Archives of Men's Health. 2024; 8(1): e9

OPEN ACCESS

ORIGINAL RESEARCH

# Proteomics in Chronic Prostatitis: Biomarker Discovery, Molecular Pathways, and Emerging Targets for Precision Medicine

**Sina Samenezhad[1], Farzad Allameh[2], Dorna Rafighi[3], Hasti Ahani[4]***

1. Urology Department, Shohada-e-Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Men's Health & Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Microbiology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
4. Department of Surgery, Razi Clinical Research Development Unit, Guilan University of Medical Sciences, Rasht, Iran.

*Received: October 2024; Accepted: November 2024; Published online: December 2024*

**Abstract:** Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common and disabling urological disorder that affects quality of life in men. Despite accounting for most prostatitis cases, its causes remain unclear, involving immune dysregulation, oxidative stress, microbial factors, and epithelial barrier dysfunction. This uncertainty complicates diagnosis and treatment.

Proteomics offers a high-throughput approach to identify proteins and pathways involved in CP/CPPS. Recent studies have profiled urine, seminal plasma, prostatic secretions, and serum to uncover biomarkers linked to inflammation, oxidative stress, and microbial virulence. These findings provide insights into molecular endotypes, guide new classifications, and point toward novel therapeutic targets such as cytokine signaling, pyroptosis pathways, and heat shock proteins. Although technical variability and small cohort sizes remain major challenges, integration of proteomics with multi-omics platforms and explainable AI may transform CP/CPPS management by enabling personalized diagnostics and targeted interventions.

**Keywords:** Chronic Pelvic Pain Syndrome (CP/CPPS), Proteomics, Biomarkers, Inflammatory Cytokines, Epithelial Barrier Dysfunction, Precision Medicine Multi-Omics

Cite this article as: Samenezhad S, Allameh F, Rafighi D, Ahani H. Proteomics in Chronic Prostatitis: Biomarker Discovery, Molecular Pathways, and Emerging Targets for Precision Medicine. Archives of Men's Health. 2024; 8(1): e9.

## 1. Introduction

Prostatitis is a common inflammatory condition of the prostate, affecting up to 50% of men at some point in their lives. It presents a broad range of symptoms, including pelvic pain, urinary difficulties, and sexual dysfunction, leading to significant reductions in quality of life. The National Institutes of Health (NIH) classifies prostatitis into four categories, with Type III chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) accounting for 90–95% of cases [1].

CP/CPPS is further divided into Type IIIa (inflammatory) and Type IIIb (non-inflammatory) based on leukocyte presence in expressed prostatic secretions (EPS). However, this subclassification is limited, as white blood cell counts do not reliably reflect symptom severity or disease course. The multifactorial etiology of CP/CPPS—including infection, immune dysregulation, oxidative stress, and psychosocial factors—has made diagnosis and treatment challenging. Current therapies are often empirical and symptom-focused, with high recurrence rates [2, 3].

In this review, we synthesize current evidence on proteomic approaches in chronic prostatitis, focusing on inflammation, oxidative stress, immune dysregulation, epithelial barrier dysfunction, and biomarker discovery. Our objective is to highlight key molecular pathways, identify emerging diagnostic and therapeutic targets, and discuss how proteomics may contribute to precision medicine strategies for CP/CPPS.

* **Corresponding Author:** Hasti Ahani; Address: Department of Surgery, Razi Clinical Research Development Unit, Guilan University of Medical Sciences, Rasht, Iran. Email: Hastyahany25@gmail.com.

## 2. Methodology

This review was conducted as a narrative review rather than a systematic review because of the wide heterogeneity of proteomic studies in chronic prostatitis, which differ in sample types, technologies, and study designs. A narrative approach allowed integration of molecular findings across diverse methodologies and facilitated synthesis of mechanistic insights rather than focusing solely on study counts.
A targeted literature search was performed in PubMed, Scopus, and Web of Science for studies published between 2000 and March 2025, using the following search terms:
"chronic prostatitis," "chronic pelvic pain syndrome", "proteomics", "biomarkers", "multi-omics", "inflammation", "oxidative stress", "epithelial dysfunction", and "molecular pathways."

### *2.1. Inclusion criteria*

• Peer-reviewed original studies or reviews applying proteomic or multi-omics methods to chronic prostatitis or related prostatic inflammation.
• Studies that investigated molecular mechanisms, biomarker discovery, or therapeutic targets.
• Both human and relevant animal model studies (distinguished in the text).

### *2.2. Exclusion criteria*

• Non-English publications, case reports, editorials, or conference abstracts without primary data.
• Studies focusing solely on prostate cancer, benign prostatic hyperplasia, or infertility, unless they provided direct insight into prostatitis-related mechanisms.
• The initial search yielded 227 articles. After screening titles and abstracts, 86 studies were selected for full-text review. Of these, 54 studies were included in the final synthesis, grouped thematically by:
• Sample type (urine, seminal plasma, prostatic fluid, serum)
• Molecular focus (inflammation, oxidative stress, tight junction proteins, microbial proteins)
• Analytical technique (LC-MS/MS, protein arrays, 2D-GE, labeling methods)

### *2.3. Inclusion criteria*

## 3. Proteomics Overview

### *3.1. Definition & Difference from Genomic*

Proteomics is the large-scale study of proteins expressed in a cell or tissue at a given time, focusing on their identity, quantity, and function. Unlike genomics, which analyzes the relatively static DNA blueprint, proteomics captures the dynamic protein landscape, reflecting real-time biological processes and disease states [4, 5].

