REVIEW ARTICLE

Review of the Application of Immunohistochemistry in the Diagnosis of Benign Prostatic Hyperplasia

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Abstract

Benign prostatic hyperplasia is a common condition in elderly men, characterized by enlargement of the prostate gland. Although benign prostatic hyperplasia is benign and does not progress to cancer, differentiating it from prostate carcinoma, particularly in early stages, is of significant clinical importance. Immunohistochemistry serves as a valuable and complementary tool in the diagnosis of benign prostatic hyperplasia, especially for distinguishing it from prostate cancer. While histopathology remains the gold standard for diagnosis, immunohistochemistry offers notable advantages that enhance diagnostic accuracy and confidence. This study aims to review the pathophysiology and histopathology of benign prostatic hyperplasia, introduce the most important and commonly used immunohistochemical markers associated with benign prostatic hyperplasia, discuss the benefits of immunohistochemistry in its diagnosis, outline the limitations and challenges of immunohistochemistry, and propose strategies to address these challenges.

Relevant literature was systematically searched in major scientific databases, including PubMed, Scopus, Google Scholar, and Web of Science, covering a ten-year period from 2015 to 2025.

The review indicated that various immunohistochemical biomarkers such as P63, HMWCK, P40, PSA, NKX3.1, CK8, Ki67, PCNA, AR, ERα, ERβ, PR, CD3, CD20, FGFs, IGFs, and HIF-1α are employed to differentiate benign prostatic hyperplasia from prostate cancer in biopsy and prostatectomy specimens. Among these, the combination of basal cell markers and AMACR significantly improves diagnostic accuracy in challenging cases.

Overall, although immunohistochemistry is not routinely used directly for benign prostatic hyperplasia diagnosis, it is highly valuable in research for better understanding benign prostatic hyperplasia pathophysiology, identifying subgroups, and distinguishing it from prostatic malignancies.

Keywords Histopathology, Hyperplasia, Immunohistochemistry, Prostate

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1 Introduction

Benign Prostatic Hyperplasia (BPH) is a common condition in elderly men, characterized by enlargement of the prostate gland. In BPH, the number of stromal and epithelial cells in the transitional zone of the prostate increases. This cellular proliferation leads to prostate enlargement, resulting in compression of the urethra that passes through the gland, which in turn causes urinary obstruction and associated lower urinary tract symptoms such as urinary frequency, urgency, weak stream, and nocturia.[1]

Although BPH is benign and does not progress to cancer, distinguishing it from prostate carcinoma particularly in the early stages is of considerable clinical importance. The diagnosis of BPH is typically based on a combination of patient medical history, digital rectal examination, serum prostate-specific antigen (PSA) testing, and transrectal ultrasound of the prostate. However, in certain cases, especially when standard test results are inconclusive or there is clinical suspicion of malignancy, a prostate biopsy is necessary for a definitive diagnosis. In biopsy specimens, pathologists employ various methods to differentiate BPH from prostate cancer.[2]

One of the most powerful and useful methods is immunohistochemistry (IHC). IHC is a laboratory technique that utilizes specific antibodies to detect particular proteins (antigens) in tissue samples. These proteins can serve as biomarkers that help distinguish benign from malignant cells.[3] IHC plays a vital role in the diagnosis of BPH and in differentiating it from prostate carcinoma. Benign prostatic cells, including those observed in BPH, possess a layer of basal cells that are lost or markedly reduced in prostate cancer. IHC markers such as P63 and high-molecular-weight cytokeratins (HMWCKs) (e.g., CK5/6) selectively stain basal cells. The presence of positive staining in a biopsy strongly supports a benign lesion such as BPH, whereas the absence or significant reduction may indicate prostate cancer.

Furthermore, other markers, such as alpha-methylacyl-CoA racemase AMACR, also known as P504S), are overexpressed in prostatic cancer cells and can aid in distinguishing cancer from BPH and other benign lesions.[4]

This study aims to elucidate how IHC can assist pathologists in achieving more accurate diagnoses, characterizing tissue features, and evaluating molecular markers associated with BPH. It also seeks to address the key question of which immunohistochemical markers are most valuable in the differential diagnosis of BPH versus prostate cancer, and how they can be applied to improve diagnostic accuracy, ultimately supporting more informed therapeutic decisions.

