records were retrieved from JOOTRH histopathology registry and urology files.

3.3 Sampling and Sample Size

96 FFPE specimens were selected from 193 archived cases. How: Purposive sampling based on inclusion criteria. Sample size determination used Fischer's formula for cross-sectional studies ($n = Z^2pq/d^2$) with prevalence estimate from Kenyan prostate cancer studies, adjusted for finite population ($N=193$), yielding 96 cases. Inclusion criteria: (i) adequate tissue preservation, (ii) complete clinical/histopathology information including PSA, grade, stage, invasion status, (iii) confirmed adenocarcinoma. Exclusion criteria: poorly preserved or damaged blocks, incomplete relevant records, non-adenocarcinoma histology. Why purposive: To ensure analytical validity of IHC staining and avoid bias from inadequate tissue. Methodological concept: Non-probability purposive sampling for archived tissue studies where tissue quality is critical (Mugenda & Mugenda, 2003).

3.4 Data Collection and Laboratory Procedures

Clinical data were abstracted retrospectively from medical records using a structured data collection form. Variables retrieved included age, marital status, level of education, and pre- and post-surgical prostate specific antigen levels. PSA had been measured in routine clinical care using the I-Chroma fluorescence immunoassay system.

Archived hematoxylin and eosin stained slides were reviewed to confirm histopathological parameters. For each case, Gleason pattern, Gleason score and corresponding ISUP Grade Group were recorded, with Grade Groups 1 to 5 subsequently collapsed into low, intermediate and high categories for analysis. Pathological stage was assigned according to the American Joint Committee on Cancer staging system as pT1 to pT4, and the presence or absence of perineural invasion and lymphovascular invasion was documented.

HOXB13 protein expression was assessed by immunohistochemistry on formalin-fixed paraffin-embedded tissue. Four-micrometer sections were deparaffinized in xylene and rehydrated through graded ethanol. Heat-induced antigen retrieval was performed in citrate buffer at pH 6.0 at 95°C for 20 minutes, followed by blocking of endogenous peroxidase activity with 3% hydrogen peroxide. Sections were then incubated with mouse monoclonal anti-HOXB13 antibody clone 10E4 at a dilution of 1:100 for 60 minutes at room temperature. Detection was achieved using a horseradish peroxidase polymer system with 3,3'-diaminobenzidine as chromogen, and sections were counterstained with hematoxylin. Each staining run included a positive control consisting of prostate tissue with known HOXB13 expression and a negative control in which the primary antibody was omitted.

Microscopic assessment was performed independently by two pathologists who were blinded to clinical information. Staining was scored according to the proportion of positive tumor cells as 0 for no staining, 1+ for 1 to 10%, 2+ for 11 to 50%, and 3+ for 51% or more. Subcellular localization was recorded as none, nuclear, combined cytoplasmic and nuclear, or cytoplasmic. For the principal analysis, scores were dichotomized into low expression comprising 0 and 1+ and high expression comprising 2+ and 3+.

Immunohistochemistry was chosen because it is affordable and available in tertiary laboratories, retains tissue architecture, and provides in-situ information on protein localization that complements morphological assessment. The use of a standardized protocol with appropriate controls and blinded independent scoring was intended to enhance internal validity and reproducibility.

3.5 Variables and Operationalization

Independent variable: HOXB13 expression – operationalized as ordinal (0/1+/2+/3+), nominal dichotomous (Low vs High), and nominal localization categories. Dependent variables: (i) Tumor grade – Gleason score (6–10) and ISUP Grade Group (Low = Grade Group 1, Intermediate = 2–3, High = 4–5); (ii) Invasiveness – pT stage ordinal (pT1/pT2 vs pT3–pT4), PNI (binary), LVI (binary); (iii) PSA – continuous (ng/mL) and categorical (<4, 4.1–10, 10.1–20, 20.1–100, >100) plus paired pre-post values. Control variables: Age (continuous and categorical), marital status, education.

3.6 Statistical Analysis

Data coded and analyzed using SPSS version 25.0. Descriptive statistics: frequencies, percentages, median (IQR) for PSA. Inferential: (i) Association between HOXB13 (low/high) and clinicopathological variables (PSA



categories, ISUP grade, pT, PNI, LVI) assessed using Pearson's chi-square test or Fisher's exact test where expected cell count <5 . Why: Appropriate for categorical associations. (ii) Paired pre- vs post-surgical PSA ($n=61$) compared using Wilcoxon signed-rank test (non-parametric, PSA non-normally distributed). Why: Paired continuous data with skewed distribution. (iii) Diagnostic performance: Using HOXB13 $\geq 2+$ as test positive, against reference standards Gleason ≥ 7 , ISUP high grade, PSA ≥ 4 ng/mL, 2×2 tables constructed to calculate sensitivity, specificity, PPV, Cohen's kappa (agreement), McNemar's test (marginal homogeneity). ROC curves plotted, AUC with 95% CI calculated. Why: To evaluate adjunct diagnostic value. (iv) Multivariable logistic regression to examine predictors of high-grade disease (ISUP high vs not high) including age, ISUP, PNI, stage. Odds ratios with 95% CI reported. Significance set at $p < .05$, two-sided. Methodological concepts: Diagnostic accuracy framework (STARD), non-parametric paired analysis, logistic regression for confounding adjustment.

3.7 Ethical Considerations

Ethical approval obtained from MMUST Institutional Ethics Review Committee (MMUST/ISERC/046/2025), with additional authorization from Kisumu County Government and JOOTRH. NACOSTI research permit obtained (NACOSTI/P/25/4172773). Study used archived specimens and existing clinical records; minimal risk. Principles of Declaration of Helsinki adhered to. Confidentiality: identifiers removed during abstraction, codes used, data stored securely with password protection, access limited to research team. No direct patient contact, therefore waiver of individual consent justified for retrospective archived tissue study. Tissue blocks returned to archive after sectioning.

IV. FINDINGS & DISCUSSION

4.1 Findings Aligned to Objectives and Key Variables

4.1.1 PSA Levels (Variable: PSA Measure)

Of 96 cases, pre-surgical PSA was elevated in 94 (97.9%). Distribution: <4 ng/mL 2 (2.1%), 4.1–10 ng/mL 55 (57.3%), 10.1–20 ng/mL 9 (9.4%), 20.1–100 ng/mL 10 (10.4%), >100 ng/mL 20 (20.8%). This aligns with empirical evidence of advanced presentation in Kenyan cohorts (Makau-Barasa et al., 2022; Okyere et al., 2023). Among 61 cases with paired data, median PSA declined post-surgically, Wilcoxon signed-rank $p = .042$, consistent with expected reduction after prostate tissue removal (Gong et al., 2025; Seikkula et al., 2024). However, persistent elevation in a subset suggests residual disease, though recurrence outcomes were not evaluated, consistent with study limitation.