### *3.2. Inclusion criteria*

**Core Techniques in Proteomics**

**3.2.1. Mass Spectrometry (MS)**

• The cornerstone of proteomics, MS enables large-scale identification and quantification of proteins in health and disease. To improve accuracy and comparability, different quantification strategies have been developed:
• Label-free quantification (LFQ) compares peptide ion intensities across runs [6].
• Stable isotope labeling by amino acids in cell culture (SILAC) – metabolic labeling for precise relative quantitation [6].
• Isobaric tags for relative and absolute quantitation (iTRAQ, TMT) – chemical labeling methods enabling multiplexing of up to 16 samples [6].
• Each method balances trade-offs in sensitivity, throughput, and reproducibility [6].

**3.2.2. 2D Gel Electrophoresis**

Separates proteins by isoelectric point and molecular weight. Although less sensitive for low-abundance proteins, it remains useful when paired with MS [7].

**3.2.3. LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)**

A widely used "bottom-up" proteomic method that digests proteins into peptides before MS analysis. It offers high sensitivity and proteome coverage, but requires robust computational pipelines to ensure reliable quantitation [8].

**3.2.4. Protein Arrays**

• High-throughput microarrays allow simultaneous measurement of thousands of proteins, enabling functional analysis, protein–protein interaction mapping, and biomarker discovery in clinical samples [9].
• A comparative summary of the major proteomic technologies, their strengths, limitations, and applications in prostatitis is provided in Table 1.

### *3.3. Sample Sources & Representative Studies in Prostatitis*

**3.3.1. Urine**

Urine proteomics, using advanced mass spectrometry-based techniques such as dimethyl leucine isobaric labeling and multidimensional protein identification technology, enables detailed profiling of metabolic and protein changes associated with prostatic inflammation and lower urinary tract symptoms. Quantitative studies in mouse models showed significant alterations in fourteen metabolites and one hundred sixty-eight proteins, including creatine, haptoglobin, immunoglobulin kappa constant, and polymeric immunoglobulin receptor, which were consistent biomarkers linked to inflammation-induced symptoms. In human samples of expressed prostatic secretions in urine, over one

thousand proteins were identified, and a panel of forty-nine prostate-enriched proteins was validated, with seven confirmed by Western blot analysis. These findings emphasize urine's value as a source of sensitive and specific biomarkers for diagnosing and understanding the mechanisms of prostatitis [10-12].

**3.3.2. Prostatic fluid**

Prostatic fluid contains proteins indicative of local inflammation. A study using tandem mass tag analysis found 377 proteins altered in fluid with abnormal lecithin bodies, many linked to immune and inflammatory pathways, relevant to chronic prostatitis. Proteomic profiling of prostasomes identified 139 mostly novel proteins, providing insight into prostate-related diseases [13, 14].

**3.3.3. Seminal plasma**

Seminal plasma, a fluid primarily secreted by the prostate and male accessory glands, serves as a critical sample source for proteomic studies related to prostate health and reproductive function. In prostatitis research, seminal plasma samples from patients revealed 1708 proteins, including 59 potential biomarkers associated with inflammation, highlighting its utility in diagnosing and monitoring prostate inflammation. In studies of male infertility, seminal plasma was analyzed to assess its antioxidant capacity [15]. Proteomic profiling of seminal plasma from men with varying oxidative stress levels identified key proteins, such as prolactin-induced protein, which were more abundant in men with elevated reactive oxygen species, indicating seminal plasma's role in protecting sperm from oxidative damage [16]. Furthermore, a detailed proteomic characterization of seminal plasma from a healthy individual identified 923 proteins, mainly extracellular and originating from accessory glands, underscoring seminal plasma as an important biological fluid for sperm viability and fertilization. These findings establish seminal plasma as a rich and informative sample source for studying prostate-related diseases and male reproductive health [17].

**3.3.4 Serum**

Serum is a critical sample source in prostatitis and prostate cancer research, enabling proteomic analyses that identify differentially expressed proteins and potential biomarkers. Advanced mass spectrometry techniques applied to serum samples reveal protein changes linked to disease stages, treatment responses, and aggressiveness, highlighting serum's value for diagnostic and predictive biomarker discovery [18]. Although each fluid provides unique insights, many share common inflammatory and oxidative stress signatures. A comparative overview of sample sources, representative biomarkers, and clinical relevance is summarized in Table 2.

## 4. Proteomics Findings in Prostatitis

### *4.1. Inflammatory and Immune Markers*

Proteomic analysis of human seminal plasma in prostatitis identified over 1700 proteins, with 59 showing significant differential expression—many linked to immune pathways, including cytokines and inflammation regulators. In murine models, prostate-specific inflammation altered urinary levels of acute-phase proteins (e.g., haptoglobin, inter--trypsin inhibitor, 1-antitrypsin), reflecting systemic innate immune activation. Additionally, human serum-derived extracellular vesicle profiling in prostate cancer revealed inflammation-related proteins (e.g., PD-L1, TGF-, Survivin) and activation of MAPK/mTOR pathways, suggesting relevance to chronic prostatic inflammation. Together, these findings indicate that while animal models highlight conserved immune mechanisms, human validation remains essential for translational application [11, 19, 20].

