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Urinary ATP as a biomarker for lower urinary tract dysfunctions - A Systematic Review

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Urinary ATP as a biomarker for lower urinary tract dysfunctions

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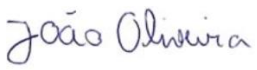
Assinatura do Estudante



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Resumo

Introdução: Os Sintomas do Trato Urinário Inferior (STUI) são uma causa frequente de consulta médica[4]. A sua prevalência aumenta com a idade e com o aumento da esperança media de vida, é esperado que seja um problema crescente causando pressão no sistema de saúde[4]. A causa destes sintomas é multifatorial e vários mecanismos fisiopatológicos podem causar os mesmos sintomas, tornado difícil o diagnóstico etiológico. O estudo urodinâmico é atualmente o exame complementar que mais informação aporta ao diagnóstico, mas apresentando, contudo, baixa sensibilidade e especificidade[4], e está associado a desconforto e a risco de infeção e hematúria. Várias substâncias químicas envolvidas na patofisiologia das disfunções miccionais têm sido investigadas como possíveis auxiliares no diagnóstico dessas disfunções, nesse contexto, o Adenosina Trifosfato (ATP) urinário emergiu como um biomarcador não invasivo em várias disfunções miccionais. Este estudo visa realizar uma revisão da literatura atual sobre o uso do ATP urinário como biomarcador diagnóstico para várias disfunções miccionais, determinar sua relação com a gravidade dos sintomas e investigar os melhores métodos de teste.

Material e métodos: Foi realizada uma revisão sistemática da literatura com recurso ao PubMed e SCOPUS, incluindo publicações desde janeiro de 2009 a abril de 2024. A nossa investigação focou-se sobre estudos em humanos que incluíam o estudo do ATP urinário em disfunções miccionais. Todos os títulos e resumos foram revistos e artigos com informação original foram incluídos.

Resultados: Níveis elevados de ATP urinário foram frequentemente observados em pacientes com bexiga hiperativa, indicando sinalização purinérgica anormal. Da mesma forma, níveis mais altos de ATP em pacientes com síndrome doloroso vesical /cistite intersticial foram associados à gravidade dos sintomas, indicando seu potencial como ferramenta de diagnóstico não invasiva. Em contraste, pacientes com bexiga hipoativa apresentaram níveis mais baixos de ATP urinário, sugerindo redução da sinalização sensorial ou da ativação do músculo detrusor. Diferentes estudos também identificaram a razão NO/ATP como um biomarcador viável para o diagnóstico de BHI, com boa sensibilidade e especificidade. O ATP está também aumentado em homens com obstrução infravesical

Conclusão: O ATP urinário é um biomarcador promissor para o diagnóstico e monitorização dos STUI, mas leituras precisas requerem técnicas padronizadas. A razão NO/ATP oferece um diagnóstico confiável para BHI, e a adição de ATP com outros biomarcadores pode melhorar a precisão diagnóstica. São necessários estudos de validação em larga escala e utilizando painéis com múltiplos biomarcadores para avaliar com segurança o potencial do ATP urinário no diagnóstico das disfunções do aparelho urinário baixo.

Palavras Chave: Foi utilizada uma lista de palavras-chave: ATP urinário, Sintomas do Trato Urinário Inferior, biomarcadores.

Abstract

Introduction: Lower Urinary Tract Symptoms (LUTS) are a common cause of medical consultation[4]. Their prevalence increases with age, and with the rising average life expectancy, they are expected to become an increasing problem, putting pressure on the healthcare system[4]. The cause of these symptoms is multifactorial, with various pathophysiological mechanisms potentially causing the same symptoms, making etiological diagnosis difficult. Urodynamic studies are currently the complementary examination that provides the most diagnostic information; however, they have low sensitivity and specificity[4] and are associated with discomfort and a risk of infection and hematuria. Various chemical substances involved in the pathophysiology of urinary dysfunctions have been investigated as potential aids in diagnosing these dysfunctions. In this context, urinary Adenosine Triphosphate (ATP) has emerged as a non-invasive biomarker in several urinary dysfunctions. This study aims to review the current literature on the use of urinary ATP as a diagnostic biomarker for various urinary dysfunctions, determine its relationship with symptom severity, and investigate the best testing methods.

Material and Methods: A systematic literature review was conducted using PubMed and SCOPUS and in Man, including publications from January 2009 to April 2024. Our investigation focused on human studies that included the study of urinary ATP in urinary dysfunctions. All titles and abstracts were reviewed, and articles with original information were included.

Results: Elevated levels of urinary ATP were frequently observed in patients with overactive bladder, indicating abnormal purinergic signaling. Similarly, higher ATP levels in patients with interstitial cystitis/bladder pain syndrome were associated with symptom severity, indicating its potential as a non-invasive diagnostic tool. In contrast, patients with underactive bladder had lower levels of urinary ATP, suggesting reduced sensory signaling or detrusor muscle activation. Different studies also identified the NO/ATP ratio as a viable biomarker for diagnosing underactive bladder, with good sensitivity and specificity. ATP is also increased in men with bladder outlet obstruction.

Conclusion: Urinary ATP is a promising biomarker for the diagnosis and monitoring of LUTS, but accurate readings require standardized techniques. The NO/ATP ratio offers a reliable diagnosis for BOO, and the addition of ATP with other biomarkers can improve diagnostic accuracy. Large-scale validation studies using panels with multiple biomarkers are needed to safely assess the potential of urinary ATP in diagnosing lower urinary tract dysfunctions.

Keywords: A list of keywords was used: urinary ATP, Lower Urinary Tract Symptoms, biomarkers.