2 Methods

This study is a narrative review based on the existing literature regarding the application of IHC in the diagnosis of BPH. The primary objective was to provide a comprehensive and analytical summary of the key findings in this field.

A systematic search of relevant articles was conducted in major scientific databases, including PubMed, Scopus, Google Scholar, and Web of Science, covering ten years from 2015 to 2025. The search utilized the following keywords: IHC, BPH, Prostate Cancer, Biomarkers, Histopathology, and Prostate Diagnosis. Among the 1,545 articles initially identified, a total of 27 full-text articles were ultimately selected and included for review.

3 Results

Pathophysiology and Histopathology of BPH

The pathophysiology of BPH is a complex process characterized by non-malignant growth and proliferation of epithelial and stromal cells within the transitional zone of the prostate. This nodular growth is primarily influenced by hormonal factors, particularly the balance between androgens, such as dihydrotestosterone (DHT) and estrogens, which shifts with aging. DHT, produced from the conversion of testosterone by the enzyme 5-alpha reductase in prostatic stromal cells, plays a key role in stimulating cellular growth and proliferation.

With advancing age, changes in hormonal balance (an increased estrogen-to-androgen ratio) and reduced apoptosis (programmed cell death) in the prostate lead to cellular accumulation and formation of hyperplastic nodules. The enlarged prostate exerts mechanical pressure on the prostatic urethra, narrowing its lumen a phenomenon known as static obstruction. In addition, increased tone of the smooth muscle within the prostatic stroma, influenced by the sympathetic nervous system, contributes to dynamic obstruction, further exacerbating lower urinary tract symptoms. This obstruction results in elevated bladder pressure, detrusor muscle hypertrophy, and eventually bladder decompensation with incomplete emptying, manifesting clinically as urinary frequency, urgency, weak stream, and nocturia.[5]

Histopathology of BPH refers to the microscopic changes observed in the prostate tissue of elderly men. The most prominent feature of BPH is the presence of multiple nodules of varying sizes, composed of differing proportions of glandular (epithelial) and stromal (connective) tissue.

Glandular nodules in BPH are primarily composed of enlarged and proliferating prostatic glands, which may appear folded or dilated. The epithelium lining these glands is bilayered, consisting of an inner layer of tall

columnar cells and an outer layer of cuboidal or squamous basal cells. Amylaceous bodies are often observed within the glandular lumens. Stromal nodules are mainly composed of dense connective tissue, fibrosis, and smooth muscle cells. In some cases, stromal proliferation may predominate, giving the cut surface of the prostate a firm, gray-white appearance.

Mixed nodules, the most common type, contain both glandular and stromal components. In normal prostate tissue, a specific ratio exists between the glandular and stromal components; in BPH, cellular proliferation increases this ratio. On average, stromal tissue constitutes approximately 60% of the volume of BPH, with a stroma-to-epithelium ratio of about 2.7. BPH almost always occurs in the transitional zone of the prostate, surrounding the urethra, which facilitates obstruction of urinary flow and the development of lower urinary tract symptoms. Non-specific chronic inflammatory cells may also be observed in BPH tissue.

The precise mechanism of BPH is not fully understood; however, an imbalance between cellular proliferation and programmed cell death (apoptosis), favoring proliferation, plays a central role. Although clinical diagnosis of BPH relies on symptoms, physical examination, and other tests, histopathology provides definitive confirmation. In cases where prostate biopsy is performed to rule out cancer, the presence of BPH changes in the pathology report indicates the benign nature of the tissue.