### *4.2. Oxidative Stress and Metabolism*

Proteomic analyses in human seminal plasma and urine have identified dysregulation of oxidative stress-related proteins, including mitochondrial stress markers and antioxidant enzymes. Elevated reactive oxygen species (ROS) contribute to epithelial damage, inflammation, and impaired sperm function in patients with CP/CPPS. Animal model studies of induced prostatitis similarly demonstrate oxidative signatures, strengthening the mechanistic link between ROS and inflammation. However, clinical translation requires cautious interpretation, as animal oxidative profiles may not fully reflect human disease heterogeneity [21-23].

### *4.3. Epithelial Barrier Dysfunction*

Recent proteomic and molecular studies implicate tight junction (TJ) disruption as a key feature in prostatitis and related prostatic disorders. Testosterone depletion—common with aging or chemical castration—leads to decreased expression of TJ proteins such as Claudin-4 and Claudin-8, compromising epithelial integrity and permitting inflammatory infiltration. Restoration of testosterone reverses these changes, reinforcing the androgen-dependent regulation of epithelial barrier function. Similarly, in benign prostatic hyperplasia (BPH), elevated TGF-1 levels suppress Claudin-1 expression via the MEK/ERK signaling pathway, increasing epithelial permeability.

From a clinical perspective, TJ proteins represent both potential biomarkers and therapeutic targets.

**4.3.1. Diagnostics**

Claudine alterations could be detected through non-invasive assays (e.g., urine- or serum-derived extracellular vesicles), enabling early identification of patients with epithelial barrier dysfunction.

#### 4.3.2. Therapeutics

Modulating TJ pathways pharmacologically—for instance, targeting TGF- signaling or using androgen restoration strategies—could help restore barrier integrity, reduce inflammation, and improve symptoms.

#### 4.3.3. Translational potential

Proteomic detection of TJ protein loss provides an avenue for risk stratification in CP/CPPS, and clinical trials incorporating TJ modulators may clarify their role in therapy.

Together, these findings highlight tight junction proteins not only as mechanistic drivers of barrier disruption but also as clinically relevant candidates for biomarker development and targeted therapy in chronic prostatitis [24-27].

### *4.4. Microbial Protein Signatures*

Proteomic profiling of expressed prostatic secretions (EPS) has revealed inflammation-associated changes in chronic prostatitis, including elevated immune mediators, oxidative stress proteins, and prostate-derived enzymes such as PSA and PAP. These alterations suggest epithelial barrier disruption and immune activation, consistent with autoimmune features observed in CP/CPPS. In bacterial prostatitis, uropathogenic E. coli strains exhibit enriched virulence protein expression, including papG allele III, foc, alpha-hemolysin (hly), and cytotoxic necrotizing factor 1 (cnf1). These microbial signatures contribute to prostatic colonization, tissue damage, and persistent inflammation. Combined host and pathogen proteomic analyses support a model in which epithelial dysfunction and microbial virulence synergize in the pathogenesis of prostatitis, offering targets for diagnostic and therapeutic development [28-31]. The key molecular pathways and proteins identified through proteomics are listed in Table 3.

The central circle represents the inflamed prostate, surrounded by four major molecular mechanisms identified via proteomics: inflammation (e.g., cytokines, TNF-), oxidative stress (e.g., ROS-related enzymes), epithelial dysfunction (e.g., Claudin proteins), and microbial virulence (e.g., bacterial toxins, PSA/PAP). This infographic reflects the integrative proteomic findings discussed in Section 3 (Figure1).

## 5. Clinical Applications

### *5.1. Diagnosis*

#### 5.1.1. Urine-Based Non-Invasive Biomarkers

Mass spectrometry-based proteomic and metabolomic analyses have identified urinary biomarkers (e.g., haptoglobin, creatine, Ig-related proteins) linked to prostatic inflammation and LUTS. Seminal plasma proteins like PAEP also show diagnostic promise. These findings support urine and seminal fluids as non-invasive sources for differentiating prostatitis subtypes [10, 32, 33].

#### 5.1.2. Seminal Plasma Biomarkers as Non-Invasive Tools for Prostatitis

Proteomic profiling of seminal plasma has revealed distinct protein alterations in patients with chronic prostatitis. Studies using mass spectrometry identified over 50 potential biomarkers, including PAEP, AZGP1, and SERPINF1, showing differential expression compared to healthy controls. These proteins, some also found in plasma, offer promise as non-invasive markers for diagnosing and monitoring chronic prostatitis. Further validation in human cohorts is needed to confirm their clinical utility [15, 32, 34].

#### 5.1.3. EPS and Post-DRE Urine Biomarkers

EPS-urine collected after DRE is rich in prostate-specific proteins, with proteomic studies identifying over 1,000 proteins and a key panel of 49 prostate-enriched markers. These proteins serve as promising non-invasive biomarkers for prostate diseases. Metabolomic analysis of EPS and plasma from CP/CPPS patients showed disrupted amino acid and energy metabolism, especially tryptophan pathways, linked to symptoms like pain and depression. These findings support EPS and post-DRE urine as valuable fluids for diagnosing and managing prostatitis [35, 36].