List of abbreviation:

ATP: Adenosine Triphosphate

AUC: Area Under the Curve

BOO: Bladder Outlet Obstruction

BPH: Benign Prostatic Hyperplasia

CMG: Cystometrogram

Cr: Creatinine

CYP3A4: Cytochrome P450 3A4

ELISA: Enzyme-Linked Immunosorbent Assay

FDV: First Desire to Void

HAM/TSP: Human T-lymphotropic Virus Type 1-Associated Myelopathy/Tropical Spastic Paraparesis

HRQoL: Health-Related Quality of Life

HTLV-1: Human T-lymphotropic Virus Type 1

IC/BPS: Interstitial Cystitis/Bladder Pain Syndrome

ICPI: Interstitial Cystitis Problem Index

ICSI: Interstitial Cystitis Symptom Index

LUTD: Lower Urinary Tract Dysfunction

LUTS: Lower Urinary Tract Symptoms

MCC: Maximal Cystometric Capacity

NGF: Nerve Growth Factor

NO: Nitric Oxide

NPV: Negative Predictive Value

NSAIDs: Nonsteroidal Anti-inflammatory Drugs

OAB: Overactive Bladder

OAB-q SF: Overactive Bladder Questionnaire Short Form

OABSS: Overactive Bladder Symptom Score

PdetQmax: Detrusor Pressure at Maximum Flow

PFS: Pressure Flow Study

PGE2: Prostaglandin E2

PPV: Positive Predictive Value

ROC: Receiver Operating Characteristic

UAB: Underactive Bladder

UDS: Urodynamic Study

UTIs: Urinary Tract Infections

VAS: Visual Analog Scale

VUF: Voided Urodynamic Fluid

WBC: White Blood Cells

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Introduction

Background and Context

Lower urinary tract symptoms (LUTS) are a common cause of medical consultation[4]. They are highly prevalent with population-based studies reporting a prevalence over 60% of men and women[22].

LUTS are classified into three main categories: storage, voiding and post-micturition symptoms. Each of these is characterized by a possible group of symptoms: storage symptoms include urinary urgency, increased daytime frequency, and nocturia; voiding symptoms include a slow urinary stream, intermittent flow, hesitation, and straining; at last, post-micturition symptoms include feelings of incomplete bladder emptying and post micturition dribble. Patients with LUTS may experience several of these symptoms at the same time and different pathophysiologic processes may cause similar symptoms, rendering an etiologic diagnosis challenging. In clinical practice the gold standard test to evaluate LUTS is the urodynamic study, however it has some invasiveness, is expensive and has limited sensitivity and specificity.

There are several lower urinary tract dysfunctions that are highly prevalent in the adult population.

Bladder outlet obstruction (BOO) caused by benign prostatic hyperplasia (BPH) is one of those dysfunctions. BOO occurs when there is an obstruction at the base of the bladder, which restricts the flow of urine into the urethra. This obstruction can lead to difficulty in starting urination, a weak urinary stream, and incomplete bladder emptying. Also its important to note that, according to European studies, BOO affects roughly 50% of individuals with LUTS caused by BPH [4 9]. However, these symptoms can also be a result of a condition outside of the urinary tract and bladder malfunction, therefore a precise diagnosis is essential for developing an efficient treatment plan.

Overactive bladder (OAB) is also highly prevalent and is a symptom complex characterized by urinary urgency [6], usually accompanied by increased frequency and nocturia, with or without urgency urinary incontinence, in the absence of proven infection or other obvious pathology. OAB affects approximately 11.8%

adults in Europe (10,8% male and 12,8% female) having normally a higher prevalence with age, especially women over 40 years old [10] [4]. Also, the pathophysiology of OAB involves abnormal activation of bladder sensory pathways and detrusor overactivity. This diagnosis is primarily clinical, based on patients' subjective symptoms and the exclusion of other bladder disorders such as interstitial cystitis, urinary lithiasis, and acute cystitis through urodynamic studies, cystoscopy, and other diagnostic methods. Despite its clinical basis, OAB's subjective diagnostic nature and reliance on invasive procedures highlights the need for simpler, less complicated, and more cost-effective diagnostic methods.

On the other hand, Underactive Bladder (UAB) is characterized by weak or absent detrusor muscle contractions during the voiding phase, leading to incomplete bladder emptying and increased post-void residual urine volume. The etiology of UAB can be neurogenic, myogenic, aging, diabetes mellitus and Bladder Outlet Obstruction. Right now, UAB can only be reliably diagnosed with invasive pressure flow studies (PFS), therefore it is important to explore less invasive and cheaper ways of making this diagnosis.

Lastly, Bladder Pain Syndrome, also known as Interstitial Cystitis, is a chronic condition characterized by bladder pain, pressure, and discomfort, often accompanied by urinary urgency and frequency. The exact cause of BPS/IC is still unknown, but it is thought to involve a combination of bladder epithelial dysfunction, abnormal sensory nerve signaling, and inflammation. Another factor to note is that there isn`t enough currently epidemiological information on IC/BPS even though it is acknowledged is more common in women rather than men. Additionaly, the European Association of Urology (EAU) outlines significant challenges in diagnosing IC/BPS which lead us to search if urinary ATP could be a possible future diagnostic tool for this purpuse.

ATP plays a crucial role in purinergic signaling, which is a key regulatory mechanism in the bladder. This signaling pathway involves the release of ATP from urothelial cells in response to bladder distension and other stimuli which, once released, binds to purinergic receptors (P2 receptors) on the surface of both urothelial cells and afferent nerves within the bladder wall. This binding initiates a cascade of events that contribute to bladder muscle contraction

(detrusor activity) and sensory signaling, which are essential for normal bladder mediating bladder sensations, particularly in pathological situations.