In histopathologic evaluation, differentiating BPH from prostate adenocarcinoma is crucial. Key distinguishing features include the following: in BPH, glands exhibit irregular but benign shapes with a well-defined bilayered epithelium, whereas in prostate cancer, glands are typically smaller, more densely packed, and lack a basal cell layer. In BPH, nuclei are small, uniform, and have inconspicuous nucleoli; in contrast, cancerous nuclei are larger, pleomorphic, and display prominent nucleoli (Figure 1).[6] BPH demonstrates a nodular and benign growth pattern, while prostate cancer exhibits a more aggressive pattern with the absence of a defined capsule.[7]

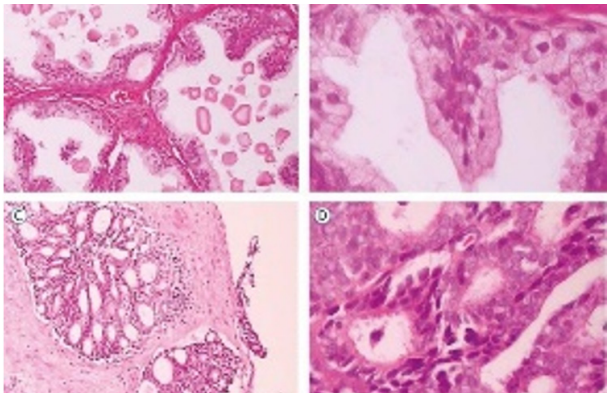

Figure 1 Histology of BPH (A and B) and prostate cancer (C and D

Immunohistochemical Markers Associated with BPH

The most commonly used immunohistochemical markers for the diagnosis and differentiation of BPH from prostate cancer are listed below (Table 1).

1. Basal Cell Markers

Basal cells form a continuous layer beneath the luminal (secretory) cells and above the basement membrane in normal and hyperplastic prostatic glands. These cells help maintain the integrity and ductal structure of the prostate glands, act as stem/progenitor cells within the prostate, possess the capacity to proliferate and differentiate into luminal cells, participate in tissue regeneration after injury or hormonal changes, and serve as a barrier between luminal cells and the stroma.[8]

One of the most important applications of IHC in the prostate is the identification of the basal cell layer. In benign prostatic tissues, including BPH, the basal cell layer remains intact and continuous. This feature is a key criterion used in the differential diagnosis from prostate cancer, as the basal cell layer is absent in most cases of invasive prostate carcinoma.[9] The most commonly used IHC markers for identifying basal cells in BPH include:

1-1. P63 (Transformation-Related Protein 63 / TP63)

P63 is a nuclear protein expressed specifically in the basal cells of the prostatic epithelium and is absent in luminal and neuroendocrine cells. The presence of p63 in glands indicates benignity. Continuous and positive p63 staining in prostatic glands reflects the presence of a basal cell layer, confirming that the lesion is benign. This marker is particularly valuable for differentiating BPH from invasive prostate cancer, where p63 is typically negative. p63 demonstrates high sensitivity and specificity for identifying basal cells.[8]

1-2. HMWCKs

These cytokeratins include CK34βE12 (also known as CK903) and CK5/6. They are cytoskeletal proteins specifically expressed in the cytoplasm of basal cells and help identify the intact basal cell layer in BPH. In BPH, basal cells are a normal and essential component of glandular architecture. The primary role of basal cell markers in prostate pathology is to distinguish benign lesions, such as BPH, from prostate cancer. Similar to p63, continuous positive staining of HMWCKs in prostatic glands indicates the presence of a basal cell layer and confirms the lesion's benign nature.[10]

1-3. P40

P40 is an isoform of p63 with higher specificity for basal cells and shows less aberrant expression in tumor cells. It is a nuclear protein and can be used as an alternative to p63 or alongside it, particularly in specific cases where better differentiation is needed.[11]

Table 1 The most important IHC markers for the diagnosis of BPH.[8,9]

Basal cell marker	Luminal cell marker	Cell proliferation marker	Hormonal receptors	Inflammatory marker	Growth factors	Other factors
P63	PSA	Ki67	AR	CD3	FGFs	HIF-1α
HMWCK	NKX3.1	PCNA	ERα	CD20	IGFs	
P40	CK8		ERβ			
			PR			

In BPH, the presence of basal cell markers in a continuous pattern assists pathologists in confirming the diagnosis of BPH and ensuring that prostate enlargement is benign. It helps distinguish BPH from prostate cancer. Conversely, the absence or discontinuous pattern of these markers in suspicious glands strongly favors a diagnosis of prostate cancer. In most cases of invasive prostate carcinoma, basal cells and therefore the expression of these markers are lost.[10]