#### 5.1.4. Serum Biomarkers

Seminal plasma proteomics identified progestagen-associated endometrial protein (PAEP) as a potential biomarker, elevated in prostatitis and varying with age. Metabolomic analysis of expressed prostatic secretions (EPS) and plasma from CP/CPPS patients revealed disrupted amino acid and lipid metabolism, especially tryptophan pathways, linked to symptoms like pain and depression, reflecting oxidative stress and immune dysfunction. In a mouse model, urinary metabolomics and proteomics found 14 metabolites and 168 proteins altered by prostate inflammation. Conserved biomarkers such as creatine, haptoglobin, and immunoglobulin kappa constantly correlated with inflammation-related LUTS. For prostate cancer, seminal plasma proteomics identified glutamine gamma-glutamyltransferase 4 (TGM4) as a prostate-specific protein outperforming PSA in diagnosis, especially in older men with elevated PSA. TGM4 is promising for inclusion in multi-marker panels [10, 32, 35, 37]. Although many proteomic studies in prostatitis have identified promising biomarkers, few provide quantitative diagnostic performance metrics such as sensitivity, specificity, or area under the receiver operating characteristic curve (AUC). This reflects the exploratory nature and small sample sizes of most current studies. The lack of validated performance data limits immediate clinical translation and underscores the need for large, prospective cohort studies to determine diagnostic accuracy.

### 5.2. Molecular Endotyping for Personalized Therapy in Prostatitis

Molecular profiling using techniques such as NanoString gene panels and metabolomics has revealed distinct expression patterns in CP/CPPS patients compared with healthy individuals. Gene expression analyses of blood and urine highlight neuroinflammatory, immune, and metabolic pathway differences, while metabolomic studies of prostatic secretions and plasma demonstrate disruptions in amino acid and energy metabolism, particularly tryptophan pathways. These molecular signatures support the classification of CP/CPPS into specific endotypes.

From a therapeutic standpoint, endotyping holds promise for tailoring treatment strategies:

• Immune-dominant endotypes (characterized by high cytokine activity, e.g., TNF-, IL-1) may respond better to immunomodulators or biologics.

• Oxidative stress–dominant endotypes (elevated ROS, mitochondrial dysfunction) could benefit from antioxidant or mitochondrial-targeted therapies.

• Neuroinflammatory endotypes (altered neuropeptide and tryptophan metabolism) may be candidates for neuromodulators or gut–brain axis interventions.

• Barrier dysfunction endotypes (loss of claudins, increased permeability) may benefit from therapies aimed at restoring epithelial integrity or modulating TGF- signaling.

• Future clinical protocols could integrate proteomic and metabolomic profiling into diagnostic workups, allowing patients to be stratified into endotypes that predict treatment response. This precision medicine approach mirrors strategies already being tested in asthma and inflammatory bowel disease, and it may guide the development of personalized, multimodal therapies for prostatitis [35, 38-41].

### 5.3. Therapeutic Targets

Several potential therapeutic targets identified through proteomic studies are still in the early stages of investigation, mostly in preclinical models. These include:

**5.3.1. Inflammatory Cytokines (TNF-, IL-1):**

Cytokine-targeting aptamers (ICTAs) have demonstrated the ability to neutralize inflammation and reduce pain in preclinical models of prostatitis. Though these agents show promise, clinical data is still limited. Early-phase clinical trials are currently underway to assess their safety and efficacy in humans.

Research Stage: Preclinical, with early-phase human trials in progress [42].

**5.3.2. Pyroptosis Pathway (Caspase-1, NLRP3):**

Inhibiting the pyroptosis pathway—an inflammatory form of programmed cell death—has proven effective in preclinical models, offering potential for reducing tissue damage and inflammation in prostatitis. These therapies remain preclinical and have not yet entered human clinical trials.

Research Stage: Preclinical [43].

Heat shock proteins, which help protect cells from oxidative stress and modulate inflammatory responses, are being explored for their potential to manage prostatitis. Though preclinical studies suggest they may be beneficial, human clinical data are not yet available.

Research Stage: Investigational (Preclinical stage) [44, 45].

Together, these findings suggest that targeting inflammatory cytokines, pyroptosis pathways, and molecular chaperones such as HSPs represents a multifaceted approach to developing effective therapies for prostatitis and related prostate diseases. Table 4 summarizes the major biomarkers identified through proteomic analysis in different biological fluids (urine, seminal plasma, EPS, serum) in CP/CPPS. While many of these biomarkers show promise for differentiating prostatitis subtypes, most studies have not reported performance metrics such as sensitivity, specificity, or AUC. These biomarkers are still being explored in clinical settings, and larger, prospective studies are necessary to validate their diagnostic utility. Clinical relevance is primarily based on their ability to reflect inflammatory, oxidative, and immune-related processes, which may assist in distinguishing chronic prostatitis from other prostate conditions or diseases.

Table 5 lists the therapeutic targets identified through proteomic studies in chronic prostatitis. These targets, including inflammatory cytokines (e.g., TNF-, IL-1), pyroptosis pathway regulators, and heat shock proteins (HSPs), are currently under investigation in preclinical models. Although they offer potential therapeutic avenues, these targets have not yet been validated in large-scale clinical trials. Most of these interventions are still at preclinical stages, with some entering early-phase human trials. Their clinical relevance lies in their ability to modulate the inflammatory and immune pathways that drive prostatitis. Future clinical trials will be crucial in determining their safety and efficacy for broader use in managing CP/CPPS.

## 6. Challenges and Limitations

Despite significant advances in proteomic technologies, their application in chronic prostatitis research and clinical management is still limited by several challenges:

### 6.1. Sample Variability

Biological samples like urine, seminal plasma, and expressed prostatic secretions can vary greatly in composition, potentially complicating protein profiling. Contamination and degradation issues further exacerbate these challenges, making reproducibility a concern across studies.

### 6.2. Small Sample Sizes

Most proteomic studies in prostatitis are based on small patient cohorts, which reduces the statistical power and generalizability of the findings. Larger, multicenter studies are needed to confirm the clinical utility of identified biomarkers.