ATP is also important in mediating detrusor contraction; in fact, it is the main responsible for the non-cholinergic detrusor contractions observed in in-vitro experiments.

In the context of urinary dysfunctions, alterations in purinergic signaling can significantly impact bladder behavior. For instance, in Overactive Bladder (OAB), elevated ATP levels lead to heightened activation of purinergic receptors, resulting in a heightened sense of urgency and possibly in unvoluntary detrusor contractions. Conversely, in Underactive Bladder (UAB), reduced ATP release can diminish detrusor muscle activity, contributing to incomplete bladder emptying.

Furthermore, in conditions such as Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC), abnormal purinergic signaling can amplify sensory pathways, leading to chronic pain and discomfort. In Bladder Outlet Obstruction (BOO), the disruption of normal ATP-mediated signaling may contribute to the compensatory mechanisms that lead to detrusor overactivity and subsequent bladder dysfunction.

Understanding the role of ATP in these pathways provides insights into the underlying mechanisms of various urinary dysfunctions and highlights its potential as a biomarker for diagnosis and monitoring. By measuring urinary ATP levels, clinicians can gain valuable information about the functional state of the bladder and the severity of the dysfunction, aiding in the development of targeted and effective treatment strategies.

Biomarkers can be indicative of the presence, severity, progression and treatment response of a disease or symptom complex. In fact, several urinary biomarkers have been studied in the context of OAB that can signal detrusor overactivity, one of which urinary ATP [10]. However, it is still uncertain whether widespread use of these biomarkers could improve OAB care. Their potential benefits are enormous, including phenotyping OAB patients, developing customized treatment plans, predicting treatment outcomes, and even establishing the cause of OAB in otherwise healthy people. In men, biomarkers may be pivotal for diagnosing BOO and guiding treatment approaches in LUTS, especially given the limits of today non-invasive diagnostic tools.

Currently, diagnosis for OAB and BOO face significant challenges. There are non-invasive techniques such as uroflowmetry and ultrasound imaging, tools that have limitations due to variability between specialists' observation, limited accuracy, and a lack of standardization. On the other hand, pressure flow studies (PFS), which are considered the gold standard for diagnosing BOO, are invasive, expensive, and not commonly available. Furthermore, urodynamic testing for detrusor overactivity, while informative, is not always trustworthy and has little clinical utility for determining the severity or prognosis of idiopathic OAB. These constraints highlight the need for new, precise, reliable, and non-invasive diagnostic methods [9].

Given ATP's critical involvement in bladder filling sensation and function, it can be useful to measure its levels in urine for diagnosis of LUTS.

With this review, we aim to summarize the existing literature on the diagnostic value of urinary ATP in lower urinary tract symptoms following the PRISMA guidelines [12] for systematic review in a comprehensive and methodological matter.

Materials and Methods

This study reports the search of available data published between the period of 1 January 2009 and 1 January 2024 with the search terms: "urinary atp" and "lower urinary tract symptoms". This search strategy identified 247 papers in all databases (143 from PubMed, 100 from Scopus, 4 from other sources). Articles that did not fulfil the inclusion criteria were not subjected to additional review (but some of them were used for introduction and discussion sections) (Table i). The selection of the articles was performed through Rayyan, which is a free web-tool that greatly speeds up the process of screening and selecting papers for academics working on systematic reviews, in three rounds. The first round consisted of a screening of all titles to exclude papers that were duplicated or unrelated to the subject, and then the included added to Rayyan for further analysis. The second round consisted of a screening of all abstracts. In the third round, the full texts of all potentially relevant studies were reviewed considering the inclusion and exclusion criteria. Potential divergences in the selection of the

study were discussed and ultimately resolved. The following information was manually extracted: (1) Database, (2) Title, (3) Population (Men, Women or Both), (4) Excluded Criteria, (5) Sampling Methods, (6) Preservation Method, (7) Analytical Methods, (8) Objective of Study, (9), Main Findings, and (10) References.

The flow diagram for selecting studies is shown in Figure 1. The initial database search yielded 247 studies, from which 108 abstracts were examined, and 45 full texts were evaluated for eligibility. A total of 28 studies were rejected after examining the inclusion and exclusion criteria, primarily because they were previous reviews of the topic. A total of 17 papers on urinary ATP were chosen. The Preferred Reporting Items for Systematic Reviews (PRISMA) checklist [3] was completed.

Results

After review of the screened articles, we divided our findings in different categories to best systematize the available information. Firstly, we distinguished the sampling methods applied across the literature and afterwards we divided our data on the results of urinary ATP testing in different LUTS complexes (OAB, BOO, UAB or ICS/BPS).

Methodologies for Measuring Urinary ATP: Sample Collection and Preservation

Across all researched studies we verified that a midstream urine collection at the peak of urination is the most frequent method used in all LUTS scenarios [13 14 15], while the quantification using bioluminescence was the most used quantification method. Using this methods, different studies tried to collect urinary ATP levels samples at what can be the most representative of bladder activity. More so, most studies including OAB, BPH/BOO, UAB, general LUTS, and IC/BPS studies agree that collecting urine at this precise time point better guarantees consistency and decreases contamination across samples. However, variations in patient hydration state and timing can still have a big impact on ATP levels.

After collection, the most significant problem is the rapid hydrolysis of ATP at room temperature, which demands fast sample handling and preservation otherwise ATP levels might plummet if samples are not rapidly processed or

frozen[15]. Most studies preserve samples at -80°C to avoid ATP breakdown which can be seen across various articles on OAB, BPH/BOO, UAB, general LUTS, and IC/BPS. The most prevalent barrier here is the logistical issue of this requirements, especially in common clinical settings where immediate freezing facilities may not always be available. Therefore, it is important to explore other storing techniques. As Kiren Gill et al [15] explored in their article exists the possibility for storing urine samples at -20°C as a more practical alternative.