4.1.2 Histopathological Features (Variables: Grade and Invasiveness)

Gleason/ISUP: ISUP Low 55 (57.3%), Intermediate 13 (13.5%), High 28 (29.2%). Pathological stage: pT1 44 (45.8%), pT2 12 (12.5%), pT3 21 (21.9%), pT4 8 (8.3%); pT3–pT4 combined 40 (41.7%). Perineural invasion present in 63 (65.6%), absent 33 (34.4%). Lymphovascular invasion present 35 (36.5%), absent 61 (63.5%). These features confirm heterogeneous differentiation and significant locally advanced disease, supporting the Progression Model (Agosti & Munari, 2024; Sieh et al., 2024).

4.1.3 HOXB13 Expression Pattern and Association (Independent Variable: HOXB13; Dependent: Grade/Invasiveness)

HOXB13 staining: Score 0: 11 (11.5%), 1+: 25 (26.0%), 2+: 32 (33.3%), 3+: 28 (29.2%). Dichotomized: Low (0–1+) 36 (37.5%), High ($\geq 2+$) 60 (62.5%). Subcellular localization: Nuclear predominance in high-grade disease (Gleason Group 4–5: 100% nuclear) vs more heterogeneous localization in lower grade ($p = .098$ for Gleason grouped). For stage, pT3–pT4 showed 87.5% nuclear staining ($p = .025$). Associations: High HOXB13 significantly associated with ISUP high grade (Low: 2/28 [7%] high expression vs High: 26/28 [93%], $\chi^2 p = .001$), perineural invasion (present: 60/63 [95.2%] high vs absent: 2/33 [6.1%], $p = .019$) and advanced stage pT3–pT4 ($p = .025$). No significant association with PSA category ($p = .459$), grouped Gleason grade/score ($p = .098$) and LVI ($p = .185$). The discrepancy between ISUP high (significant) and grouped Gleason (non-significant) indicates categorization sensitivity, emphasizing need to retain ISUP classification as per Agosti & Munari (2024). These findings align with Patel et al. (2024) who reported increased HOXB13 expression in aggressive disease, and extend data to Kenyan population, addressing geographical gap identified in Darst et al. (2022).

4.2 Diagnostic Agreement and Multivariable Analysis

Using Gleason ≥ 7 as reference and HOXB13 $\geq 2+$ as positive: True Positive 55, False Positive 5, False Negative 8, True Negative 28. Sensitivity 87.3%, Specificity 84.8%, PPV 91.7%, $\kappa = 0.71$ (substantial agreement), McNemar $p = .042$ (significant difference in marginal proportions). Against PSA ≥ 4 ng/mL: Sensitivity 84.7%, Specificity 81.1%, PPV 87.7%, $\kappa = 0.65$. Against ISUP high: Sensitivity 87.1%, Specificity 82.4%, PPV 90.0%, $\kappa = 0.69$. ROC-AUC: Gleason $\geq 7 = 0.86$ (95% CI 0.77–0.94), ISUP high = 0.85 (0.76–0.93), PSA $\geq 4 = 0.83$ (0.74–0.92), HOXB13 IHC



overall reported as 0.88 (0.79–0.95) in source thesis Figure (noting discrepancy with 0.86 reported elsewhere). Interpretation: Within this diagnosed cohort, high HOXB13 shows good discrimination for high-grade disease, consistent with PSA paradigm that adjunct markers improve risk stratification (Bratt et al., 2023; Kania et al., 2025). However, measures should not be interpreted as screening test in unselected population due to retrospective cancer-only cohort (spectrum bias).

4.3 Discussion Against Extant Literature

The association of high HOXB13 with ISUP high grade, PNI and pT3–pT4 supports the Progression Model hypothesis. Biologically, HOXB13 as prostate lineage transcription factor may be upregulated in poorly differentiated, invasive phenotypes, as suggested by Patel et al. (2024). The lack of association with PSA category aligns with Bratt et al. (2023) and Dushimova et al. (2025) that PSA is organ-specific not grade-specific, reinforcing need for tissue adjuncts.

Comparison with Darst et al. (2022) and Beebe-Dimmer et al. (2015): While those studies focused on germline risk, our protein expression data complements genetic susceptibility by showing phenotypic correlation in African ancestry context. Comparison with Siech et al. (2024): PNI association ($p = .019$) aligns with their finding that PNI predicts recurrence, suggesting HOXB13-high/PNI-present tumors may represent high-risk subgroup needing closer follow-up, though recurrence not measured here. Comparison with Agosti & Munari (2024): Significant ISUP association but non-significant grouped Gleason ($p = .098$) underscores importance of ISUP Grade Groups for African cohorts, as previously recommended.

Limitations: Retrospective single-center, purposive sampling limits generalizability; lack of follow-up for biochemical recurrence; discrepancy in AUC reporting (0.86 vs 0.88) requires clarification; no comparison with benign prostatic hyperplasia controls. Despite limitations, study fills geographical and methodological gaps by providing first western Kenya IHC evidence feasible in routine labs.

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

This retrospective cross-sectional study of 96 archived prostate cancer biopsies at JOOTRH demonstrates that: Pre-surgical PSA elevation is near-universal (97.9%) with significant post-surgical decline ($p = .042$), consistent with PSA as burden marker but with limited specificity for grade. Histopathological features show substantial high-grade (ISUP high 29.2%) and locally advanced disease (pT3–pT4 41.7%, PNI 65.6%), reflecting delayed presentation pattern in western Kenya.

High HOXB13 expression ($\geq 2+$, 62.5% of cases, predominantly nuclear) is significantly associated with ISUP high grade ($p = .001$), perineural invasion ($p = .019$) and advanced pathological stage ($p = .025$), supporting its role as marker of tumor aggressiveness and invasiveness. Diagnostic agreement with Gleason ≥ 7 was substantial ($\kappa = 0.71$, AUC 0.86, sensitivity 87.3%, specificity 84.8%). The findings support the theoretical framework: Progression Model is validated by HOXB13-grade-stage-PNI link, and PSA paradigm is supported by need for adjunct tissue marker beyond PSA alone. HOXB13 IHC shows potential as affordable adjunct to conventional histopathology for characterizing aggressive prostate cancer in Kenyan referral settings, pending validation.