### 6.3. High Cost and Accessibility in Low-Resource Settings

Advanced proteomic techniques, such as mass spectrometry and high-throughput protein arrays, are often cost-prohibitive. These technologies require specialized equipment, trained personnel, and significant financial investment, which are not available in many low-resource settings. This poses a major barrier to the widespread adoption of proteomics for routine diagnostics, especially in regions where healthcare funding is limited.

### 6.4. Standardization and Protocol Variability

The lack of standardized protocols for sample collection, processing, and data analysis hampers cross-study comparisons. This variability complicates efforts to translate proteomic findings into clinically actionable biomarkers or therapeutic targets.

Overcoming these challenges will require international collaboration, standardized protocols, and innovative solutions to make proteomic platforms more cost-effective and accessible in clinical settings, particularly in underfunded regions [46-49].

## 7. Future Directions

Advances in multi-omics technologies—including genomics, transcriptomics, proteomics, metabolomics, and macrobiotics—offer a more comprehensive understanding of chronic non-bacterial prostatitis (CNP). Integration of these platforms can reveal complex molecular interactions underlying inflammation, immunity, metabolism, and the gut–prostate axis, as shown in recent studies linking CNP to changes in gut microbiota, gene expression, and DNA methylation. Longitudinal proteomic studies and urinary biomarker profiling hold promise for non-invasive diagnostics and monitoring of disease progression. Meanwhile, AI and deep learning are increasingly used to analyze multi-omics data, though challenges in model interpretability persist. The application of explainable AI (xAI) may improve clinical relevance by making these models more transparent and actionable [11, 50-54].

Together, these developments pave the way for personalized medicine in prostatitis, shifting research toward integrative, data-driven approaches that enable precision diagnostics and targeted therapies.

## 8. Conclusion

Proteomics has shown great potential in uncovering the molecular mechanisms underlying chronic prostatitis, particularly in identifying biomarkers linked to inflammation, oxidative stress, immune dysregulation, and epithelial barrier dysfunction. These findings open the door to personalized diagnostics and targeted therapies, offering hope for better disease management.

However, despite the promising results, the clinical utility of these proteomic biomarkers remains limited due to several factors:

• Small sample sizes and a lack of large validation cohorts hinder the ability to draw definitive conclusions about their diagnostic accuracy and therapeutic potential.

• Standardization issues in sample collection, processing, and analysis further complicate their integration into routine clinical practice.

• While early studies suggest the utility of biomarkers in stratifying patients and guiding treatment, these results need to be validated in larger, multicenter studies before proteomics can be confidently applied in clinical settings.

Thus, while proteomics offers exciting possibilities, it is important to approach its application in chronic prostatitis with caution until more robust data become available from well-powered clinical trials. Future research should focus on standardizing protocols, improving reproducibility, and conducting large-scale studies to confirm the clinical relevance and efficacy of these biomarkers in diverse patient populations.

## 9. Appendix

### 9.1. Acknowledgment

The authors have no acknowledgments to declare.

### 9.2. Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

### 9.3. Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### 9.4. Authors contributions

All authors were involved in the conception, drafting, and revision of the manuscript. All authors read and approved the final version of the manuscript.