1-4. Diagnostic Cocktails

Pathologists often use a combination of markers, typically p63/HMWCK, together with AMACR. In this cocktail, p63 and HMWCK, which indicate benign basal cells, are positive, whereas AMACR, which is expressed in prostate cancer, is negative or minimally expressed in BPH. This combination enhances diagnostic accuracy, particularly in challenging or atypical cases.[11]

2. Luminal Cell Markers

In BPH, luminal (secretory) cells constitute the main component of the glandular tissue. These cells are responsible for producing prostatic fluid and proteins such as PSA. Similar to basal cell markers, luminal cell markers are primarily used in IHC to differentiate BPH from prostate cancer, rather than for the direct diagnosis of BPH itself. These markers are expressed in the luminal (secretory) cells of the prostate.[12]

2.1. PSA

PSA is a glycoprotein produced almost exclusively in the nuclei of prostatic luminal (secretory) epithelial cells. PSA expression in luminal cells is strong and widespread in BPH, making it an excellent marker for confirming the prostatic origin of a tissue, for example in metastases of unknown origin. IHC for PSA can help distinguish prostatic lesions benign or malignant from other tissues or tumors not originating from the prostate.

Although PSA is expressed in BPH, it is also expressed in prostate cancer, albeit with potentially different levels and staining patterns. Therefore, PSA alone cannot differentiate BPH from prostate cancer. However, in poorly differentiated tumors with ambiguous morphology, PSA positivity can aid in identifying prostatic origin. Serum PSA levels are elevated in both BPH and prostate cancer, but tissue expression assessed by IHC can be useful in research and for distinguishing prostatic lesions from other malignancies not derived from the prostate.[13]

2.2. Alpha-Methylacyl-CoA Racemase

AMACR is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched-chain fatty acids. It is one of the most important markers for diagnosing prostate cancer. In prostate cancer cells, particularly adenocarcinomas, AMACR expression is increased. In contrast, luminal cells in benign prostatic tissue, including BPH, are negative for AMACR or show only weak and focal expression. Therefore, AMACR serves as a positive marker for prostate cancer and a negative marker for BPH. This characteristic makes it highly valuable in IHC panels for distinguishing prostate cancer from BPH and other benign lesions, such as adenosis or atypical atrophy.[8]

2.3. NKX3.1

NKX3.1 is a homeodomain transcription factor whose expression is largely restricted to the prostate and is regulated by androgens. It functions as a tumor suppressor in the prostate and is strongly expressed in the nuclei of luminal epithelial cells in both normal prostate tissue and BPH. This marker can aid in identifying the prostatic origin of metastatic tumors, as its expression is rare in other tumors (with few exceptions, such as certain breast cancers). However, reduced or lost expression of NKX3.1 is also observed in some prostate cancers, making it a potential prognostic marker as well.[14]

2.4. Cytokeratin 8 (CK8)

CK8 is a low-molecular-weight cytokeratin typically expressed in the cytoplasm of prostatic luminal (secretory) epithelial cells, as well as in other glandular epithelia. It is used to identify luminal cells in prostatic tissue. In benign tissues such as BPH, CK8 is widely expressed in luminal cells. When used in combination with basal cell markers (e.g., CK5/6 or p63), CK8 can help confirm the bilayered structure (basal and luminal) of benign epithelium and distinguish it from prostate cancers, which usually lack a basal layer and display a single proliferative luminal layer.[15]

3. Markers Related to Cell Proliferation

In BPH, cell proliferation plays a central role in prostate enlargement. Proliferation-related markers in immunohistochemistry serve as tools to assess the rate and extent of cell division in prostatic tissue. Although these markers are not routinely used for diagnosing BPH, they are highly valuable in research studies for better understanding the pathophysiology of BPH, evaluating disease progression, and assessing the effects of treatments.[16]

3.1. (MIB-1) Ki-67

Ki-67 is a cell proliferation marker expressed in the nuclei of dividing cells. In BPH, Ki-67 expression is generally lower than in prostate cancer. It can be used to assess the rate of cell proliferation in BPH and its relationship with disease progression. Ki-67 is a nuclear protein expressed during all active phases of the cell cycle (G1, S, G2, M) but absent in resting cells (G0). Therefore, the percentage of Ki-67-positive cells serves as an index of the proliferative fraction in a tissue.

