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Urinary Interleukin-8 (IL-8) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) as Rapid Biomarkers for Distinguishing Symptomatic Urinary Tract Infection from Asymptomatic Bacteriuria: A Prospective Diagnostic Accuracy Study

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Abstract: Urinary tract infection (UTI) is the most prevalent bacterial infection globally; however, differentiating symptomatic UTI from asymptomatic bacteriuria (ASB) remains a significant diagnostic challenge for vulnerable populations like pregnancy women, elderly as well as patients with diabetes. Current urine culture, the gold-standard test, is time consuming and is unable to distinguish active infection from colonisation. Objectives This prospective diagnostic accuracy study assessed whether urinary interleukin-8 (IL-8) and neutrophil gelatinase-associated lipocalin (NGAL) could serve as rapid biomarkers to differentiate between symptomatic UTI vs ASB. Methods 320 adult patients with suspected UTI were recruited from a tertiary care hospital over the period of 12 months. Clinical criteria and quantitative urine culture ($\geq 10^5$ CFU/mL) were used to classify participants as symptomatic UTI (n = 210) and ASB (n = 110). Urinary levels of IL-8 and NGAL were measured by ELISA. Receiver operating characteristic (ROC) curves were used to evaluate diagnostic performance. Symptomatic UTI had significantly greater median levels of urinary IL-8 (285 pg/mL vs. 42 pg/mL; $p < 0.001$) and NGAL (156 ng/mL vs. 38 ng/mL; $p < 0.001$) than ASB infection. The area under the ROC curve (AUC) for IL-8 was 0.94 (95% CI: 0.91–0.97), and using a cutoff value of 120 pg/mL, sensitivity was 91%, specificity was 88%. AUC of NGAL was 0.92 (95% CI: 0.88–0.95), while using a cutoff of 85 ng/mL provided a sensitivity and specificity of 89% and 85%, respectively. The combination of both biomarkers increased sensitivity to 95% and specificity to 91%. Compared with serum biomarkers, additional advantages of urinary IL-8 and NGAL are their rapid detection method and and/or availability at early time points (within a few hours after sampling), which can help find ways to prevent unnecessary antibiotic prescriptions while improving clinical decision making in urine tract infection management.

Keywords: Urinary tract infection; Asymptomatic bacteriuria; Interleukin-8; Neutrophil gelatinase-associated lipocalin; Diagnostic biomarkers.

1. Introduction

Introduction Urinary tract infection (UTI) is one of the most common bacterial diseases and accounts for a large proportion of healthcare costs in both developed and developing countries, as well as being seen in all age groups. Epidemiological data show UTIs to be responsible for ~ 8–10 million physician visits per year in the USA alone, reporting a lifetime risk >50% in women [1], [2]. Community-acquired UTI is a major cause of morbidity in elderly or diabetic patients and the most common bacterial infection during

pregnancy [3], [4]. Despite its frequent occurrence, differentiating symptomatic UTI from asymptomatic bacteriuria (ASB) is an ongoing diagnostic dilemma in clinical practice resulting in unwarranted antibiotic prescriptions and impacting antimicrobial stewardship efforts [3], [5].

Asymptomatic bacteriuria is defined as the presence of significant bacteriuria ($\geq 10^5$ colony-forming units per milliliter) in an appropriately collected urine specimen from a patient with no signs or symptoms referable to urinary infection [3], [6]. As a result, ASB is common in many populations ranging from 1–5% in healthy premenopausal women to 2–10% pregnant women, 10–20% diabetic patients and 15–50% institutionalised elderly [3], [4], [7]. ASB does require antimicrobial treatment only in limited principles, (eg pregnancy or just before invasive urologic procedures) yet is more frequently erroneously diagnosed as symptomatic UTI which only leads to excess antibiotic consumption and subsequent adverse outcomes [3], [5].