## References

1. He, H., et al., Preclinical models and evaluation criteria of prostatitis. Frontiers in Immunology, 2023. Volume 14 -

2023.
2. Han, C.-L., et al., Comparison of cytokine levels in prostatic secretion between the IIIa and IIIb subtypes of prostatitis. Asian Journal of Andrology, 2024. 26(1).
3. Maeda, K., K. Shigemura, and M. Fujisawa, A review of current treatments for chronic prostatitis/chronic pelvic pain syndrome under the UPOINTS system. International Journal of Urology, 2023. 30(5): p. 431-436.
4. McArdle, A.J. and S. Menikou, What is proteomics? Archives of disease in childhood - Education & practice edition, 2021. 106(3): p. 178.
5. Lawrance, I.C., B. Klopcic, and V.C. Wasinger, Proteomics: An Overview. Inflammatory Bowel Diseases, 2005. 11(10): p. 927-936.
6. Ankney, J.A., A. Muneer, and X. Chen, Relative and Absolute Quantitation in Mass Spectrometry-Based Proteomics. Annu Rev Anal Chem (Palo Alto Calif), 2018. 11(1): p. 49-77.
7. Magdeldin, S., et al., Basics and recent advances of two dimensional- polyacrylamide gel electrophoresis. Clinical Proteomics, 2014. 11(1): p. 16.
8. Karpievitch, Y.V., et al., Liquid Chromatography Mass Spectrometry-Based Proteomics: Biological and Technological Aspects. The annals of applied statistics, 2010. 4 4: p. 1797-1823.
9. LaBaer, J. and N. Ramachandran, Protein microarrays as tools for functional proteomics. Curr Opin Chem Biol, 2005. 9(1): p. 14-9.
10. Wei, P., et al., Urinary Metabolomic and Proteomic Analyses in a Mouse Model of Prostatic Inflammation. Urine (Amst), 2019. 1: p. 17-23.
11. Hao, L., et al., Quantitative proteomic analysis of a genetically induced prostate inflammation mouse model via custom 4-plex DiLeu isobaric labeling. Am J Physiol Renal Physiol, 2019. 316(6): p. F1236-f1243.
12. Principe, S., et al., Identification of prostate-enriched proteins by in-depth proteomic analyses of expressed prostatic secretions in urine. J Proteome Res, 2012. 11(4): p. 2386-96.
13. Zhang, Y.D., et al., [Differential proteins in the prostate fluid with abnormal lecithin bodies and immunologic mechanism of chronic prostatitis patients]. Zhonghua Nan Ke Xue, 2021. 27(7): p. 609-615.
14. Utleg, A.G., et al., Proteomic analysis of human prostasomes. Prostate, 2003. 56(2): p. 150-61.
15. Kagedan, D., et al., Characterization of the seminal plasma proteome in men with prostatitis by mass spectrometry. Clin Proteomics, 2012. 9(1): p. 2.
16. Sharma, R., et al., Proteomic analysis of seminal fluid from men exhibiting oxidative stress. Reprod Biol Endocrinol, 2013. 11: p. 85.
17. Pilch, B. and M. Mann, Large-scale and high-confidence proteomic analysis of human seminal plasma. Genome Biol, 2006. 7(5): p. R40.
18. Hamza, G.M., et al., Proteomics of prostate cancer serum and plasma using low and high throughput approaches. Clinical Proteomics, 2024. 21(1): p. 21.
19. Signore, M., et al., Diagnostic and prognostic potential of the proteomic profiling of serum-derived extracellular vesicles in prostate cancer. Cell Death & Disease, 2021. 12(7): p. 636.
20. Kagedan, D., et al., Characterization of the seminal plasma proteome in men with prostatitis by mass spectrometry. Clinical Proteomics, 2012. 9(1): p. 2.
21. Roumeguère, T., et al., Oxidative stress and prostatic diseases. Mol Clin Oncol, 2017. 7(5): p. 723-728.
22. Ihsan, A.U., et al., Role of oxidative stress in pathology of chronic prostatitis/chronic pelvic pain syndrome and male infertility and antioxidants function in ameliorating oxidative stress. Biomedicine & Pharmacotherapy, 2018. 106: p. 714-723.
23. Kaltsas, A., et al., Oxidative Stress in Benign Prostatic Hyperplasia: Mechanisms, Clinical Relevance and Therapeutic Perspectives. Diseases, 2025. 13(2): p. 53.
24. Meng, J., et al., Testosterone regulates tight junction proteins and influences prostatic autoimmune responses. Horm Cancer, 2011. 2(3): p. 145-56.
25. Bhat, A.A., et al., Tight Junction Proteins and Signaling Pathways in Cancer and Inflammation: A Functional Crosstalk. Frontiers in Physiology, 2019. Volume 9 - 2018.
26. Wang, K., et al., Tight junction protein claudin-1 is downregulated by TGF-1 via MEK signaling in benign prostatic epithelial cells. The Prostate, 2020. 80(14): p. 1203-1215.
27. Shang, Y., D. Cui, and S. Yi, Opening tight junctions may be key to opening the blood-prostate barrier. Med Sci Monit, 2014. 20: p. 2504-7.
28. Drake, R.R., et al., Clinical collection and protein properties of expressed prostatic secretions as a source for biomarkers of prostatic disease. J Proteomics, 2009. 72(6): p. 907-17.
29. Deng, A.-Q., et al., The role of microbiota in the chronic prostatitis/chronic pelvis pain syndrome: a review. Frontiers in Microbiology, 2025. Volume 16 - 2025.
30. Lipsky, B.A., I. Byren, and C.T. Hoey, Treatment of Bacterial Prostatitis. Clinical Infectious Diseases, 2010. 50(12): p. 1641-1652.
31. Mitsumori, K., et al., Virulence characteristics of Escherichia coli in acute bacterial prostatitis. J Infect Dis, 1999. 180(4): p. 1378-81.
32. Rokka, A., et al., Mass spectrometric identification of diagnostic markers for chronic prostatitis in seminal plasma by analysis of seminal plasma protein clinical samples. Cellular and Molecular Biology, 2017. 63(7): p.