In BPH, Ki-67 expression is typically low, indicating slow and controlled growth compared with prostate cancer, which exhibits a higher Ki-67 index. Ki-67 may be used to investigate how various factors such as hormones, inflammation, and growth factors affect cell proliferation in BPH. It can also be employed to evaluate reductions in proliferation following medical treatments, such as 5-alpha reductase inhibitors. In some cases, the Ki-67 index can aid in distinguishing BPH from suspicious lesions, such as high-grade prostatic intraepithelial neoplasia or low-Gleason-grade prostate cancer, although it is not definitive on its own.[17]

3.2. PCNA

Proliferating cell nuclear antigen (PCNA) is a nuclear protein involved in DNA synthesis and repair, expressed during the S, G1, and G2 phases of the cell cycle. Similar to Ki-67, it is used to assess the proliferative fraction of cells. Although Ki-67 is generally preferred due to its stronger association with active cell division, PCNA has also been employed in BPH research to evaluate proliferation.[18]

These markers help researchers quantify growth rates and cellular changes in BPH and identify influencing factors, which can contribute to the development of new therapeutic strategies. Assessing changes in proliferation indices following medical treatments for BPH, such as 5-alpha reductase inhibitors, can demonstrate the cellular-level effectiveness of these therapies.

Due to the benign nature of BPH and the availability of standard clinical and histopathological diagnostic methods (e.g., hematoxylin and eosin staining), proliferation markers are not routinely used for clinical diagnosis. Their primary application is in distinguishing BPH from malignancies and in research studies. In prostate cancer, a higher Ki-67 index may be associated with poorer prognosis. Proliferation can vary across different regions of BPH, necessitating careful evaluation and adequate sampling.[17,18]

4. Hormone Receptors

Hormones play a central role in the pathogenesis and growth of BPH. The prostate is a hormone-dependent gland, and its growth is primarily influenced by androgens and estrogens. Receptors for these hormones are present in prostatic cells, through which hormones exert their effects. In IHC studies of BPH, examining hormone receptors can provide insights into disease mechanisms, its association with age, and the potential responsiveness to hormonal therapies, such as 5-alpha reductase inhibitors.[19]

4.1. Androgen Receptor (AR)

Androgens play a key role in prostate growth. The expression of ARs has been observed in both the epithelial and stromal cells of the prostate and can be examined in studies investigating the mechanisms of BPH and responses to hormonal therapies. AR is a nuclear protein that binds to androgenic hormones such as testosterone and DHT. Upon binding, AR is translocated into the cell nucleus, where it regulates the transcription of genes associated with prostate growth and function.

DHT is the most potent androgen in the prostate and plays a central role in stimulating its growth. The expression of AR in epithelial and stromal cells is evident in BPH, and its level and distribution may be influenced by age and hormonal status. IHC studies have investigated the relationship between changes in AR expression and BPH progression as well as clinical symptoms.

AR serves as the main therapeutic target in the treatment of BPH (e.g., 5-alpha-reductase inhibitors that reduce DHT production) and prostate cancer (androgen deprivation therapies). Evaluating AR expression by IHC can provide insights into the mechanisms of action of these drugs. Although AR is expressed in both BPH and prostate cancer, variations in expression patterns such as intensity or distribution may occur in different lesions. However, AR alone is not considered a differential diagnostic marker for distinguishing BPH from prostate cancer.[20]

4.2. Estrogen Receptors

Estrogens also play an important role in the pathophysiology of BPH, and studying the expression of their receptors can provide valuable insights into the underlying disease mechanisms. There are two main types of estrogen receptors: ER-alpha (ERα) and ER-beta (ERβ).