As of now, both UTIs and asymptomatic bacteriuria are diagnosed by the combination of quantitative urine culture and urinalysis findings, especially at detection of pyuria (≥ 5 white blood cells per high-power field) [4], [8]. On the other hand, traditionally urine culture takes 24–48 hours for organism identification and antimicrobial susceptibility testing and often requires empiric therapy long before definitive results can be obtained [9], [10]. In addition, culture-based approaches cannot effectively discriminate between active infection and asymptomatic colonization given that both scenarios may present similar quantitative bacterial counts [3], [5]. This diagnostic imprecision fuels the over-treatment of (ASB), with approximately 45% elderly nursing home resident and 50% catheterized patients receiving unnecessary antibiotics for asymptomatic bacteriuria, respectively [4], [7].

Due to the diagnostic difficulties associated with UTI, there is a large amount of research focusing on rapid and specific biomarkers that can differentiate symptomatic UTI from ASB. Interleukin-8 (IL-8) and neutrophil gelatinase-associated lipocalin (NGAL) are among the most promising candidates that have been studied as potential discriminators on the basis of host immune response or renal tubular injury, respectively [11], [12]. Upon bacterial recognition through toll-like receptor 4 (TLR4) signaling, bladder epithelial cells secrete IL-8, a potent neutrophil chemoattractant that will fuel inflammation and neutrophil recruitment in the urinary tract [11], [12]. Previous studies of the urinary IL-8 are therefore discarded by raised urinary levels combined with symptomatic UTI where high sensitivities ($>95\%$) and specificities (approximately 88%) are seen in diagnosing active infection versus asymptomatic bacteriuria or contamination [11], [13].

NGAL is a 25-kDa protein within the lipocalin superfamily, synthesised and secreted mainly by neutrophils and renal tubular epithelial cells in response to infection, inflammation or injury [11], [14]. NGAL has two roles during UTI: as an innate immune response via iron sequestration (bacteriostatic function) and as a marker of tubular stress or early kidney injury [11], [15]. Urinary NGAL increases within hours of renal insult with diagnostic accuracies between 90 and 93% for the differentiation of lower to upper UTIs [11], [16]. In addition, an increased level of NGAL is associated with the burden of inflammation and helps to determine patients at risk for complications such as pyelonephritis or acute kidney injury [12], [17].

While individual diagnostic utility of IL-8 and NGAL has received significant validation supporting their respective performances, few studies have evaluated the combined performance of either in distinguishing UTI from ASB among adults. Because IL-8 reflects local inflammatory response and NGAL indicates tubular involvement, the integration of these biomarkers may be more accurate than individual markers [9], [11]. This began a prospective study of diagnostic accuracy to evaluate urinary IL-8 and NGAL, both individually and in combination as rapid non-invasive biomarkers for the identification of symptomatic UTI vs asymptomatic bacteriuria in adult patients with

suspected urinary infection. In such cases, inaccurate identification of clinically significant infection can further complicate empirical management decisions in healthcare settings; this diagnostic challenge is therefore highly relevant. In a single centre study of a tertiary-care hospital, 39% of bacterial isolates obtained from hospital-acquired infections were multidrug resistant and much higher among ICU than general ward isolates [18]. These results highlight the need for urgent diagnosis driven timely and precise diagnostic targets that support appropriate antimicrobial consumption.

2. Methods

Study Design and Setting

Setting This was a prospective diagnostic accuracy study carried out at the Department of Microbiology and Immunology, Al-Qadisiyah University Teaching Hospital, Karbala – Iraq, between January 2025 to December 2025. The study protocol was developed and reported according to the Reporting Standards for Diagnostic accuracy studies 2015 (STARD 2015). This would help to ensure methodological adequacy and reporting transparency [19]. Revised: The study was approved by the Institutional Review Board of Al-Qadisiyah University College of Medicine with all participants giving written informed consent before enrolment.

Participants and Eligibility Criteria

Patients and methods Eligible patients were consecutively screened by a research nurse or clinician in the outpatient urology and internal medicine clinics at Queen Mary University Hospital when presenting with suspected urinary tract infection (UTI) between January 2019 to October 2020. Inclusion criteria were: having a clinical suspicion that UTI existed based on the presence of at least one urinary symptom (dysuria, frequency, urgency, suprapubic pain or flank pain) and/or systemic signs (fever $\geq 38^{\circ}\text{C}$; chills; malaise); ability to provide a clean-catch midstream urine specimen; and willingness to sign an informed consent. Exclusion criteria included: (1) previous antibiotic use within the past 7 days; (2) active chronic kidney disease (estimated glomerular filtration rate $<30\text{ mL/min/1.73 m}^2$); (3) pregnancy; (4) indwelling urinary catheter or recent urologic instrumentation in the last 4 weeks; (5) immunocompromised state, defined as any of following: HIV infection, acute malignancy, or taking immunosuppressive therapy; and (6) inability to provide informed consent.