Urinary ATP in Urological Conditions

8 articles reported data on urinary ATP concentration in OAB patients.

Silva Ramos M. et al, evaluated urinary ATP in women with detrusor overactivity and in asymptomatic controls and found that urinary ATP levels were significantly (P,0.05) higher in women with detrusor overactivity compared to controls (7004 +/- 1228 pM vs 2647 +/- 403 pM p<0,001). A significant difference was also shown when ATP concentration was normalized either to urine creatinine levels or voiding volume (27,5 +/- 8,3 pmol/mg vs 7,2 +/-1,7 pmol/mg p=0,022 and 1556,1 +/- 290,9 pmol vs 707,8 +/- 139,6 pmol p=0,012 respectively)[10]. The discriminator value of ATP was significant, with an AUC of 0.741(95% CI 0.62 to 0.86; P,0.001). In the same study, it was also noted that women were subdivided in wet and dry OAB accordingly if they had urgency with or without incontinence respectively. After this it was tested the correlation with ATP/Cr level which found out that it was significantly (P,0.05) higher than controls in women with OAB, despite they have been characterized as OAB dry or OAB wet. Also, water intake was shown to alter ATP measurements, increasing its sensitivity.

In 2 of Firouzmand S. et al studies[7,8], positive correlations were seen between the urinary levels of ATP/Cr and the total ICIQ-OAB symptom score (p=0,007) and the frequency score (p< 0,001) as well as in the ratio ATP/NO.

Sugaya K. et al article[16] also shown a significantly positive correlation between urinary ATP and OAB patients' symptom severity scores in different tests, including the OAB-q SF, HRQoL, and OABSS[16]

Despite this, some studies like the one from Suh YS. et al[17] had different results when testing the utility of urinary level of ATP/Cr level in diagnosing OAB. Despite

having higher urinary ATP levels than control groups, OAB patients did not show statistically significant difference between the two groups. In this case, the discriminatory value was not significant, having an AUC of 0.608 (95% CI, 0.505–0.711; P=0.058).

Cheng Y. et al noted in their articles[18] [19] that there was an inverse correlation between ATP concentration in VUF and FDV but not MCC when combined with urine pH, contrary to what was seen in control groups. These findings may suggest that ATP could be an important factor for the initial perception of the need to urinate, in patients with OAB.

Firouzman S. et al most recent study[6] measured ATP in 95 human urine samples and found that urinary ATP by itself was not suitable to identify initial stages of OAB development. However, the combination of Age + gender + IL-5 + ATP was shown to have the highest predictive power for OAB diagnosis with p=0.045, showing a significant discriminator value with AUC of 0,727 ± 0,056 as well as the highest PPV (21%, 67% for female and 17%, 50% for male) and NPV (96%, 75% for female and 97%, 86% for male) values compared to the other logistic models. Considering these results, an equation was constructed to predict the likelihood of the identified high OAB symptomatic score group ($P=1/1+e^{-x}$, where X= -3.09 +5.393 x subject's age[b] +1.797 x Gender (Female 1, Male 0) +34.767 x [IL-5/Cr][b] + (-562.743) x [ATP/Cr][b]).

3 articles reported data on urinary ATP concentration in BPH and BOO patients.

Silva-Ramos M. et al study[9] found that urinary ATP concentration was significantly higher in BOO patients than in asymptomatic controls levels (2.62 +/- 0.19 nM vs 1.03 +/- 0.18 nM; p value < 0,0001) which was also verified when urinary ATP was normalized to urine creatinine levels to account for fluctuations in urine concentration (3.99 +/- 0.48 pmol/mg vs 1.11 +/- 0.30 pmol/mg; p value < 0,0001), with significant discriminatory value with an AUC of 0.91 (95%CI 0.86–0.96; P < 0.001) and 0.88 (95%CI 0.81–0.94; P < 0.001) respectively representing high-sensitive biomarkers for discriminating symptomatic BOO patients.

This also applied in other studies like Chen Z. et al study [14], which shown urinary ATP levels of 118.54±7.18 nM in BOO patients vs 47.67±2.42 nM in controls before normalized and 126.20±7.99 vs. 51.8±2.63 after normalized to urine creatinine levels, being significantly higher in both situations (p<0,0001). The

AUC value was 0.83 (95% CI: 0.78–0.88, P<0.001) and 0.82 (95% CI: 0.77–0.88, P<0.001) respectively, showing was no difference between the two variables (P>0.05).

It was also taken into consideration that BOO patients voided smaller (P < 0.001) volumes of urine compared to control individuals [9]. Because of this, in Silva Ramos M. et al study [9] it was corrected urinary ATP by the corresponding voided volume which was shown to still be significantly higher in BOO patients than in asymptomatic controls (208,8 +/- 34,62 nmol vs 456 +/- 36 nmol ; p value < 0,0001) and also that there is a positive correlation between the ATP amount in urine samples and voided volumes both in asymptomatic men and BOO patients. Lastly, both voided volume (VV, r = -0.517, P < 0.001) and bladder volume (VV + PVR, r = -0.556, P < 0.001), but not the post-void residual urine (PVR, r = -0.097, P = 0.509), positively correlated with urinary ATP.

