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URINARY HOST IMMUNE RESPONSE BIOMARKERS IN URINARY TRACT INFECTIONS: ASSOCIATIONS WITH URINE CULTURE AND CLINICAL OUTCOMES

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ABSTRACT

Objective. To examine associations of urinary host immune response proteins with urine culture, symptomatic urinary tract infection diagnosis, and subsequent clinical outcomes in adults. Methods. Available full texts were critically appraised within a prespecified protocol. PubMed, eLibrary, ClinicalTrials.gov and WHO ICTRP were searched, with targeted full-text retrieval and reference checking. Scopus and Web of Science searches could not be performed; Cochrane records could not be exported and Google Scholar access was blocked. Of 4,778 combined records, 238 cross-database duplicates were automatically merged. Screening of the remaining 4,540 records is incomplete; this manuscript does not represent a completed systematic review. Results. Eleven core full-text reports were examined across nine report families. Three reports shared the same symptomatic sample. Diagnostic performance varied with the population and reference definition. Diagnostic discrimination did not establish prediction of recurrence. Serial biomarker changes were distinguished from baseline prognostic associations. Aggregate-data reanalysis of one selected sample gave 84.0% sensitivity and 91.2% specificity for the ≥2-marker rule. These are study-specific estimates, not pooled results. Conclusion. Urinary immune response proteins may complement microbiological testing, but the assessed evidence does not establish a universal threshold or a validated standalone predictor of treatment failure. External validation and evaluation of incremental clinical value are required.

Keywords: urinary tract infection; urine culture; host response; cytokines; interleukin-8; lipocalin-2; recurrence; biomarkers.

INTRODUCTION

Urine culture and assessment of the host response address different questions. Culture detects microorganisms under specific cultivation conditions, whereas host-response assessment characterizes inflammation in the urinary tract. A positive culture without compatible clinical findings cannot automatically be equated with symptomatic infection, and an elevated cytokine concentration does not establish a bacterial cause of symptoms. Clinical interpretation requires consistency between microbiological findings, symptoms, sampling conditions, and previous treatment [2, 13].

Interleukin-8 (IL-8), or CXCL8, participates in neutrophil recruitment; lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL), contributes to restricting bacterial access to iron; and heparin-binding protein (HBP) is released by neutrophils. These mechanisms explain interest in urinary markers but do not guarantee specificity for infection: the signal depends on the cellular composition of urine, renal tissue status, and local inflammation [10, 11, 17].

Existing reviews already address biomarker-based UTI diagnosis and the biological mechanisms underlying recurrence. The contribution of the present analysis therefore lies in distinguishing three questions: association with microbial growth, recognition of symptomatic infection, and prediction of an event that has not yet occurred. A claim that this is the first review on the topic would be unjustified [5, 15].

This study aims to compare these questions using assessed full texts and to define the limits of clinical interpretation.

MATERIALS AND METHODS

Protocol version 1.0 was prepared on November 4, 2026, after preliminary examination of the literature and before formal selection; it was not registered. The principal eligibility criteria were adults aged at least 18 years with suspected or confirmed symptomatic UTI; measurement of a soluble urinary immune or inflammatory protein; and an association with urine culture, a diagnostic classification incorporating culture, or a subsequent clinical outcome. Asymptomatic bacteriuria was eligible as a comparator. Studies confined to children, animals, or serum markers, individual case reports, and protocols without results were excluded from the primary synthesis. The prespecified principal clinical outcome was treatment failure at days 7–14. Recurrence after recovery within 30 and 90 days was distinguished from persistence. Prognostic interpretation required biomarker measurement before the event. Existing bacteremia was considered a concurrent indicator of severity,

and post-treatment measurements were treated as serial measurements. Full-text availability was mandatory; abstracts were not used to transfer numerical results into the synthesis.

The PubMed search yielded 3,909 records, and 36 eLibrary queries yielded 869 unique identifiers. Deduplication used normalized titles, publication years, and first authors while preserving links between sources. Potential matches that did not satisfy all conditions were retained for checking. Multiple reports from the same sample were sought separately: a shared cohort does not justify deleting a substantively different publication but does preclude counting participants repeatedly.

Four ClinicalTrials.gov queries yielded 69 unique registrations; six WHO ICTRP records added no new identifiers.

Full-text analysis of the core set extracted study design, sample size, analytical material, infection definition, measurement timing, and estimates of diagnostic accuracy or associations with outcomes. The minimum age in Liu et al. was not reported; these findings were retained for critical discussion. Available reviews, a protocol, and technological studies were considered separately. The authors conducted the assessment jointly.