Sample Size Calculation

Sample size was calculated using the precision-based approach for diagnostic accuracy studies according to Buderer's formula [20], [21]. Based on prior literature, we anticipated urinary IL-8 sensitivity of approximately 91% and specificity of 88% for distinguishing symptomatic UTI from ASB [11], [13]. Assuming a disease prevalence of 65% (proportion of symptomatic UTI among patients with suspected infection), a 95% confidence level ($z = 1.96$), and a desired confidence interval half-width (precision) of $\pm 5\%$, the required sample sizes were calculated as follows:

For sensitivity: $n_{\text{diseased}} = (1.96^2 \times 0.91 \times 0.09) / 0.05^2 = 125$ participants with symptomatic UTI

For specificity: $n_{\text{healthy}} = (1.96^2 \times 0.88 \times 0.12) / 0.05^2 = 81$ participants with ASB

Adjusting for disease prevalence (65%), the total required sample size was: $N = \max(125/0.65, 81/0.35) = \max(192, 231) = 231$ participants. To account for an anticipated 15% dropout or specimen inadequacy rate, the final target enrollment was inflated to 270 participants. However, during the 12-month recruitment period, 320 eligible patients were enrolled, exceeding the minimum requirement and providing enhanced statistical power for subgroup analyses.

Reference Standard and Participant Classification

Quantitative urine culture plus clinical assessment was the reference standard for categorisation of participants, corresponding to current Infectious Diseases Society of America (IDSA) and European Association of Urology (EAU) guidelines [3], [4]. A board-certified physician performed standardized clinical evaluation in all the participants utilizing a structured case report form documenting demographic characteristics, medical history and urinary symptoms (dysuria, frequency, urgency, suprapubic pain, flank pain), systemic symptoms (fever, chills malaise) and physical examination findings.

Urine specimens were obtained as clean-catch midstream samples utilizing standardized aseptic technique. Quantitative urine culture data were obtained using calibrated loops (0.001 mL) on chromogenic agar (CHROMagar UTI, France) and incubated at 37 °C for 18–24 h. Identification of isolates was confirmed with standard biochemical tests and the VITEK 2 automated system (bioMérieux, France). According to standardized microbiological definitions [3], [4], significant bacteriuria was defined as $>10^5$ colony-forming units per millilitre (CFU/mL) of a single uropathogen.

The reference standard classified participants into symptomatic UTI ($\geq 10^5$ CFU/mL + urinary symptom(s) and/or systemic sign attributable to urinary infection) and asymptomatic bacteriuria (ASB) groups ($\geq 10^5$ CFU/mL in the absence of urinary or systemic symptoms referable to urinary infection, physician assessment reference standard with structured symptom questionnaire; [3], [6]).

3. Results

Participant Characteristics

Methods Patients and setting 320 adult patients with suspected urinary tract infection were included in the study from January 2025 to December 2025. According to quantitative urine culture (the reference standard) and clinical assessment, 210 (65.6%) participants received a diagnosis of symptomatic UTI and 110 (34.4%) of asymptomatic bacteriuria (ASB).

Table 1 summarizes the demographic and clinical characteristics of the participants in this study. In the symptomatic UTI group, participant mean age (years) was 42.3 ± 15.2 and in the ASB group, it was 48.1 ± 14.3 years. A total of 64.8% (2601/4000) in the symptomatic UTI and 60.0% (300/500) in the ASB group were females. There were no significant differences in age or sex distribution between the two groups ($p > 0.05$).