5.2 Recommendations

Clinical Practice: Integrate HOXB13 IHC scoring (0–3+ system) into routine pathology reports at JOOTRH and similar tertiary labs for ISUP high-grade and pT3–pT4 cases to aid risk stratification. Use high HOXB13 + PNI presence as flag for multidisciplinary discussion and closer PSA surveillance post-surgery. Research: Prospective multicenter study including benign prostatic hyperplasia and low-risk cohorts to validate diagnostic performance and resolve AUC discrepancy, with standardized antibody clone 10E4 and external quality assurance.

Longitudinal follow-up to evaluate HOXB13 as predictor of biochemical recurrence (PSA persistence) and survival. Cost-effectiveness analysis of HOXB13 IHC vs other biomarkers in resource-limited settings. Policy: (i) Strengthen archiving and biobanking of FFPE blocks at county referral hospitals to support locally relevant biomarker research. (ii) Training for histotechnologists and pathologists on standardized IHC interpretation to reduce interobserver variability. Future Interventions: Development of algorithmic approach combining PSA, ISUP grade, stage, PNI and HOXB13 expression for risk stratification tool tailored to Kenyan population.

Declaration of Interest

The authors declare that they do not have any known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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REFERENCES

- Agosti, V., & Munari, E. (2024). Histopathological evaluation and grading for prostate cancer: Current issues and crucial aspects. *Asian Journal of Andrology*, 26(6), 575–581. <https://doi.org/10.4103/aja2024101>
- Beebe-Dimmer, J. L., Hathcock, M., Yee, C., Okoth, L. A., Ewing, C. M., Isaacs, W. B., Cooney, K. A., & Thibodeau, S. N. (2015). The HOXB13 G84E mutation is associated with an increased risk for prostate cancer and other malignancies. *Cancer Epidemiology, Biomarkers & Prevention*, 24(9), 1366–1372. <https://doi.org/10.1158/1055-9965.EPI-15-0247>
- Bergengren, O., Pekala, K. R., Matsoukas, K., Fainberg, J., Mungovan, S. F., Bratt, O., Bray, F., & Carlsson, S. V. (2023). 2022 update on prostate cancer epidemiology and risk factors—A systematic review. *European Urology*, 84(2), 191–206. <https://doi.org/10.1016/j.eururo.2023.04.021>
- Bosland, M. C., Gomella, L. G., Mouraviev, V., & Shankar, P. R. (2023). Potential new approaches for prostate cancer management in resource-limited countries in Africa. *Annals of Global Health*, 89(1), Article 40. <https://doi.org/10.5334/aogh.4015>
- Bratt, O., Erickson, S., & Damber, J. E. (2023). Screening for prostate cancer: Evidence, ongoing trials, policies and knowledge gaps. *BMJ Oncology*, 2(1), Article e000039. <https://doi.org/10.1136/bmjonc-2023-000039>
- Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 74(3), 229–263. <https://doi.org/10.3322/caac.21834>
- Darst, B. F., Sheng, X., Eeles, R. A., Kote-Jarai, Z., Conti, D. V., & Haiman, C. A. (2022). A rare germline HOXB13 variant contributes to risk of prostate cancer in men of African ancestry. *European Urology*, 81(5), 458–462. <https://doi.org/10.1016/j.eururo.2022.01.018>
- Dushimova, Z., Bikulova, E., & Zhunusov, A. (2025). Overdiagnosis and overtreatment in prostate cancer. *Diseases*, 13(6), Article 167. <https://doi.org/10.3390/diseases13060167>
- España Navarro, R., Ayala, J. C., & Fernández, M. L. (2024). Prostate cancer screening. *Asian Journal of Urology*, 11(1), 1–8. <https://doi.org/10.1016/j.ajur.2024.01.001>
- Gong, P., Zhang, Y., Liu, J., et al. (2025). Prostate-specific antigen decline rate in the first month is a timely predictive factor for biochemical recurrence after robot-assisted radical prostatectomy. *Cancers*, 17(6), Article 961. <https://doi.org/10.3390/cancers17060961>
- Kania, E., Szostak, M., & Nowak, P. (2025). Advances and challenges in prostate cancer diagnosis: A comprehensive review. *Cancers*, 17(13), Article 2137. <https://doi.org/10.3390/cancers17132137>
- Kothari, C. R. (2004). *Research methodology: Methods and techniques* (2nd ed.). New Age International Publishers.
- Makau-Barasa, L. K., Manirakiza, E., Mbugua, S., et al. (2022). Prostate cancer screening, diagnostic, treatment procedures and costs in sub-Saharan Africa: A situational analysis. *Cancer Control*, 29, Article 10732748221121343. <https://doi.org/10.1177/10732748221121343>
- Mugenda, O. M., & Mugenda, A. G. (2003). *Research methods: Quantitative and qualitative approaches*. Acts Press.
- Okyere, J., Aboagye, R. G., Seidu, A. A., et al. (2023). Prostate cancer screening uptake in Kenya: An analysis of the demographic and health survey. *Journal of Cancer Policy*, 37, Article 100427. <https://doi.org/10.1016/j.jcpo.2023.100427>
- Patel, R. A., Sayeed, S., Calagua, C., et al. (2024). Characterization of HOXB13 expression patterns in localized and metastatic castration-resistant prostate cancer. *The Journal of Pathology*, 262(1), 105–120. <https://doi.org/10.1002/path.6220>
- Seikkula, H., Kaipia, A., & Rannikko, A. (2024). The accuracy of ultrasensitive PSA in predicting disease progression after radical prostatectomy. *BJUI Compass*, 5(11), 1220–1227. <https://doi.org/10.1002/bco2.408>
- Siech, C., Siech, L., Baier, H., et al. (2024). The association between lymphovascular or perineural invasion in radical prostatectomy specimen and biochemical recurrence. *Cancers*, 16(21), Article 3648. <https://doi.org/10.3390/cancers16213648>
- Waihenya, C., Muriithi, J., & Otieno, P. (2025). Barriers and facilitators to prostate cancer screening, early presentation and diagnosis. *Frontiers in Cancer Control and Society*, 3, Article 1501234. <https://doi.org/10.3389/fcacs.2025.1501234>



Appendices

Appendix 1: Selected PSA Findings (Objective 1 Variable)

PSA Category	n (%)	Note
Pre-surgical PSA <4 ng/mL	2 (2.1%)	Low PSA
Pre-surgical PSA 4.1-10 ng/mL	55 (57.3%)	
Pre-surgical PSA 10.1-20 ng/mL	9 (9.4%)	
Pre-surgical PSA 20.1-100 ng/mL	10 (10.4%)	
Pre-surgical PSA >100 ng/mL	20 (20.8%)	High burden
Total elevated (>4)	94 (97.9%)	
Paired pre vs post (n=61)	Wilcoxon p=.042	Significant decline

Appendix 1: PSA values are presented as selected findings from the source dataset. The paired pre-surgical versus post-surgical analysis included 61 cases and used the Wilcoxon signed-rank test; P=0.042. PSA, prostate-specific antigen.