ERα is primarily expressed in stromal cells and, to a

lesser extent, in epithelial cells of the prostate. It is believed to promote stromal growth and, consequently, contribute to the development of BPH. In contrast, ERβ is predominantly expressed in epithelial cells (both luminal and basal) as well as in stromal cells, and it is generally considered a suppressor of prostate growth or a tumor suppressor.

With aging, the balance between testosterone and estrogen shifts, leading to a relative increase in estrogen levels. Through their receptors, estrogens can stimulate cell proliferation and inhibit apoptosis in the prostate, thereby contributing to the pathogenesis of BPH. IHC studies have explored the expression patterns of ERα and ERβ in BPH tissues to better understand their roles in disease progression. Given the involvement of estrogens, targeting estrogen receptors particularly ERα may represent a potential therapeutic strategy for managing BPH.[21]

4.3. Progesterone Receptor (PR)

Although progesterone is primarily known as a female sex hormone, its receptors can also be found in certain male tissues, including the prostate. The expression of progesterone receptors (PR) in the male prostate has been less extensively studied compared to estrogen and ARs, and their role in the pathogenesis of BPH remains less clearly defined.

Some studies have suggested that PR may be expressed in stromal cells of the prostate and, together with androgens and estrogens, may influence prostate growth. However, its role appears to be less central than that of AR and ER. IHC can be used to evaluate the presence and distribution of PR in BPH tissues.[22]

5. Other Factors

5.1. Inflammatory Markers

Chronic inflammation plays a significant role in the pathophysiology of BPH. CD3 and CD20 are markers of T and B lymphocytes, respectively, and can be used to assess lymphocytic infiltration in prostatic tissues affected by BPH.[23]

5.2. Growth Factors

Growth factors such as fibroblast growth factors (FGFs) and insulin-like growth factors (IGFs) are involved in the regulation of cellular growth within the prostate. Alterations in their expression may contribute to the development and progression of BPH.[24]

5.3. Hypoxia-Inducible Factor Alpha (HIF-α)

This protein is expressed in response to hypoxia (oxygen deficiency) and has been investigated for its role in the mechanisms of BPH, particularly regarding its association with inflammation and angiogenesis.[25]

Advantages of Using IHC in the Diagnosis of BPH

IHC provides a highly valuable adjunct to conventional histopathology in distinguishing BPH from malignant prostatic lesions, particularly adenocarcinoma. One of its major advantages lies in the accurate differentiation between BPH and prostate cancer through the assessment of the basal cell layer. In benign lesions such as BPH, the glandular structures are surrounded by a continuous or nearly continuous basal cell layer, whereas this layer is absent in prostatic adenocarcinoma. The use of antibodies against basal cell markers such as p63 and HMWCKs, including 34βE12 or CK5/6 facilitates this distinction. Continuous, positive staining for these markers supports a benign diagnosis, while absent or discontinuous staining suggests malignancy. In contrast, AMACR typically shows strong positivity in prostate cancer cells but is negative or minimally expressed in benign tissues. The combined use of AMACR and basal cell markers in a multiplex IHC panel significantly enhances diagnostic accuracy by providing a clear immunoprofile for differentiating BPH from cancer.

IHC is particularly valuable in challenging or ambiguous cases, such as when biopsy specimens are limited in size or when morphological overlap exists between benign and malignant lesions. Certain benign entities, including atypical adenomatous hyperplasia (AAH), post-atrophic hyperplasia, and inflammatory changes, may mimic carcinoma under light microscopy. In such contexts, IHC staining patterns offer critical diagnostic clarity and help prevent overdiagnosis or misclassification. Moreover, by clarifying uncertain histopathological findings, IHC reduces the frequency of indeterminate diagnoses such as atypical small acinar proliferation (ASAP), thereby minimizing the need for repeat biopsies and improving diagnostic confidence.