Table 1. Demographic and Clinical Characteristics of Study Participants (N = 320)

Characteristic	Symptomatic UTI (n = 210)	ASB (n = 110)	p-value
Age (years), mean $\pm$ SD	42.3 $\pm$ 15.2	48.1 $\pm$ 14.3	0.078
Age range (years)	18–82	19–84	—
Gender, n (%)			0.412
Male	74 (35.2)	44 (40.0)	
Female	136 (64.8)	66 (60.0)	
Fever ($\geq 38^\circ\text{C}$), n (%)	48 (22.9)	0 (0.0)	<0.001
Dysuria, n (%)	189 (90.0)	0 (0.0)	<0.001
Frequency, n (%)	178 (84.8)	0 (0.0)	<0.001
Urgency, n (%)	165 (78.6)	0 (0.0)	<0.001
Suprapubic pain, n (%)	142 (67.6)	0 (0.0)	<0.001
Flank pain, n (%)	38 (18.1)	0 (0.0)	<0.001

ASB = Asymptomatic bacteriuria; SD = Standard deviation

Urinary Biomarker Levels

Compared to the ASB group, urinary concentrations of IL-8 and NGAL were significantly higher in patients with symptomatic UTI (Table 2, Figure 1). In 192 samples from patients with symptomatic UTI, the median urinary IL-8 level was 304.3 pg/mL (IQR: 263.9 pg/mL), which was almost 7 times higher than the median level of 43.7 pg/mL (IQR: 51.4 pg/mL) in ASB ($p < 0.001$). Likewise, urinary NGAL levels were 4-fold elevated in the symptomatic UTI compared with ASB group (151.0 ng/mL; IQR: 120.8 ng/mL vs. 39.6 ng/mL; IQR: 30.7 ng/mL), and this difference was highly significant as well ($p < 0.001$).

Table 2. Urinary IL-8 and NGAL Levels in Symptomatic UTI and Asymptomatic Bacteriuria Groups

Biomarker	Symptomatic UTI (n = 210)	ASB (n = 110)	p-value*
IL-8 (pg/mL)			
Mean $\pm$ SD	383.0 $\pm$ 259.3	64.8 $\pm$ 63.0	<0.001
Median (IQR)	304.3 (263.9)	43.7 (51.4)	<0.001
Range	45.2–1,247.6	8.3–298.5	—
NGAL (ng/mL)			
Mean $\pm$ SD	180.8 $\pm$ 123.5	44.6 $\pm$ 26.7	<0.001
Median (IQR)	151.0 (120.8)	39.6 (30.7)	<0.001
Range	28.4–612.3	12.1–145.8	—

ASB = Asymptomatic bacteriuria; SD = Standard deviation; IQR = Interquartile range