On the other hand Chen Z. et al study [14], noted that void volume (VV, 180.1±12.1 mL) in BOO patients demonstrated an inverse correlation with urinary ATP levels (P=0.006), while the Bladder Outlet Obstruction Index (64.06±2.51, P<0.001), Pdet@Qmax (75.26±2.79 cmH2O, P<0.001), and MaxPdet (80.95±3.12 cmH2O, P=0.002) demonstrated a positive correlation with ATP measured in urine samples. Qmax (8.13±0.47 mL/s) tended to be correlated with urinary ATP, but it did not reach significance (P>0.05). With a BOOI >40 cutoff point to separate BOO from non-BOO patients, ROC curves showed high discriminatory values of AUC for both ATP and ATP/Cr at 0.77 (95% CI: 0.67–0.87) and 0.76 (95% CI: 0.66–0.87) for ATP/Cr respectably, being this finding statistically significant (P<0.001).

Lastly, Sugaya K. et al article[16] also shown that urinary ATP/ creatinine ratio was significantly lower (P = 0.009) in normal male controls than in BPH patients.

Chen Z. et al study [14] also gave us another important finding. In patients with Acute Urinary Retention (AUR) or Detrusor Obstruction (DO) history it was noted that urinary ATP concentration ((162.35±18.47 nM, n=34) and (169.38±20.64 nM, n=28) respectably) were significantly higher compared with patients without history (114.46±8.75 nM, n=65, P=0.006) indicating that increased bladder pressure and inflammation may trigger a greater release of ATP from the urothelium.

There were also 2 articles reporting data on urinary ATP concentration in UAB and ICS/BPS patients, having each 1.

In Krishnan et al study[1], we found that urinary levels of ATP were significantly decreased in UAB patients compared to controls (546.1 ± 37.3 pg/µl vs. 610.7 ± 24.9 pg/µl, p value < 0,0001) which could indicate the potential of urinary ATP as a biomarker for UAB. We also found that urinary NO/ATP ratio showed excellent discrimination between UAB patients and controls (2.26±0.2 vs. 1.84±0.18, p value < 0,0001) with an area under the curve of 0.91. At a cut-of level of 2.06, NO/ATP ratio high sensitivity and specificity in diagnosing UAB with an accuracy of 88.5%, 88.9% and 88.6% respectively. On the other hand, it was not found a significant correlation between urinary ATP or NO levels with UDS parameters such as PVR, PDet Qmax and BCI.

In Wu Y. et al study [2], we found that urinary ATP levels were significantly higher in IC/BPS patients (62.99 ± 7.97 nM vs 53.26 ± 7.82 nM, p value < 0,0001), respectively compared to the control group. It was then made a ROC curve test to evaluate the diagnostic efficacy of urinary ATP for IC/BPS which had an AUC value of 0.811 (95% CI 0.730–0.892; P < 0.0001) for a threshold value for ATP concentration at 56.6 nM. Based on those results, it was investigated the potential correlation between IC/BPS patients and symptom scores[20], where Visual analog score (r = 0.6070; P < 0.0001), Interstitial cystitis symptom index (r = 0.5822; P < 0.0001), and Interstitial cystitis problem index (r = 0.5234; P < 0.0001) from the O'Leary-Sant score shown a positive correlation with urinary ATP levels in IC/BPS patients. In spite these findings, this study still found some variation in ATP concentrations. This heterogeneity could be attributed to a multitude of factors such as patient hydration, sample collection duration, and individual variances in ATP metabolism.

Lastly, we found 3 studies that tested urinary ATP in a broader spectrum of LUTS conditions.

Cheng Y. et al found a significant inverse correlation between MCC per 100ml and urinary ATP in patients with idiopathic DO (r^2=0.267, p=0.002) [18].

Gill K. et al study[15] linked high urinary ATP levels to inflammation, especially when pyuria surpassed 10 WBC/µL. However, ATP failed to distinguish successfully at

lower levels of pyuria, implying that it has limited diagnostic utility for milder inflammation.

McLatchie L. found that women and voiding symptoms were associated with greater urinary ATP levels[21]. This research implies that there are gender differences in symptom expression in LUTS. However, this biomarker was unaffected when comparing with age or the frequency and severity of other LUTS symptoms.

Discussion

Overview

According to previous studies urinary ATP has gained significant interest as a biomarker for diagnosing and monitoring a variety of urological conditions. Focusing mainly on his utilization in LUTS, these biomarkers were measured in a variety of different clinical trials to test their importance as a clinical tool allowing for non-invasive diagnosis of LUTS existence, progression, and therapy response.

A significant observation from the reviewed studies is the consistency in the method of urine collection. Most studies opted for the midstream urine collection at the peak of urination and used bioluminescence to quantify the levels of urinary ATP which we agree as the best sampling and quantifying methods of urinary ATP. This method of urine collection is thought to be most representative of bladder activity, ensuring that ATP levels reflect the physiological state of the bladder during peak function, helping minimize contamination, which can otherwise lead to variability in ATP readings. Moreover, bioluminescence is used because is the most straightforward, cost-effective and exceedingly dependable technique for quantifying ATP levels[2]. Despite this, the studies indicate us that factors such as patient hydration status and the exact timing of sample collection can significantly impact ATP levels, to combat this we advise for a need for standardized protocols to ensure consistency across different clinical and research settings. Another critical challenge identified is the rapid hydrolysis of ATP at room temperature, necessitating immediate handling and preservation of samples to prevent degradation. Most studies emphasize the importance of rapid

processing or freezing to maintain ATP integrity, with the standard practice being storage at -80°C. However, just like in Kiren Gill et al [15] study, we identify a logistical barrier for this preservation method in the difficulty of maintaining such low temperatures, particularly in typical clinical settings where immediate freezing facilities may not always be available. Having this in mind and knowing that ATP hydrolysis presented the same rate at -80 and -20ºC, we considered that urine samples storing at -20°C can be a more practical alternative.