35-39.
33. Hao, L., et al., Quantitative proteomic analysis of a genetically induced prostate inflammation mouse model via custom 4-plex DiLeu isobaric labeling. American Journal of Physiology-Renal Physiology, 2019. 316(6): p. F1236-F1243.
34. Cao, Z., D.T. Sloper, and N. Nakamura, Identification of Altered Proteins in the Plasma of Rats With Chronic Prostatic Inflammation Induced by Estradiol Benzoate and Sex Hormones. ACS Omega, 2021. 6(22): p. 14361-14370.
35. Zhang, F.X., et al., Chronic prostatitis/chronic pelvic pain syndrome induces metabolomic changes in expressed prostatic secretions and plasma. Asian J Androl, 2025. 27(1): p. 101-112.
36. Principe, S., et al., Identification of Prostate-Enriched Proteins by In-depth Proteomic Analyses of Expressed Prostatic Secretions in Urine. Journal of Proteome Research, 2012. 11(4): p. 2386-2396.
37. Drabovich, A.P., et al., Multi-omics Biomarker Pipeline Reveals Elevated Levels of Protein-glutamine Gamma-glutamyltransferase 4 in Seminal Plasma of Prostate Cancer Patients. Mol Cell Proteomics, 2019. 18(9): p. 1807-1823.
38. Shoskes, D.A., et al., Neuroinflammatory gene expression in chronic prostatitis/chronic pelvic pain syndrome patients: insights into etiology and phenotype biology. Transl Androl Urol, 2021. 10(8): p. 3340-3347.
39. Al-Daffaie, F.M., et al. Metabolomics and Proteomics in Prostate Cancer Research: Overview, Analytical Techniques, Data Analysis, and Recent Clinical Applications. International Journal of Molecular Sciences, 2024. 25, DOI: 10.3390/ijms25105071.
40. Hachem, S., et al., Contemporary Update on Clinical and Experimental Prostate Cancer Biomarkers: A Multi-Omics-Focused Approach to Detection and Risk Stratification. Biology (Basel), 2024. 13(10).
41. Sadeesh, N., M. Scaravilli, and L. Latonen Proteomic Landscape of Prostate Cancer: The View Provided by Quantitative Proteomics, Integrative Analyses, and Protein Interactomes. Cancers, 2021. 13, DOI: 10.3390/cancers13194829.
42. Ho, D.-R., et al. Beneficial Effects of Inflammatory Cytokine-Targeting Aptamers in an Animal Model of Chronic Prostatitis. International Journal of Molecular Sciences, 2020. 21, DOI: 10.3390/ijms21113953.
43. Zhao, M., et al., Relationship between pyroptosis-mediated inflammation and the pathogenesis of prostate disease. Frontiers in Medicine, 2023. Volume 10 - 2023.
44. Ikwegbue, P.C., et al., Interplay between heat shock proteins, inflammation and cancer: a potential cancer therapeutic target. Am J Cancer Res, 2019. 9(2): p. 242-249.
45. Ikwegbue, P.C., et al. Roles of Heat Shock Proteins in Apoptosis, Oxidative Stress, Human Inflammatory Diseases, and Cancer. Pharmaceuticals, 2018. 11, DOI: 10.3390/ph11010002.
46. Vaezzadeh, A.R., et al., Proteomics and opportunities for clinical translation in urological disease. J Urol, 2009. 182(3): p. 835-43.
47. Tonry, C.L., et al. The Role of Proteomics in Biomarker Development for Improved Patient Diagnosis and Clinical Decision Making in Prostate Cancer. Diagnostics, 2016. 6, DOI: 10.3390/diagnostics6030027.
48. Lee, K.H., et al., Addressing statistical challenges in the analysis of proteomics data with extremely small sample size: a simulation study. BMC Genomics, 2024. 25(1): p. 1086.
49. Frantzi, M., A. Bhat, and A. Latosinska, Clinical proteomic biomarkers: relevant issues on study design & technical considerations in biomarker development. Clinical and Translational Medicine, 2014. 3(1): p. e7.
50. Liu, J., et al., Multi-Omics Analysis Reveals Changes in the Intestinal Microbiome, Transcriptome, and Methylome in a Rat Model of Chronic Non-bacterial Prostatitis: Indications for the Existence of the Gut-Prostate Axis. Front Physiol, 2021. 12: p. 753034.
51. Resurreccion, E.P. and K.-w. Fong The Integration of Metabolomics with Other Omics: Insights into Understanding Prostate Cancer. Metabolites, 2022. 12, DOI: 10.3390/metabo12060488.
52. Gómez-Cebrián, N., et al., Metabolic Phenotyping in Prostate Cancer Using Multi-Omics Approaches. Cancers (Basel), 2022. 14(3).
53. Hussein, A., M. Prasad, and A. Braytee, Explainable AI Methods for Multi-Omics Analysis: A Survey. arXiv preprint arXiv:2410.11910, 2024.
54. Xin, L., et al., Artificial Intelligence for Central Dogma-Centric Multi-Omics: Challenges and Breakthroughs. arXiv preprint arXiv:2412.12668, 2024.