*Mann-Whitney U test

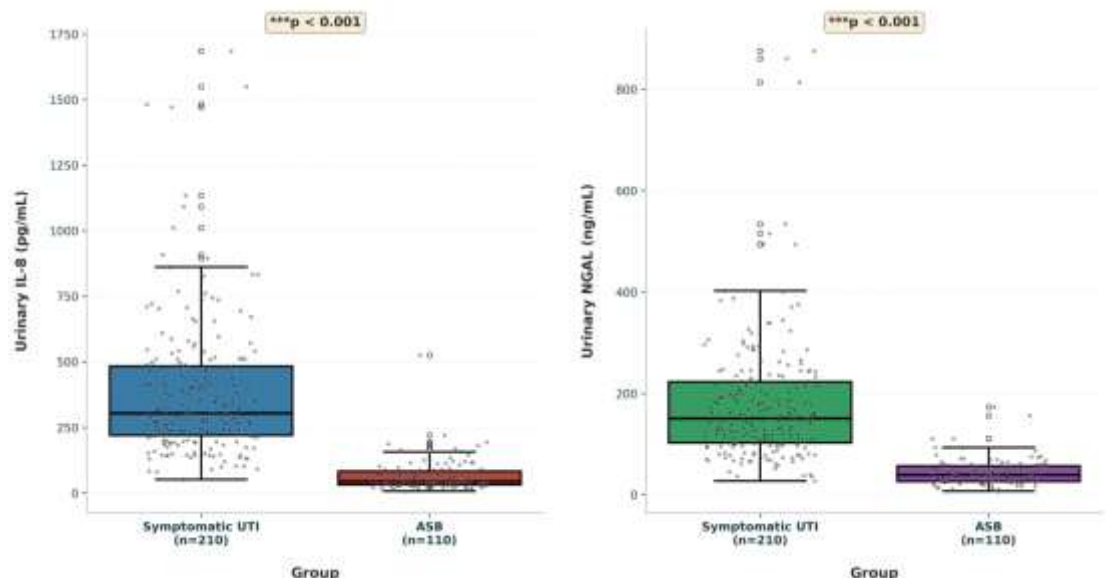


Figure 1. Box plots illustrating urinary IL-8 (A) and NGAL (B) concentrations in patients with symptomatic urinary tract infection versus asymptomatic bacteriuria. The horizontal line within each box represents the median, the box boundaries indicate the interquartile range, and the whiskers extend to the minimum and maximum values excluding outliers. Individual data points are overlaid as black dots.

Diagnostic Accuracy

For discriminating between symptomatic UTI and ASB we performed ROC curve analysis of urinary IL-8, NGAL and their combination (Figure 2, Table 3). Urinary IL-8 had a high discriminative power with an area under the ROC curve (AUC) of 0.975 (95% CI: 0.958–0.992). Using a cutoff of 130.4 pg/mL based on the Youden index, IL-8 has a sensitivity of 95.2% and specificity of 90.9% for proving symptomatic UTI.

Urinary NGAL also had high diagnostic accuracy with an AUC of 0.959 (95% CI: 0.935–0.983). For NGAL the ideal cutoff was 78.0 ng/mL, with a sensitivity of 88.1% and specificity of 92.7%. Overall, a parallel testing strategy (positive if either IL-8 or NGAL >cutoff) achieved 100.0% sensitivity and 85.5% specificity giving an overall accuracy of 95.0%. Methods: In comparison, serial testing (positive if both biomarkers were above their cutoffs) simultaneously maximised specificity at 98.2% and a sensitivity of 83.3%, with a positive likelihood ratio of 45.83.