In terms of its application, urinary ATP levels have shown correlations with various urological conditions, some more than others, especially its inverse results in OAB and UAB which could indicate some degree of affinity for this type of conditions. Across most studies we found that urinary ATP had a high correlation with OAB compared to control groups, which was consistent when normalized to urine creatinine, while the contrary was found between UAB patients and control groups where we found significantly lower urinary ATP levels in UAB patients compared to controls. This suggests that ATP release is associated with the detrusor muscle agreeing with current literature, highlighting urinary ATP's potential as a biomarker for both of this condition.

Regarding BOO patients and ICS/BPS, urinary ATP levels were significantly higher in patients these conditions compared to controls, while it was also noted a correlation between urinary ATP levels and their corresponding symptom severity scores not only in both BOO and IC/BPS patients but also in OAB patients. Both these findings support its role as a biomarker not only for diagnosis but also for monitoring disease severity in these conditions.

Despite this, there were found some conflicting results across multiple studies in OAB and BOO patients. This discrepancy highlights the need for further investigation into the factors influencing urinary ATP levels and their diagnostic accuracy, like for example, water intake, which was suggested to potentially increase the sensitivity of ATP measurements across multiple studies, indicating that hydration status may influence urinary ATP levels.

Regarding UAB and IC/BPS, unfortunately there are still very few studies that test the application of urinary ATP as a biomarker. Because of this, we need to have in consideration that the results we found may not be completely reliable becoming

increasingly important to develop more studies regarding urinary ATP as a biomarker of this conditions in the future.

Across studies, firstly, regarding OAB studies, we created a comparison table of all available OAB studies with urinary ATP concentrations compared between OAB patients and control with the following results (Table ii), being excluded from this table, all Firouzmand S. et al studies which did not provide the urinary ATP concentrations measured in their articles. Secondly, comparing now across all types of LUTS conditions, we found that, for the most part, urinary ATP levels were increased in BOO and ICS/BPS when compared with OAB. This can be attributed to the significant mechanical stress and inflammation associated with these conditions. In BOO, the physical blockage causes increased bladder pressure and mechanical stress, which may trigger greater ATP release from the urothelial cells, while in IC/BPS, chronic inflammation of the bladder wall can lead to enhanced ATP release as a pro-inflammatory mediator. In contrast, OAB primarily involves abnormal bladder muscle contractions with less cellular stress and inflammation, resulting in comparatively lower ATP release and explaining this urinary ATP difference. Lastly UAB presents itself as the lowest urinary ATP values observed which agrees with our finding that UAB inversely correlates with urinary ATP values.

Finaly, one of the most important findings we made was the fact that while urinary ATP by itself appears to have some conflicting findings regarding its diagnostic potential for lower urinary tract dysfunctions, it has shown across all types of conditions that it can be a part of a reliable multi marker diagnostic tool. As a result, urinary ATP should be seen as part of a larger diagnostic panel rather than a single sign.

Conclusion:

After reviewing previous studies, we reached the conclusion that urinary ATP may have promise as a diagnostic biomarker for lower urinary tract dysfunctions either as a solo biomarker, or in combination with others, albeit further research is needed in numerous areas before it can be completely utilized in clinical practice. Therefore, future study should focus on the following areas to improve our understanding and application of urine ATP in diagnosing and controlling LUTS.

It is important that there are reliable and reproducible results across all studies before we even think about using urinary ATP as a biomarker in clinical practice. On this note, we advise future research to concentrate on developing standardized techniques for sample collection, handling, and preservation in which variability in ATP concentrations caused by factors such as patient hydration status, sample collection time, and storage conditions should be minimized. To achieve this goal researchers should establish best practices, such as appropriate sample storage conditions and the use of preservatives to prevent ATP deterioration.

Secondly, we should thrive to perform studies that involve a wide range of patient populations to confirm the diagnostic accuracy and reliability of urinary ATP in various lower urinary tract dysfunctions, including overactive bladder (OAB), benign prostatic hyperplasia (BPH), bladder outlet obstruction (BOO), underactive bladder (UAB), and interstitial cystitis/bladder pain syndrome (IC/BPS).

There is literature evidence that suggests that combining urinary ATP with additional biomarkers (e.g., NGF and PGE2, NO) or with other discriminating factors (e.g., age, gender) may improve diagnosis accuracy. It is then of clinical relevance important to investigate the diagnostic potential of these combinations, which can provide a more complete picture with high specificity and sensitivity results of bladder function and inflammation.

Another important factor for future clinical application is the development of easy, available and cost-effective testing methods for urinary ATP levels while being able to provide reliable and real time data for its use in diagnosis of LUTS conditions.

It also has clinical value to study the effectiveness of urinary ATP in monitoring disease development and therapy response to providing insights into treatment efficacy and assisting in the development of tailored management strategies for LUTS patients.

Lastly to effectively integrate urinary ATP as a diagnostic tool, clinical guidelines need be developed based on strong evidence gathered from studies following above specifications.

In conclusion, in this review, we considered that urinary ATP may be a promising biomarker for diagnosing and managing lower urinary tract problems. While there are already examples of its potential use there need to be further research to address current limitations and establish its therapeutic utility. The need for standardizing techniques, verifying findings across varied populations, investigating multi-marker approaches, establishing point-of-care tests, and conducting long-term investigations are all important steps toward one day being able to incorporate urinary ATP into routine clinical practice. As research continues, urinary ATP may dramatically improve LUTS diagnosis, monitoring, and treatment, thereby increasing patient outcomes and quality of life.