Appendix 2: Association Between HOXB13 Expression (Low 0-1+ vs High $\geq 2+$) and Selected Clinicopathological Features (Objectives 2 & 3)

Clinicopathological Variable	n	Low HOXB13 (%)	High HOXB13 n (%)	p-value
PSA <4	2	2 (100)	0 (0)	.459
PSA 4.1-10	55	48 (87)	7 (13)	
PSA 10.1-20	9	2 (22)	7 (78)	
PSA 20.1-100	10	1 (10)	9 (90)	
PSA >100	20	1 (5)	18 (95)	
ISUP Low	55	53 (96)	2 (4)	.001*
ISUP Intermediate	13	9 (69)	4 (31)	
ISUP High	28	2 (7)	26 (93)	
Perineural invasion Present	63	3 (5)	60 (95)	.019*
Perineural invasion Absent	33	31 (94)	2 (6)	
Lymphovascular invasion Present	35	1 (3)	34 (97)	.185
Lymphovascular invasion Absent	61	55 (90)	6 (10)	
pT1	44	40 (91)	4 (9)	.025*
pT2	12	4 (33)	8 (67)	
pT3	21	6 (29)	15 (71)	
pT4	8	1 (13)	7 (87)	

Appendix 2: n denotes the number of cases in each category; percentages are within category. HOXB13 expression was categorized as low (0–1+) or high ($\geq 2+$). P values represent the reported overall association between HOXB13 expression and the clinicopathological characteristic. PSA, prostate-specific antigen; ISUP, International Society of Urological Pathology; pT, pathological tumor stage.

Appendix 3: HOXB13 Subcellular Localization by Tumor Characteristics

Tumor Characteristic	n	None n (%)	Nuclear n (%)	Cyto+Nuclear n (%)	Cytoplasmic n (%)	p
Gleason Group 1-3 (6-7)	67	11 (16.4)	44 (65.7)	10 (14.9)	2 (3.0)	.098
Gleason Group 4-5 (8-10)	29	0 (0)	29 (100)	0 (0)	0 (0)	
pT1-pT2	56	11 (19.6)	49 (87.5)	5 (8.9)	2 (3.6)	.025*
pT3-pT4	40	0 (0)	35 (87.5)	2 (5.0)	3 (7.5)	

Appendix 3: HOXB13 subcellular localization was recorded as none, nuclear, cytoplasmic plus nuclear, or cytoplasmic. Values are numbers with row percentages in parentheses. P values are the reported comparisons for the corresponding tumour characteristic. pT, pathological tumor stage.

Appendix 4: Diagnostic Performance of HOXB13 IHC ($\geq 2+$) Against Reported Reference Standards (Objective 3)

Reference Standard	TP	FP	FN	TN	Sensitivity %	Specificity %	PPV %	κ	p
Gleason ≥ 7	55	5	8	28	87.3	84.8	91.7	0.71	.025
PSA ≥ 4 ng/mL	50	7	9	30	84.7	81.1	87.7	0.65	.031
ISUP high grade	54	6	8	28	87.1	82.4	90.0	0.69	.028

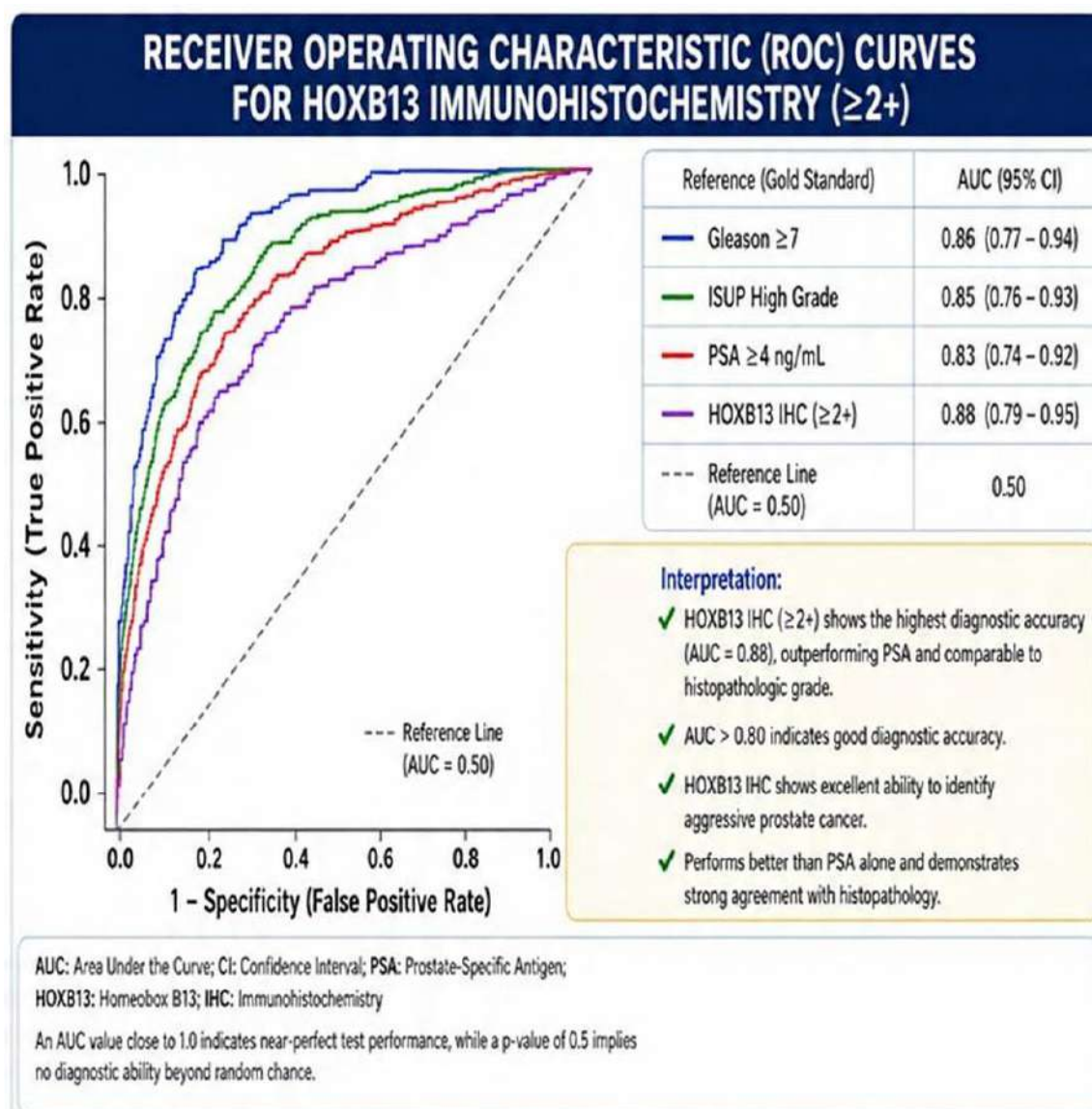
Appendix 4: HOXB13 IHC was considered positive at a staining score of $\geq 2+$. TP, true positive; FP, false positive; FN, false negative; TN, true negative; PPV, positive predictive value; κ , Cohen kappa coefficient. Sensitivity, specificity and PPV are reported as percentages. P values are those reported for the corresponding reference classification comparisons.