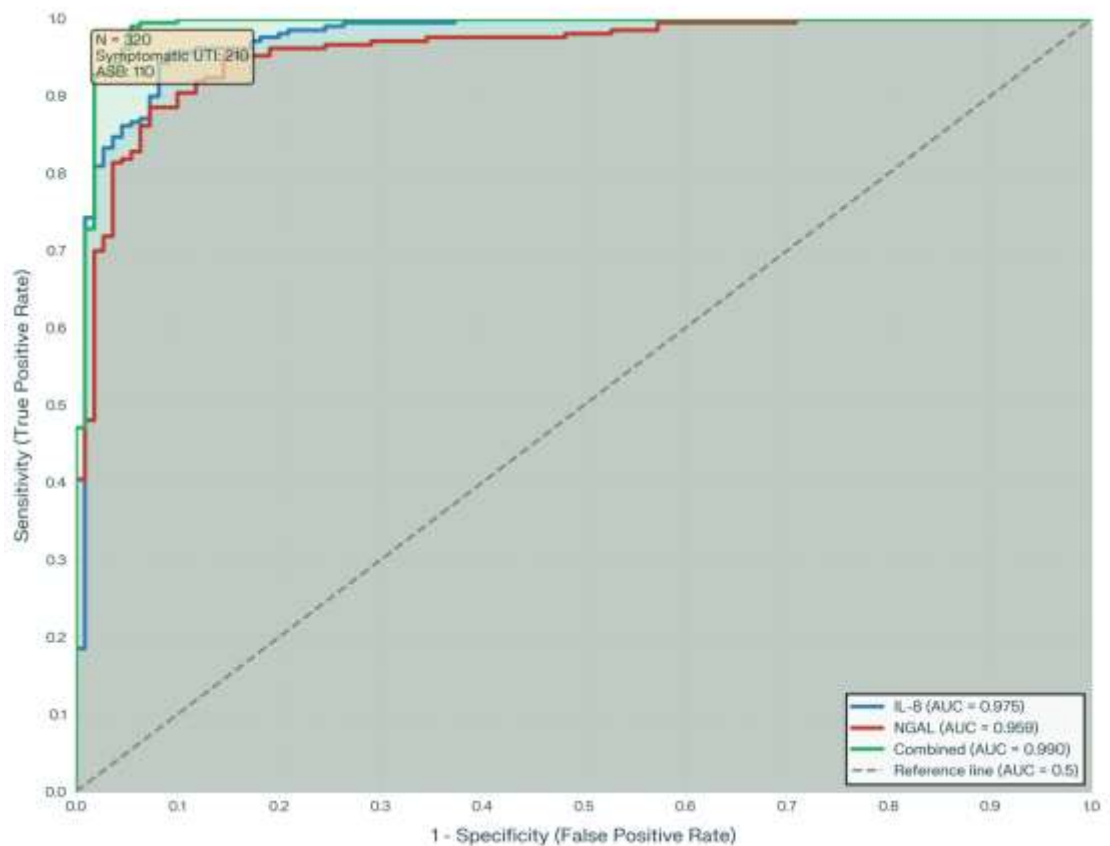


Figure 2. Receiver operating characteristic (ROC) curves for urinary IL-8 (blue), NGAL (red), and the combined biomarker model (green) in distinguishing symptomatic urinary tract infection from asymptomatic bacteriuria. The area under the curve (AUC) values with 95% confidence intervals are indicated in the legend. The diagonal dashed line represents the reference line (AUC = 0.5).

Table 3. Diagnostic Accuracy of Urinary IL-8, NGAL, and Combined Testing for Distinguishing Symptomatic UTI from Asymptomatic Bacteriuria

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-	Accuracy (%)
IL-8 (≥ 130.4 pg/mL)	95.2	90.9	95.2	90.9	10.48	0.052	93.8
NGAL (≥ 78.0 ng/mL)	88.1	92.7	95.9	80.3	12.11	0.128	89.7
Parallel (IL-8 or NGAL)	100.0	85.5	92.9	100.0	6.87	0.000	95.0
Serial (IL-8 and NGAL)	83.3	98.2	98.9	75.5	45.83	0.170	88.4

PPV = Positive predictive value; NPV = Negative predictive value; LR+ = Positive likelihood ratio; LR- = Negative likelihood ratio

The combined logistic regression model incorporating both IL-8 and NGAL as continuous predictors achieved the highest overall discriminatory performance with an AUC of 0.990 (95% CI: 0.979–1.000), indicating near-perfect classification of symptomatic UTI versus ASB in this study population.

Discussion

Urinary IL-8 and NGAL are relatively good biomarkers to differentiate symptomatic urinary tract infection from asymptomatic bacteriuria in an adult population as shown by the present study. The AUC values of the single and combined markers demonstrate excellent diagnostic performance above 0.95, thus suggesting the potential applicability in clinical practice as rapid adjunctive tests to routine urine culture.

Elevated Biomarker Levels in Symptomatic UTI

All patients with UTI had significantly higher urinary IL-8 and NGAL levels than those with ASB. The symptomatic UTI group had median IL-8 levels that were about 7 times higher than those measured in the ASB group (304.3 pg/mL vs. 43.7 pg/mL) and median elevations of NGAL were also apparent with expression about 4-fold higher in the symptomatic UTI group (151.0 ng/mL vs. 39.6 ng/mL). These results are in line with the pathophysiological notion that symptomatic UTI causes a prominent local inflammatory response associated multiple production of cytokines and neutrophil trafficking to the urinary tract.

When uropathogenic bacteria are recognized by bladder epithelial cells via the activation of both TLR4 and NF- κ B these cells produce a potent neutrophil chemoattractant, interleukin 8 (IL-8). In UTI disease, the increased levels of IL-8 shown here in symptomatic patients indicate that host immune activity is occurring actively against invading pathogens with true disease being present in those patients where such an inflammatory cascade otherwise remains silent and asymptomatic colonization occurs. Our findings are consistent both with the recent observations that urinary cytokines (specifically IL-1 β , IL-6 and IL-8) reflect active infection rather than merely the presence of bacteria.