Published 2 × 2 tables were reanalyzed to calculate sensitivity (Se), specificity (Sp), and exact Clopper–Pearson 95% confidence intervals (CIs). Logarithmic intervals were used for likelihood ratios (LRs) and diagnostic odds ratios (DORs). The nested rules requiring at least two positive markers or all three positive markers were compared using exact McNemar tests and Holm adjustment for two comparisons. Separate proportions of persistence and recurrence, extreme scenarios for two missing outcomes, and illustrative post-test probabilities were calculated. These additional analyses were developed after examination of the data and were exploratory. Subgroups with repeated episodes were analyzed descriptively only. Diagnostic estimates were preliminarily assessed against QUADAS-3 domains, and prognostic associations against QUIPS domains.

RESULTS AND DISCUSSION

The clearest association with microbial density was reported in a series of publications involving 583 samples from symptomatic patients aged 60 years or older. Median NGAL and IL-8 concentrations increased across the categories of no growth, 10^4–$<10^5$, and $\geq 10^5$ CFU/mL. However, the Parnell report, Haley comparative analysis, and Akhlaghpour study with additional asymptomatic controls used the same original symptomatic sample: recruitment dates, sample sizes, laboratory, and patient characteristics matched. These represent three reports from one research program rather than three independent confirmations [2, 9, 14].

In Akhlaghpour et al., the diagnostic comparison comprised 351 symptomatic cases positive by both culture and multiplex polymerase chain reaction (M-PCR) and 228 asymptomatic participants. A rule requiring at least two positive markers had 84.0% sensitivity and 91.2% specificity (Table 1). The remaining symptomatic samples were excluded from the principal accuracy comparison. Such uncertain cases often require additional testing in practice; the performance of this selected comparison therefore cannot be extrapolated without validation to all patients with dysuria [2].

Table 1. Selected diagnostic estimates from assessed full texts

Study	Target and sample	Result	Limit of applicability
Akhlaghpour 2024 [2]	Symptomatic UTI vs asymptomatic controls; 351/228	≥2 markers: Se 84.0%; Sp 91.2%	Cases positive by both culture and M-PCR
Gadalla 2019 [7]	Culture; 183 women; test subset 55	MMP9/NGAL/CXCL8/IL-1β: AUC 0.81 (95% CI 0.68–0.94)	Internal evaluation
Ebrahimzadeh 2024 [4]	Current recurrent UTI; 23 + 23	IL-8: Se 73.9%; Sp 95.7%; AUC 0.885	Controls without UTI history
Kjölvmark 2014 [11]	Definite UTI vs no UTI	HBP 30 ng/mL: Se 89.2%; Sp 89.8%	45 probable UTI cases excluded from ROC comparison
Hrbáček 2025 [10]	Pyelonephritis/complicated UTI vs cystitis	Lcn2/Cr + Cu/Cr: Se 78%; Sp 65%	Small groups; data-derived thresholds

Note. Se, sensitivity; Sp, specificity; AUC, area under the receiver operating characteristic curve; Lcn2, lipocalin-2; Cr, creatinine; Cu, copper. Estimates are not pooled and address different diagnostic targets.

In the Haley analysis, several markers were also elevated in samples positive by M-PCR but negative by standard culture. This supports the hypothesis of clinically relevant inflammation in some culture-negative cases but does not establish causation for each detected microorganism. Competing explanations, including previous treatment, cultivation conditions, colonization, and noninfectious inflammation, require separate examination [9].

In the POETIC diagnostic analysis, Gadalla et al. studied 183 women with symptoms of uncomplicated UTI. A four-marker combination distinguished positive from negative cultures in a small held-out subset of the same sample. Changing culture interpretation rules changed the optimal set of immunological predictors. No external validation was performed. Internal evaluation reduces the risk of optimism but does not replace validation in another institution and a different clinical population [7].

Among postmenopausal women, Ebrahimzadeh et al. reported high specificity for an IL-8 model identifying a current recurrent episode. However, controls were women without a history of UTI, and the multiplex diagnostic component included only 23 patients per group. This design assesses discrimination between relatively contrasting groups and may