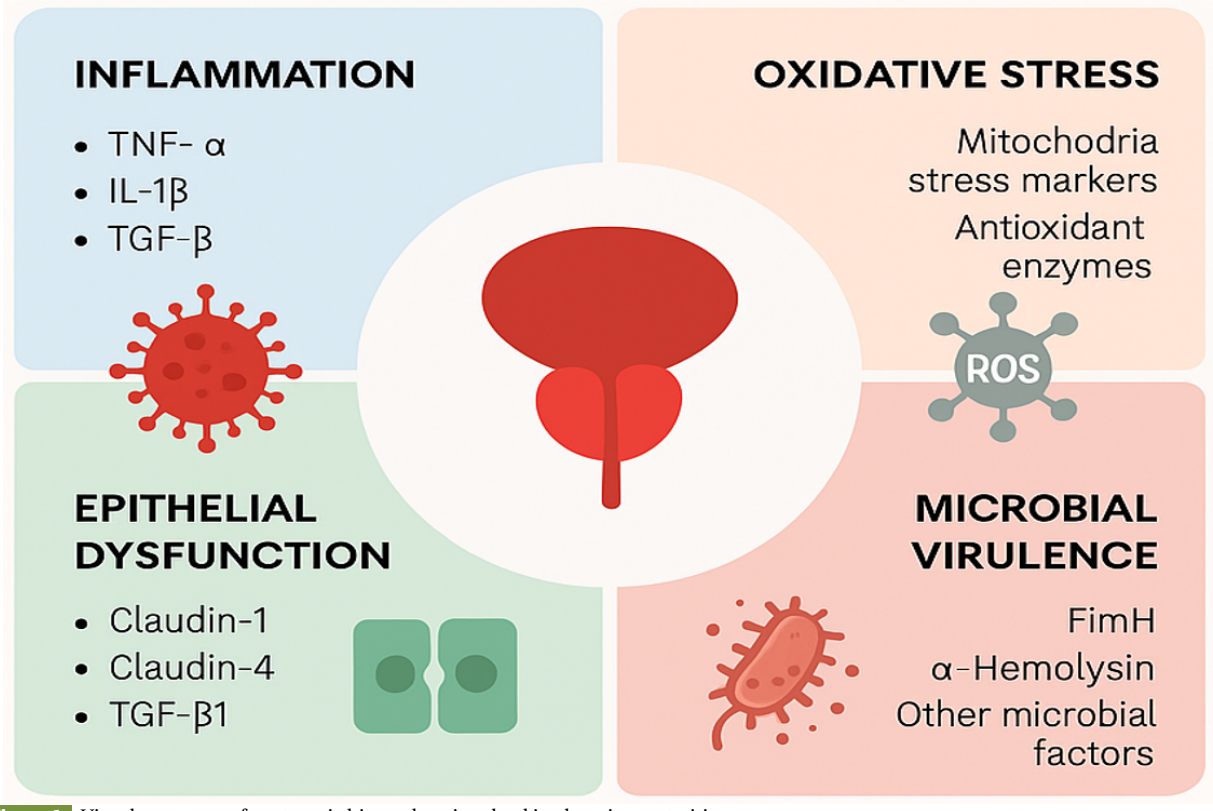


**Figure 1:** Visual summary of proteomic biomarkers involved in chronic prostatitis.

**Table 1:** Core Proteomic Technologies and Their Applications

| Technique | Principle | Strengths | Limitations | Applications in Prostatitis |
|---|---|---|---|---|
| Label-free MS | Peptide ion intensity comparison | Simple, no labeling needed | Sensitive to technical variability | Biomarker discovery in urine, serum |
| SILAC | Incorporation of isotopes in cell culture | Accurate, reproducible | Limited to cultured cells | Mechanistic pathway analysis |
| iTRAQ/TMT | Chemical isobaric tagging | High multiplexing, quantitative | Expensive, ratio distortion possible | Comparative analysis across patient groups |
| 2D-PAGE | Separation by pI and MW | Resolves protein isoforms | Low sensitivity for rare proteins | Seminal plasma profiling |
| LC-MS/MS | Peptide separation + MS/MS | High sensitivity, broad coverage | Complex data analysis | Major tool for clinical proteomics |
| Protein Arrays | Antibody-based microarrays | High-throughput, functional | Requires validated antibodies | Screening inflammatory cytokines, HSPs |

**Table 2:** Sample Sources and Key Biomarkers Identified via Proteomics

| Sample Source | Key Biomarkers Identified | Associated Mechanisms | Clinical Relevance |
|---|---|---|---|
| Urine | Haptoglobin, Creatine, IgG, IgA, polymeric Ig receptor | Inflammation, LUTS, oxidative stress | Non-invasive, reflects local and systemic inflammation |
| Prostatic Fluid | PSA, PAP, Lecithin body proteins | Local immune activation, epithelial changes | Directly reflects prostatic milieu |
| Seminal Plasma | PAEP, AZGP1, SERPINF1, Prolactin-induced protein | Inflammation, oxidative stress, infertility | Biomarkers of oxidative damage and reproductive impact |
| Serum | Cytokines (IL-6, TNF-), HSP70, HSP90, extracellular vesicle proteins | Systemic immune activation, stress response | Useful for systemic biomarker panels and prognostic models |

**Table 3:** Key Molecular Pathways Identified in CP/CPPS

| Biological Process | Molecular Players/Proteins Identified | Role in Pathogenesis | References |
|---|---|---|---|
| Inflammation | TNF-, IL-1, TGF-, PD-L1, Survivin | Drives chronic immune response | [19, 20, 42] |
| Oxidative Stress | ROS, Antioxidant enzymes Mitochondrial stress markers | Tissue damage, sperm dysfunction | [21-23] |
| Tight Junction Dysfunction | Claudin-1, Claudin-4, TGF-1 | Barrier breakdown, immune infiltration | [24-27] |
| Pyroptosis Pathway | Caspase-1, NLRP3, IL-18 | Inflammatory cell death, tissue injury | [43] |
| Heat Shock Proteins | HSP70, HSP90 | Inhibit ROS, stabilize proteins, reduce inflammation | [44-45] |

**Table 4:** Clinical Application of Proteomics in Prostatitis

| Diagnostic Source | Key Biomarkers | Diagnostic Utility | References |
|---|---|---|---|
| Urine | Haptoglobin, Creatine | Non-invasive diagnosis of inflammation | [10, 32] |
| Seminal Plasma | PAEP, AZGP1 | Subtype differentiation | [15, 34] |
| EPS/Post-DRE Urine | 49 protein panel | Prostate-specific diagnosis | [35, 36] |
| Serum | TGM4, PAEP | Risk stratification, age-specific insights | [37] |

Note: While the table highlights key biomarkers and their potential diagnostic roles, most studies did not report detailed performance metrics (e.g., AUC, sensitivity, specificity). These gaps should be considered when interpreting clinical utility.

**Table 5:** Therapeutic Targets Emerging from Proteomic Studies

| Target Class | Example Molecules | Mode of Action | Research Stage |
|---|---|---|---|
| Inflammatory Cytokines | TNF-, IL-1 | Neutralized by aptamers to reduce inflammation | Preclinical |
| Pyroptosis Pathway | Caspase-1, NLRP3 | Suppression prevents cell death and cytokine storm | Experimental |
| Heat Shock Proteins | HSP70, HSP90 | Reduce ROS, protect cells from stress | Investigational |

Note: Most of the therapeutic targets listed are at preclinical stages, with some undergoing early-phase human trials.
Larger clinical studies are needed to determine the effectiveness and safety of these interventions in CP/CPPS patients.