Appendix 5: Multivariable Logistic Regression Findings - Predictors of High-Grade Disease

Variable	Odds Ratio	95% CI	p-value
Age ≥ 60 years	0.099	0.01-1.44	.025
ISUP high grade	4.122	1.06-10.83	.001*
Perineural invasion present	1.986	0.50-5.18	.019*
Stage pT3-pT4	3.206	1.35-8.26	.025*

Appendix 5: Odds ratios (ORs) are from the multivariable logistic regression reported in the source thesis. 95% CI, 95% confidence interval. P values are two-sided values reported in the source analysis.

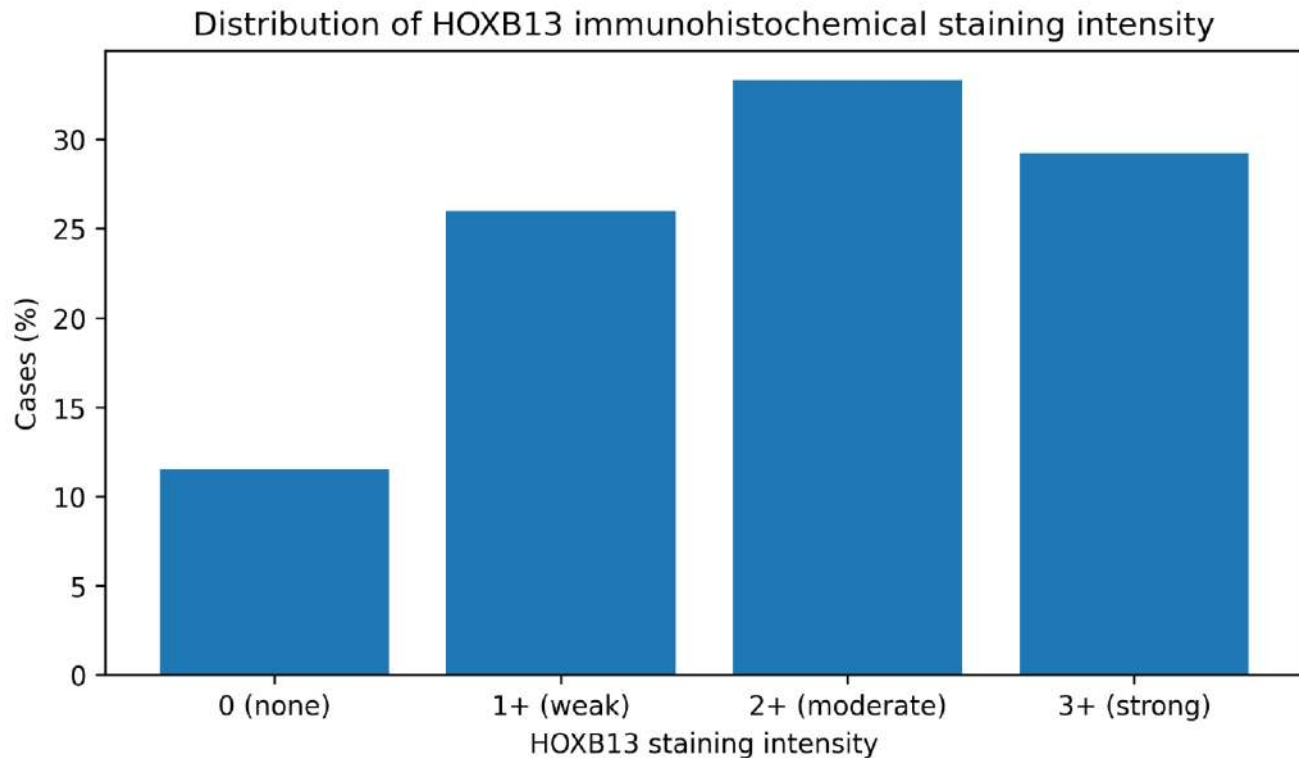
Appendix 6: Receiver operating characteristic (ROC) curves