Appendix

Table i. Inclusion and exclusion criteria in the articles selected[5]

Inclusion Criteria	Exclusion Criteria
Articles published in the English language	Articles published in other languages
Articles published from 1 January 2009 to 1 January 2024	Articles published prior to 2009
Articles reporting findings from any country	Articles with non human test subjects
Articles related to urinary ATP as a biomarker for lower urinary tract symptoms	Articles related exclusively to urothelium ATP
	Articles related to urinary ATP as a biomarker for upper urinary tract symtpoms
Original scientific articles on the topic	Abstracts of congress, reports, reviews/state of the art articles

Table ii. Urinary ATP obtained in OAB patients vs control groups

Reference	OAB Patients (Men or Woman)	Urinary ATP (pM) in OAB patients	Urinary ATP/Cr (pmol/mg) in OAB patients	Urinary ATP (pM) in controls	Urinary ATP/Cr (pmol/mg) in controls	P value
Silva-Ramos M. et al[10]	Woman	7004 +/- 1228	27,5 +/- 8,3	2647 +/- 403	7.2 +/- 1.7	0,001[a]; 0,022[b]
Suh YS.et al[17]	Both		23.1 +/- 6.7		3,7 +/- 0,9	0,058
Cheng Y. et al[18]	Woman	148000		129000		0,58
Sugaya K. et al[16]	Woman		14,6 +/- 15,4			

a: Comparison in concentration of Urinary ATP (pM) in OAB patients and controls

b: Comparison in concentration of Urinary ATP/Cr (pmol/mg) in OAB patients and controls

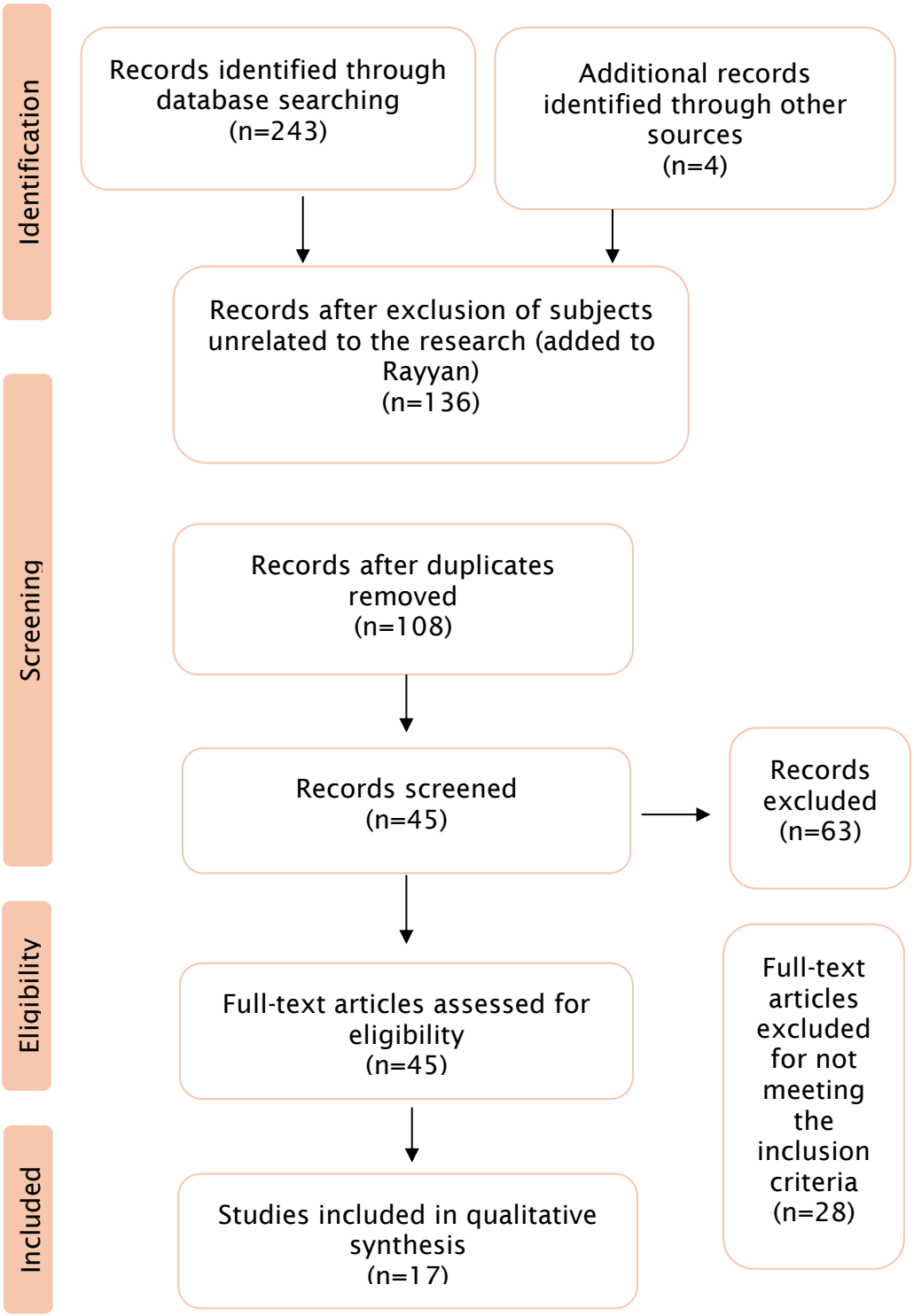

Figure 1: PRISMA Flowchart [3]