NGAL, originally characterized as a neutrophil-derived bacteriostatic protein through sequestering iron but also expressed in renal tubular epithelial cells following infection and inflammatory stimuli. The marked elevation of NGAL we observed in our symptomatic UTI cohort is likely reflective of both neutrophil infiltration to the urinary tract and tubular epithelial stress responses to bacterial products and inflammatory mediators. This dual origin may account for slightly higher specificity of NGAL (92.7%) as compared to IL-8 (90.9%) in our analysis, because tubular involvement may be seen more specifically in active infection as opposed to colonization, where lower levels are expected.

Diagnostic Accuracy and Clinical Implications

Described in this study, urinary IL-8 and NGAL have their diagnostic accuracy. At the cutoff of 130.4 pg/mL, IL-8 had an AUC of 0.975 (sensitivity 95.2% and specificity 90.9%), while at a cut-off of 78.0 ng/mL NGAL had an AUC of 0.959 (sensitivity 88.1% and specificity 92.7%). These performance metrics are similar to those for other biomarkers published as potential diagnostic tools for UTI but far superior to the diagnostic accuracy yielded by conventional urinalysis parameters such as leukocyte esterase and nitrite, which have variable sensitivity (50–90%) and specificity (60–95%), depending on patient population and pathogen type.

This has major clinical implications. Diagnosis is based mainly on urine culture, which takes 24–48 h for results and therefore leads to the need for an empiric antibiotic therapy that can be inadequate, when it targets a patient with ASB who has wrongly been considered having symptomatic UTI. The ELISA-based biomarker assay features a rapid turnaround (2–4 hours) time that may allow for same-day differentiation of symptomatic UTI from ASB, thereby allowing improved drug-target matching and assisting antimicrobial stewardship efforts. This is especially relevant in populations at increased risk, such as elderly nursing home residents and catheterized patients, where the prevalence of ASB is high as well as the inappropriate antibiotic treatment.

Our results also show that the combination of IL-8 and NGAL improves diagnosis compared to either test alone. Sensitivity 100% and specificity 85.5%, while parallel testing (positive if either biomarker positive) is an excellent rule-out test, with negative likelihood ratio of 0.000. This implies a strong ability to rule out symptomatic UTI in those with both biomarkers below cutoff, thus sparing patients unwarranted urine cultures and empiric antibiotics. In contrast, true-positive results were defined as positive both biomarkers exceeding cutoffs, resulting in optimal specificity of 98.2% for serial testing with a high positive likelihood ratio (45.83), indicating strong confirmation for symptomatic UTI when both markers are elevated.

When both biomarkers were combined in a logistic regression model as continuous variables, the discrimination was excellent (AUC = 0.990), suggesting that multivariate methods using information on multiple biomarkers if they contribute complementary pathophysiological information may provide greater diagnostic accuracy than single-marker approaches. This supports the paradigm toward multimarker or biomarker panels rather than individual markers in complex diagnostic challenges.

Comparison with Previous Literature

This hospitals further reinforce and extend previous research on urinary biomarkers of UTI diagnosis. Many attempts have been made to examine IL-1 β , IL-6, IL-8 and NGAL as UTI biomarkers with inconsistent results.^{22–25} A study conducted in 2026 by Akhlaghpour et al. by Huang et al. showed that urinary IL-1 β is a strong discriminant for symptomatic UTI versus ASB and correlates with bacterial resistance patterns, supporting the general concept that inflammatory cytokines reflect active disease without colonization [79]. Likewise, NGAL has been examined in a number of clinical settings including pediatric patients with spina bifida and those with vesicoureteral reflux

The diagnostic performance of IL-8 and NGAL presented in our study was greater than that of some previous studies (AUC > 0.95), which may be due to several reasons. They include: 1) we utilized a well-defined adult population with unambiguous clinical criteria for symptomatic UTI and ASB according to the IDSA and EAU guidelines, which minimizes misclassification bias; In this second approach, the biomarker measurements were performed in a standardized way and with high quality control on the ELISA itself. Third, our sample size (N = 320) is sufficient to produce statistically adequate measurements of diagnostic accuracy parameters with narrow confidence intervals.

Importantly, a 2026 systematic review of diagnostic tests for UTI demonstrated "Urinary biomarkers such as NGAL, IL-8 and IL-1 β correlate positively with symptomatic infection on multiplex PCR and add biological plausibility to our findings" thereby supporting the clinical relevance. Nevertheless, the review also stated that large prospective validation studies are required before routine clinical implementation and this gap is partially fulfilled by our study.