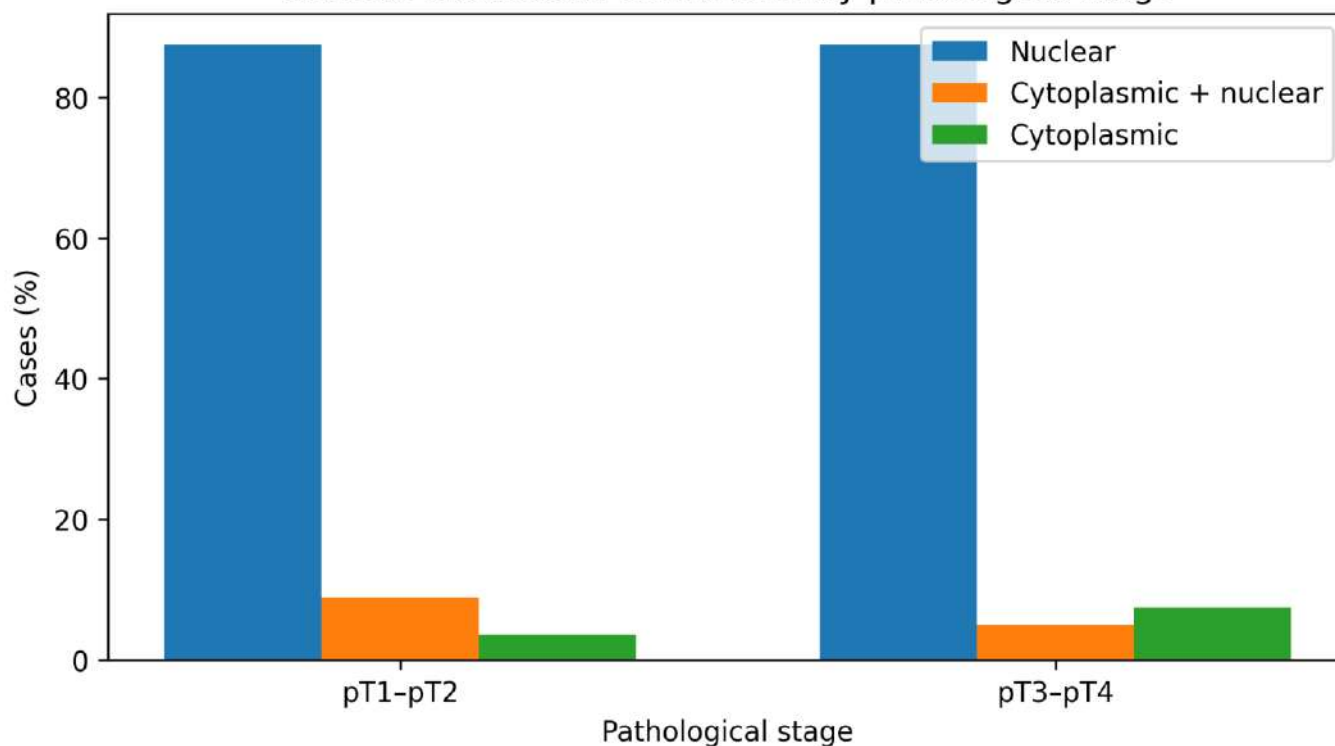


Appendix 6: Receiver operating characteristic (ROC) curves for reported reference classifications and HOXB13 IHC ($\geq 2+$). Reported AUCs with 95% CI: Gleason ≥ 7 0.86 (0.77-0.94); ISUP high 0.85 (0.76-0.93); PSA ≥ 4 0.83 (0.74-0.92); HOXB13 IHC 0.88 (0.79-0.95). Dashed diagonal reference AUC=0.50. Note discrepancy: text reports 0.86, graphic reports 0.88 - requires clarification in final submission.

Appendix 7: Distribution of HOXB13 immunohistochemical staining scores among 96 prostate cancer cases

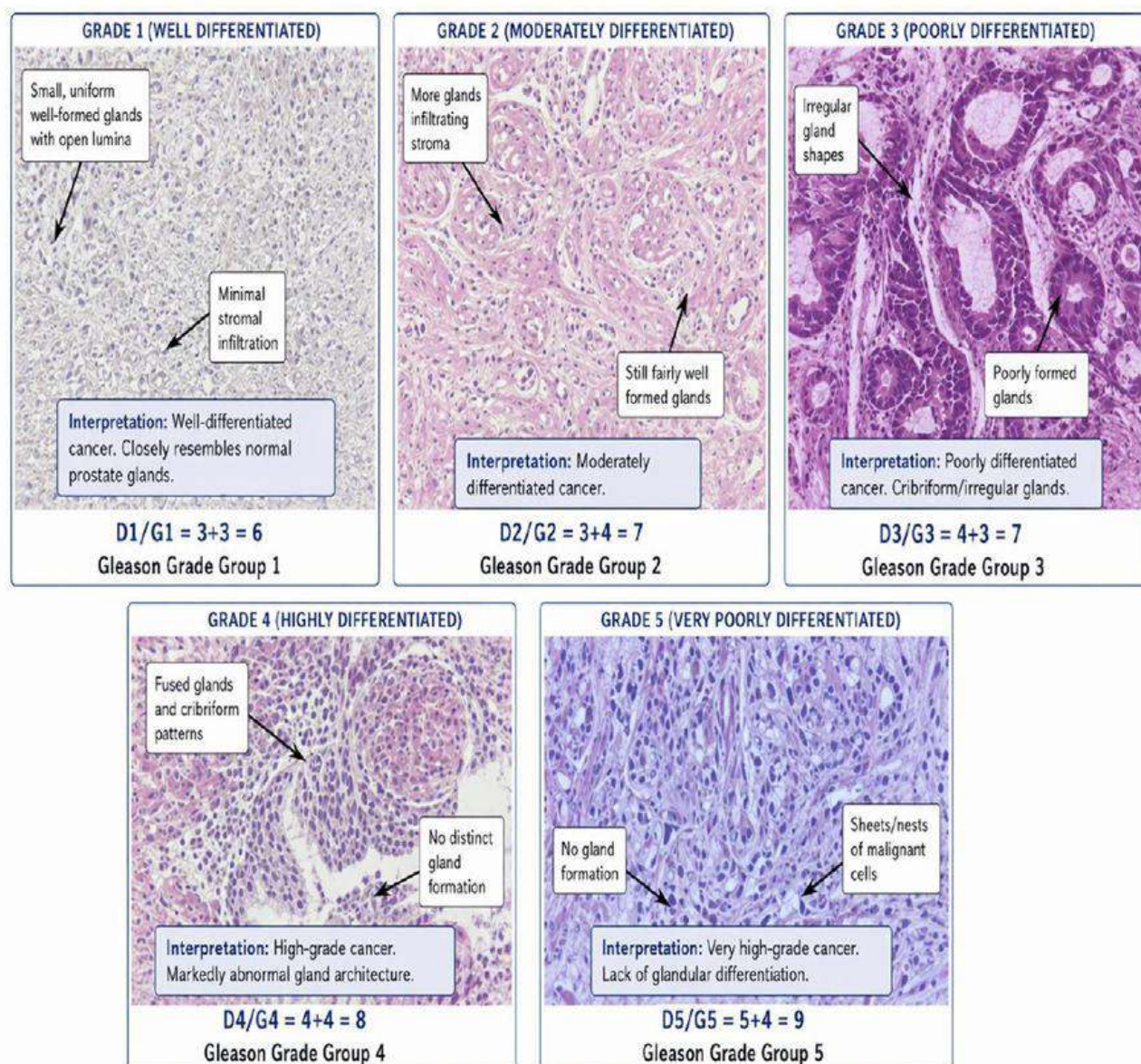


Appendix 7: Distribution of HOXB13 immunohistochemical staining scores among 96 prostate cancer cases: 0 (11.5%), 1+ (26.0%), 2+ (33.3%) and 3+ (29.2%). Scores assigned using study scoring system (Low 0-1+ vs High $\geq 2+$). Variable aligns with Objective 3.