Bibliography

1. Krishnan A, Sharma G, Devana SK, et al. Urinary adenosine triphosphate and nitric oxide levels in patients with underactive bladder: a preliminary study. *World J Urol*. Dec 2021;39(12):4421-4425. doi:10.1007/s00345-021-03784-5
2. Wu Y, He Y, Qi J, Wang S, Wang Z. Urinary ATP may be a biomarker of interstitial cystitis/bladder pain syndrome and its severity. Article. *Biomol Biomed*. 2024;24(1):170-175. doi:10.17305/bb.2023.9694
3. Salameh J-P, Bossuyt PM, McGrath TA, et al. Preferred reporting items for systematic review and meta-analysis of diagnostic test accuracy studies (PRISMA-DTA): explanation, elaboration, and checklist. *bmj*. 2020;370
4. Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. *Eur Urol*. Dec 2006;50(6):1306-14; discussion 1314-5. doi:10.1016/j.eururo.2006.09.019
5. McInnes MD, Moher D, Thombs BD, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. *Jama*. 2018;319(4):388-396.
6. Firouzmand S, Ajori L, Young JS. New participant stratification and combination of urinary biomarkers and confounders could improve diagnostic accuracy for overactive bladder. *Sci Rep*. Feb 20 2020;10(1):3085. doi:10.1038/s41598-020-59973-6
7. Firouzmand S, Ajori L, Towse J, et al. Investigating the associations of mucosal P2Y6 receptor expression and urinary ATP and ADP concentrations, with symptoms of overactive bladder. *Neurourol Urodyn*. Mar 2020;39(3):926-934. doi:10.1002/nau.24322
8. Firouzmand S, Young JS. A pilot study to investigate the associations of urinary concentrations of NO, ATP and derivatives with overactive bladder symptom severity. *Exp Physiol*. Jun 2020;105(6):932-939. doi:10.1113/ep088450
9. Silva-Ramos M, Silva I, Oliveira JC, Correia-de-Sá P. Increased Urinary Adenosine Triphosphate in Patients With Bladder Outlet Obstruction Due to Benign Prostate Hyperplasia. *Prostate*. Nov 2016;76(15):1353-63. doi:10.1002/pros.23207
10. Silva-Ramos M, Silva I, Oliveira O, et al. Urinary ATP may be a dynamic biomarker of detrusor overactivity in women with overactive bladder syndrome. *PLoS One*. 2013;8(5):e64696. doi:10.1371/journal.pone.0064696
11. Antunes-Lopes T, Cruz CD, Cruz F, Sievert KD. Biomarkers in lower urinary tract symptoms/overactive bladder: a critical overview. *Curr Opin Urol*. Jul 2014;24(4):352-7. doi:10.1097/mou.0000000000000064
12. Şalvarlı Şi, Griffiths MD. Internet gaming disorder and its associated personality traits: A systematic review using PRISMA guidelines. *International Journal of Mental Health and Addiction*. 2021;19:1420-1442.
13. Matsuo T, Miyata Y, Nakamura T, Satoh K, Sakai H. Prosultiamine for treatment of lower urinary tract dysfunction accompanied by human T-lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis. *Int J Urol*. Jan 2018;25(1):54-60. doi:10.1111/iju.13468

14. Chen Z, Liu Y, Zhao M, et al. Urinary ATP may be a biomarker for bladder outlet obstruction and its severity in patients with benign prostatic hyperplasia. Article. *Transl Androl Urol*. 2020;9(2):284-294. doi:10.21037/tau.2020.02.18
15. Gill K, Horsley H, Kupelian AS, et al. Urinary ATP as an indicator of infection and inflammation of the urinary tract in patients with lower urinary tract symptoms. *BMC Urol*. Feb 21 2015;15(1):7. doi:10.1186/s12894-015-0001-1
16. Sugaya K, Nishijima S, Kadekawa K, Miyazato M, Mukouyama H. Relationship between lower urinary tract symptoms and urinary ATP in patients with benign prostatic hyperplasia or overactive bladder. *Biomed Res*. Oct 2009;30(5):287-94. doi:10.2220/biomedres.30.287
17. Suh YS, Ko KJ, Kim TH, et al. Potential Biomarkers for Diagnosis of Overactive Bladder Patients: Urinary Nerve Growth Factor, Prostaglandin E2, and Adenosine Triphosphate. *Int Neurourol J*. Sep 2017;21(3):171-177. doi:10.5213/inj.1732728.364
18. Cheng Y, Mansfield KJ, Allen W, Millard RJ, Burcher E, Moore KH. Correlation between cystometric volumes, ATP release, and pH in women with overactive bladder versus controls. *Neurourol Urodyn*. Sep 2013;32(7):969-73. doi:10.1002/nau.22344
19. Cheng Y, Mansfield KJ, Allen W, Walsh CA, Burcher E, Moore KH. Does adenosine triphosphate released into voided urodynamic fluid contribute to urgency signaling in women with bladder dysfunction? *J Urol*. Mar 2010;183(3):1082-6. doi:10.1016/j.juro.2009.11.028
20. Pires MV, de Lima CJ, Carvalho HC, Moreira LH, Fernandes AB. Effectiveness of intravesical ozone in interstitial cystitis by the O'Leary–Sant symptom index. *International Urogynecology Journal*. 2023;34(7):1437-1446.
21. McLatchie L, Sahai A, Caldwell A, Dasgupta P, Fry C. ATP shows more potential as a urinary biomarker than acetylcholine and PGE(2) , but its concentration in urine is not a simple function of dilution. *Neurourol Urodyn*. Mar 2021;40(3):753-762. doi:10.1002/nau.24620
22. Coyne, K. S. et al. The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: results from the Epidemiology of LUTS (EpiLUTS) study. BJU Int. 104, 352–360 (2009).