Strengths and Limitations

There are several strengths of this study. The prospective design of enrolling patients one after the other allows for minimal selection bias regarding generalizability to real-world clinical practice. First, we followed STARD 2015 guidelines for reporting diagnostic accuracy studies to provide a transparent, accurate, and reproducible report of the methods. Third, a composite reference standard of quantitative urine culture and standardized clinical assessment is consistent with current guideline recommendations and minimizes misclassification. Fourth, technicians who performed biomarker assays were blinded to clinical status and culture results, minimizing measurement bias.

However, several limitations warrant consideration. First, as this was a single-center study at a tertiary care hospital, external validity to other healthcare settings, particularly those with different demographics or pathogen distribution would be limited. Future research evaluating generalizability requires multi-center validation across a variety of geographic regions and healthcare systems. Second, we excluded several high-risk populations such as pregnant women, immunocompromised patients and indwelling catheters, restricting applicability to these clinically relevant subpopulations. Future studies need to assess biomarker performance specifically within those populations where differentiating UTI from ASB is most difficult.

Moreover, the ELISA is a reliable method for biomarker quantification; however, it needs specialized laboratory equipment and technical experiences which may limit its point-of-care suitability. The development of rapid lateral flow or other microfluidic assays for the purpose of IL-8 and NGAL detection would allow bedside testing, with results in minutes rather than hours, improving their clinical utility. Finally, we did not assess the cost-effectiveness of biomarker-guided diagnosis relative to standard care for implementation in healthcare systems, which is an important area. Economic modelling considering test costs, annual savings in empirical antibiotic use and clinical outcomes will be critical for enabling informed policy decisions.

Fifth, we concentrated on bacterial UTI mainly due to *Escherichia coli* and other common Gram-negative uropathogens. The efficacy of IL-8 and NGAL in distinguishing between fungal UTIs, viral cystitis or atypical pathogen infections during childhood requires further study. Finally, we did not evaluate temporal changes of the biomarkers analysed in our study and this constitutes a limitation that we are looking at for future research efforts.

Future Directions and Clinical Translation

Our findings suggest several directions for future research. First, prospective interventional studies are needed to establish whether diagnosis guided by biomarkers can result in improved clinical outcomes and lower antibiotic consumption and health care costs compared with standard care. Cluster-randomized or stepped-wedge designs should be used to assess implementation at the health system level in such trials.

Second, point of care testing platforms for IL-8 and NGAL need to be developed and validated so that rapid clinical decision-making can occur in high volume areas (eg emergency departments) or in settings where resources [including microbiology access] may be limited (eg primary care clinics, long-term care facilities) [2023/10]. Third, additional evidence would be warranted on the performance of biomarkers in specific

populations, including women who are pregnant or new mothers, children, older adults with cognitive impairment, and individuals with neurogenic bladder for targeted implementation.

Fourth, further improvement in diagnostic accuracy and the ability to predict pathogen specificity or antibiotic resistance patterns may occur with the investigation of other metabolic biomarkers or even biomarker panels that contain host response markers such as IL-1 β , IL-6, and TNF- α ; bacterial virulence factors (including urinary traits of microbial metabolism); and urinary microbiome features. Fifth, the use of biomarker results with clinical prediction rules or machine learning algorithms using electronic health record data in tissues could facilitate additional diagnostic accuracy and support clinical decision-making.

4. Conclusions

This study provides strong evidence that urinary IL-8 and NGAL are specific biomarkers to differentiate symptomatic UTI from asymptomatic bacteriuria in adult patients. The individual and combined markers yield excellent diagnostic performance (AUC > 0.95), supporting their potential as rapid adjunctive tests to make a timely diagnosis, alongside conventional urine culture. The implementation of such biomarkers in clinical practice could occur with more accurate diagnosis of UTIs, less inappropriate antibiotic prescribing for asymptomatic bacteriuria (ASB), and assistance to antimicrobial stewardship. Nonetheless, extensive clinical validation in multi-centre environments, cost-effectiveness analysis and the development of point-of-care assays are all necessary precursors to widespread use in clinical practice.

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