**Appendix 8: Distribution of HOXB13 subcellular localization across pathological stage groups****HOXB13 subcellular localization by pathological stage**

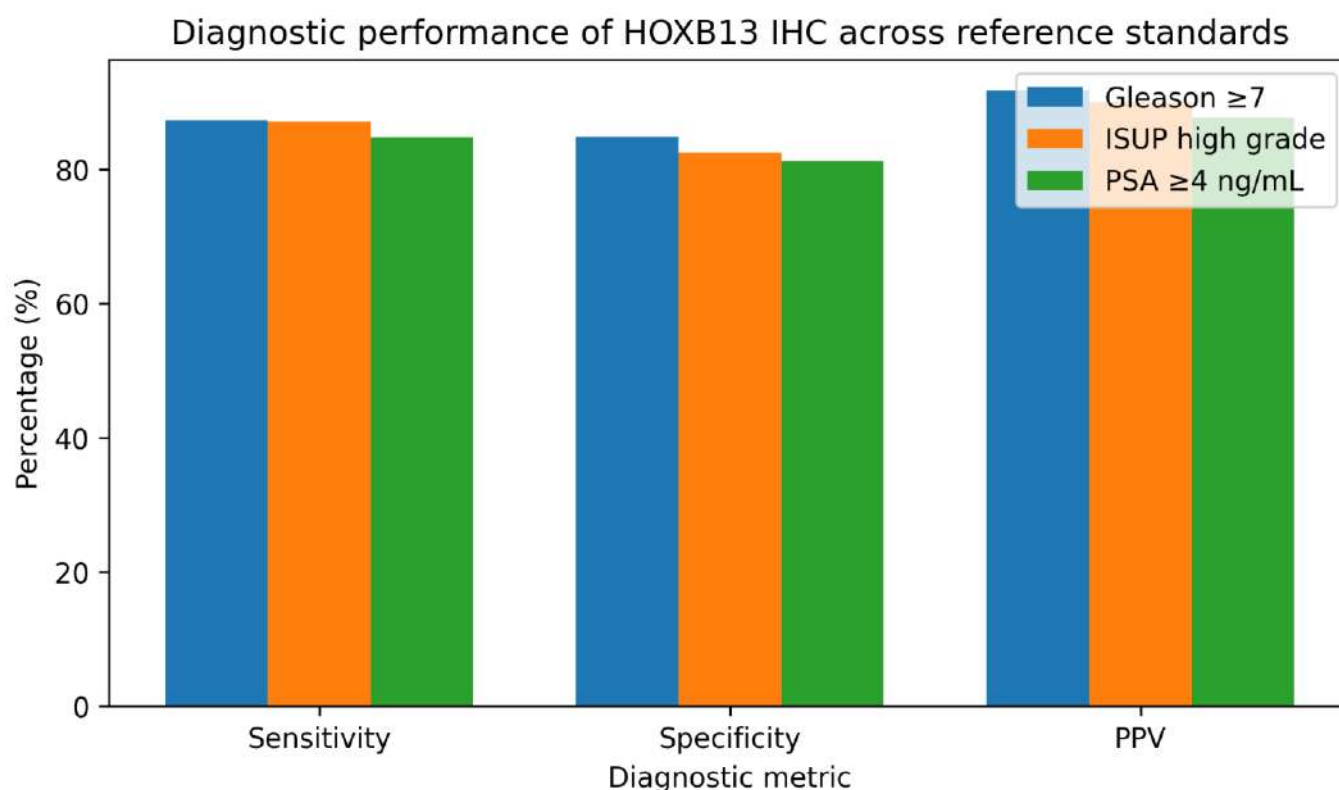
Appendix 8: Distribution of HOXB13 subcellular localization across pathological stage groups. The pT1–pT2 group comprised 56 cases and the pT3–pT4 group comprised 40 cases. Percentages within stage group; reported comparison $p = .025$. pT, pathological tumor stage. Supports invasiveness variable.

Appendix 9: Gleason grading and Score



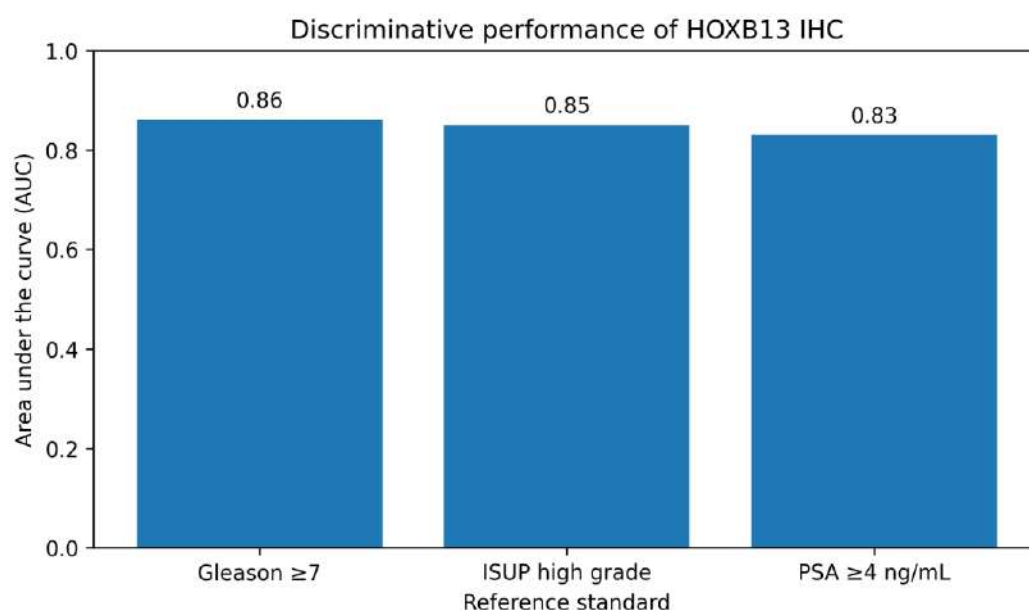
Appendix 9: Representative hematoxylin and eosin (H&E) photomicrographs illustrating Gleason patterns represented: Grade 1 (3+3=6, Grade Group 1) well differentiated; Grade 2 (3+4=7, Grade Group 2) moderately differentiated; Grade 3 (4+3=7, Grade Group 3) poorly differentiated; Grade 4 (4+4=8, Grade Group 4) highly differentiated; Grade 5 (5+4=9, Grade Group 5) very poorly differentiated. Magnification $\times 100$. Variable for Objective 2.