Beyond differentiation of benign from malignant conditions, IHC also assists in confirming the prostatic origin of metastatic lesions in men with cancers of unknown primary site. Markers such as PSA and prostate-specific acid phosphatase can confirm prostatic origin even when the primary tumor is not readily apparent. In addition, while less commonly applied for BPH, IHC can support assessment of therapeutic response in patients undergoing radiotherapy or hormone therapy for prostate cancer. Overall, the integration of IHC into diagnostic workflows enhances both the precision and reliability of prostate pathology, ensuring more accurate distinction between BPH and carcinoma and thereby informing appropriate clinical management and treatment decisions.[26]

Despite its substantial diagnostic value, the use of IHC in differentiating BPH from malignant lesions is not without limitations and challenges. One of the most significant difficulties arises in distinguishing BPH from atypical or morphologically variant forms of prostate

cancer, particularly in small or fragmented biopsy samples. While the absence of the basal cell layer is a hallmark of prostatic adenocarcinoma, its preservation indicates benign pathology. IHC markers such as p63 and HMWCKs are commonly employed to identify basal cells; however, incomplete or discontinuous staining can occasionally lead to misinterpretation. Similarly, AMACR, which is typically positive in malignant prostatic epithelium and negative in BPH, may exhibit variable expression some benign lesions may show focal positivity, while certain cancers may lack AMACR expression altogether, complicating diagnostic certainty. [8]

Another challenge involves the overlap in marker expression between benign and malignant tissues. Although expression patterns and staining intensities generally differ, the presence of shared immunoreactivity may yield ambiguous results that necessitate cautious interpretation or additional confirmatory tests. Furthermore, the quality of tissue fixation and processing exerts a crucial influence on IHC outcomes. Inadequate fixation can cause epitope degradation and weak or false staining, while artifacts such as tissue compression or necrosis may obscure cellular detail and further hinder accurate evaluation.[27]

The intrinsic histological heterogeneity of BPH also adds complexity to IHC interpretation. As BPH comprises glandular, stromal, and inflammatory elements in varying proportions, marker expression can differ across tissue regions, occasionally leading to inconsistent staining and interpretive difficulty. Moreover, certain benign but atypical lesions such as adenosis AAH and focal atrophy can closely mimic prostate carcinoma both morphologically and immunohistochemically. In such cases, reliance on IHC alone may be insufficient, emphasizing the need for comprehensive correlation with histomorphological features and clinical findings to achieve an accurate diagnosis.[7]

Strategies to Reduce Challenges

To mitigate the diagnostic challenges associated with BPH and improve the accuracy of differentiation from malignant lesions, several strategies have been proposed. One of the most effective approaches is the use of antibody panels that combine multiple markers with complementary diagnostic value. In particular, a triple-antibody cocktail consisting of high-molecular-weight cytokeratin (HMWCK), p63, and AMACR is widely utilized to enhance diagnostic precision and reduce interpretive uncertainty.[11]

Equally important is the interpretation of IHC findings within the broader histopathological and clinical context. IHC results should never be considered in isolation; rather, they must be evaluated alongside tissue morphology and other clinical data to avoid misdiagnosis and ensure accurate pathological classification.[7] The expertise of the interpreting pathologist also plays a decisive role, as accurate diagnosis depends on the ability to integrate morphological assessment with nuanced interpretation of IHC staining patterns. Experience and familiarity with the spectrum of prostatic lesions are therefore critical for reliable evaluation.[27]

Finally, ensuring technical accuracy in IHC procedures requires the consistent use of internal and external positive and negative controls. These controls help verify staining quality, antibody specificity, and reproducibility, thereby maintaining the reliability of diagnostic results and minimizing technical errors.[27] Collectively, these strategies strengthen the diagnostic value of IHC and support its effective application in differentiating BPH from prostate cancer.

4 Discussion

BPH is an age- and androgen-dependent disease, influenced by additional factors such as estrogens, chronic inflammation, and complex cellular interactions. Understanding these factors is essential for developing strategies for prevention and treatment. Most IHC markers discussed in BPH studies are primarily used to differentiate BPH from prostate cancer. Direct clinical use of IHC solely for diagnosing or classifying BPH is less common, as BPH diagnosis mainly relies on clinical evaluation, PSA testing, and standard histopathological assessment with H&E staining. However, in research, these markers provide deeper insights into the molecular and cellular aspects of BPH.