Appendix 10: Diagnostic performance of HOXB13 IHC ($\geq 2+$) across three reference classifications



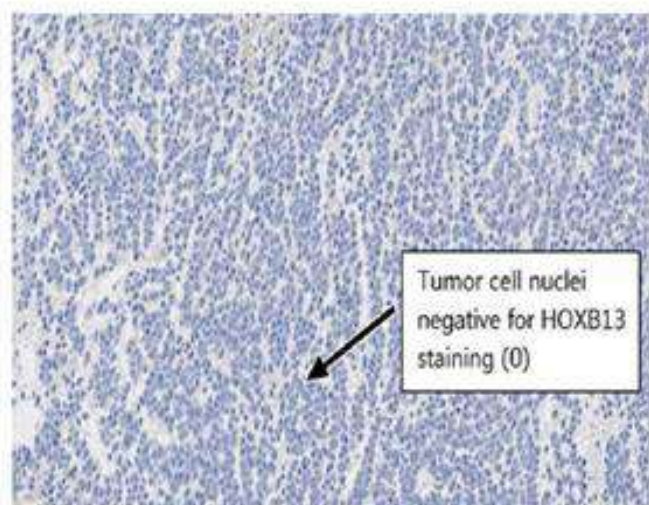
Appendix 10: Diagnostic performance of HOXB13 IHC ($\geq 2+$) across three reference classifications. Values: Gleason ≥ 7 (87.3%, 84.8%, 91.7%); ISUP high grade (87.1%, 82.4%, 90.0%); PSA ≥ 4 ng/mL (84.7%, 81.1%, 87.7%). Sensitivity, specificity, PPV.

Appendix 11: AUC values for HOXB13 IHC ($\geq 2+$) across three reference classifications

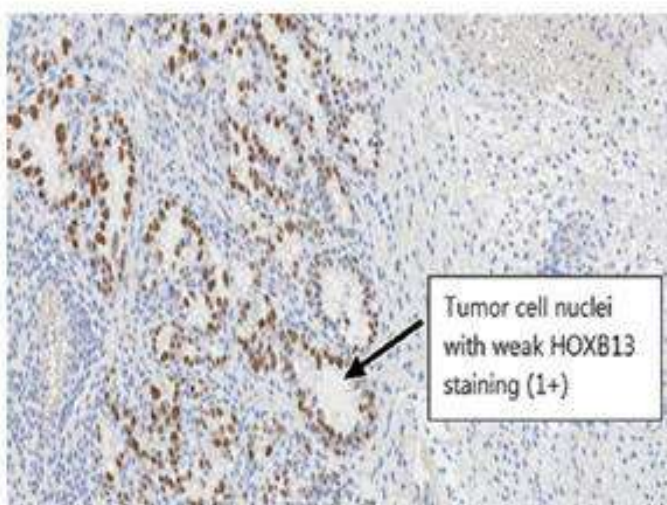


Appendix 11: Area under the ROC curve (AUC) values reported for HOXB13 IHC against reference classifications: Gleason ≥ 7 (0.86), ISUP high grade (0.85), PSA ≥ 4 ng/mL (0.83). AUC indicates good discrimination (>0.80).

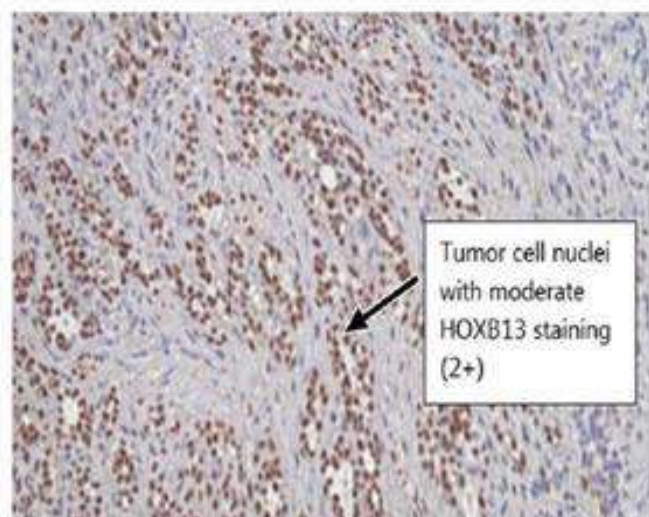
Appendix 12: Expression of HOXB13 in archived prostatic biopsy tissues



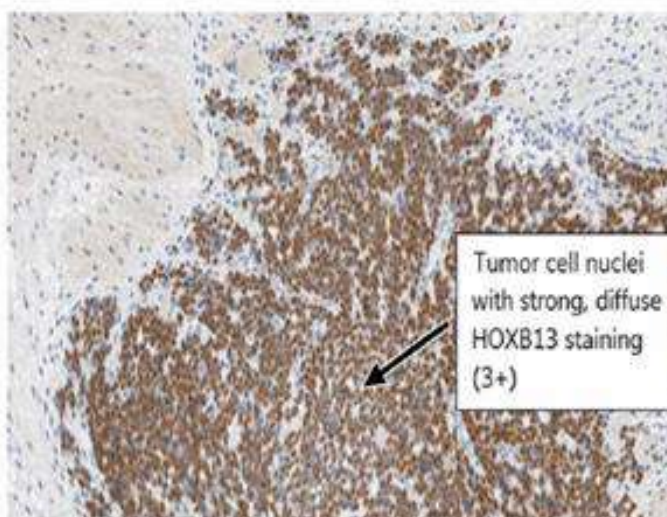
D6/ H 0. Staining (Negative)



D7/ H 1+. Staining (Weak)



D8/ H 2+. Staining (Moderate)



D9/ H 3+. Staining (Strong)

Appendix 12: Representative HOXB13 immunohistochemical staining in prostate adenocarcinoma: (A) score 0, no tumor-cell nuclear staining; (B) score 1+, weak staining; (C) score 2+, moderate staining; and (D) score 3+, strong/diffuse staining. Original magnification $\times 100$.