Basal and Luminal Markers

Basal cell markers (e.g., p63, HMWCKs) are critical for confirming benignity and the presence of an intact basal cell layer. Luminal markers such as PSA and NKX3.1 are useful for confirming the prostatic origin of a lesion, whether benign or malignant. AMACR, also a luminal marker, is mainly used in the context of ruling out malignancy: absent or very low AMACR expression supports benign lesions like BPH and argues against prostate cancer. Therefore, in pathology, IHC panels typically combine basal cell markers (p63/HMWCKs) with luminal markers (AMACR) to provide a more definitive diagnosis in challenging prostate cases, particularly for differentiating BPH from prostate cancer.

Proliferation Markers: Markers such as Ki-67 and PCNA are powerful tools for understanding cellular dynamics in BPH. Their role is mainly research-oriented or in distinguishing BPH from premalignant or malignant lesions and they are not routinely used as diagnostic markers for BPH itself.

Hormonal Receptors

Androgen and estrogen receptors, especially AR, ERα, and Erβ, play key roles in BPH pathogenesis. Studying their expression via IHC provides valuable insights into disease biology and therapeutic approaches. IHC allows localization and quantification of these receptors in different prostate cell types (epithelial, stromal, basal, luminal), helping to understand how hormones influence BPH growth at the cellular level. Evaluating hormonal receptors can also aid in predicting patient responses to hormonal therapies (e.g., finasteride or dutasteride, which inhibit 5-alpha-reductase) and identifying potential new therapeutic targets.

Overall, IHC serves as a complementary and valuable diagnostic tool in prostate pathology, particularly in the diagnosis and differentiation of BPH from prostate cancer. By detecting specific molecular markers in tissue, IHC enables pathologists to provide more accurate diagnoses, leading to appropriate therapeutic management and improved patient outcomes. Ongoing advancements in new markers and IHC techniques hold the potential to further enhance diagnostic accuracy, but this requires precise knowledge of markers, proper technical execution, and careful interpretation in the clinical and morphological context.

While narrative reviews can provide a useful overview of the application of IHC in BPH diagnosis, they have notable limitations due to their qualitative and non-systematic nature. These reviews often lack a comprehensive and transparent search protocol, which can lead to selection bias by potentially overlooking key studies. Furthermore, the quality assessment of included studies is rarely performed formally in narrative reviews, raising questions about the reliability of their findings.

Most importantly, the absence of a quantitative synthesis method, such as meta-analysis, prevents the statistical combination of results, making it impossible to derive an overall effect size or definitive conclusions regarding the diagnostic value of IHC. Consequently, the findings of narrative reviews on IHC use in BPH are often limited to the subjective interpretation of the authors and may not fully capture inconsistencies present in the scientific literature. These limitations make it challenging to apply the results of such reviews for clinical decision-making or to guide future research directions.

5 Conclusion

It is clear that while histopathology remains the diagnostic gold standard for BPH, IHC is an indispensable complementary tool, primarily for the crucial task of differentiating BPH from prostate cancer. This review confirmed the utility of a wide panel of biomarkers including P63, HMWCK, and particularly the combination of basal cell markers with AMACR which significantly enhances diagnostic confidence and accuracy in clinically challenging scenarios. Therefore, although IHC is not typically used for the direct, routine diagnosis of BPH, its high value lies in refining the differential diagnosis of prostatic lesions and, critically in advancing research to better understand the pathophysiology, and ultimately improve the management strategy for men presenting with symptoms related to prostatic enlargement.

Declarations

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All of this manuscript were drafted by the authors and subsequently reviewed, edited, and definitively approved by the authors with the assistance of AI [ChatGPT-4]

Authors' Contributions

Tayebeh Mohammadi primarily developed the methodology (search strategy) and performed the literature investigation, as well as preparing the original draft of the manuscript. Zahra Minoush Siavosh Haghighi contributed to the strategic direction (conceptualization) and was primarily responsible for the critical review, editing, and finalization of the manuscript. Both authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Consent for Publication

Not applicable.

Ethical Considerations

The study is a review and does not require Ethics approval.

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