overestimate utility in more complex differential diagnosis. Combinations with PGE2 were considered separately because PGE2 is a lipid mediator rather than a protein marker covered by the protocol [4].
The emergency department study by Middelkoop provides a different picture: 176 patients contributed 196 episodes, with urinary markers available for 179 episodes. At similar sensitivity, these markers did not show a consistent advantage over routine tests. Findings differed according to catheterization and leukopenia. High accuracy in selected cases compared with healthy controls therefore does not establish incremental value in a clinically heterogeneous population [13].
Distinguishing cystitis from upper urinary tract involvement requires separate validation. In the study of 95 adults by Hrbáček et al., combining lipocalin-2 with copper produced moderate performance in distinguishing pyelonephritis/complicated UTI from cystitis. Lipocalin-2 alone did not reliably separate these clinical forms. The findings illustrate the potential value of combining host response with another biological characteristic but derive from small groups with different age and sex distributions [10].
The Russian study by Pavlov and Korenkov demonstrates that IL-8 depends on the urine sampling site: bladder and ureteral urine were assessed separately in women with nephrolithiasis. This is relevant to biomarker interpretation but does not justify extrapolating the observed concentrations to routine midstream urine. The full text does not adequately describe how classification was linked to culture; the study was therefore not used as evidence of diagnostic accuracy under the criteria of the primary synthesis [1].
Calprotectin also requires a distinction between bacteriuria and symptomatic infection. In Waldecker-Gall et al., the AUC was 0.70 for calprotectin and 0.71 for pyuria. The absence of a statistically significant difference between AUCs does not demonstrate noninferiority: this requires a prespecified margin and an appropriate design. Ambiguities in the infection definition and age distribution leave the final eligibility of this publication unresolved [18].
Prognostic interpretation is particularly vulnerable to conflating the event with the timing of the clinical visit. Czaja et al. followed 104 women daily and recorded 38 E. coli recurrences; a complete cytokine series was available for 18 episodes. IL-6 and IL-8 rose sharply on the day of the recurrence visit rather than consistently over preceding days. Some participants already had symptoms before the visit. These findings therefore describe an evolving episode rather than demonstrate the ability to identify a risk group in advance [3].
In Ebrahimzadeh et al., subsequent symptomatic, culture-positive episodes were monitored for 12 months. IL-8 and IL-13 were not significantly associated with a new recurrence despite diagnostic discrimination between groups. This directly illustrates why a diagnostic AUC cannot serve as a measure of future risk. The longer follow-up period is also not equivalent to the prespecified 30- and 90-day windows [4].
Liu et al. followed 40 women for up to three months. Nine experienced persistence and ten experienced recurrence; baseline NGAL was highest in those with persistence. The study does not justify combining these conditions into a new outcome for this review. Some participants received antibiotics before the initial visit, and early repeat events were not always confirmed by a new culture. Persistently elevated NGAL after treatment may accompany an ongoing process; this is not equivalent to pretreatment baseline prediction. The minimum participant age requires clarification, so inclusion in a strictly adult synthesis remains provisional [12].

Table 2. Timing of biomarker measurement relative to clinical events

Report	Timing and outcome	Supported interpretation
Czaja 2009 [3]	Daily samples; cytokines before and on the recurrence visit	Dynamics of an evolving episode; no validated prediction
Ebrahimzadeh 2024 [4]	Baseline IL-8/IL-13; recurrence over 12 months	No significant association; small cohort
Liu 2024 [12]	Baseline, week 1, months 1 and 3; persistence distinguished from recurrence	Exploratory NGAL finding; pretreatment and outcome definitions limit prediction
van der Starre 2015 [17]	Proteins measured in the presence of existing bacteremia	Concurrent association with severity, not a future outcome

The study by van der Starre further illustrates the limits of extrapolating local responses to systemic complications. Among patients with febrile E. coli UTI, urinary IL-6, IL-8, and LL-37 did not differ significantly between those with and without bacteremia. Measurements characterized an already existing condition; even an observed association could not have been interpreted as prediction of subsequent bacteremia [17].
The principal limitations of diagnostic estimates were selection of contrasting cases and controls, derivation of thresholds in the same data, exclusion of indeterminate cases from ROC analyses, and lack of independent validation. Prognostic findings were limited by small samples, pretreatment, incomplete control of confounding, and inconsistent definitions of recurrence. Commercial affiliations or funding do not themselves demonstrate bias; specific design and analytical decisions must be assessed [2, 4, 7, 11, 12].
In a qualitative robustness assessment, the three PATH583 reports were treated as one group; serial measurements were excluded from arguments about baseline prediction; and bacteremia at inclusion was distinguished from subsequent events. After these distinctions, support for established prediction of treatment failure was weaker, whereas the observation that several markers are associated with local inflammation remained. Numerical comparisons of nested diagnostic rules and missing-outcome scenarios were also performed; no pooled between-study effect was calculated.
Devices reproducing concentrations of several markers require an independent clinical reference standard. In USENSE, training labels were linked to ELISA and thresholds of the biomarkers themselves. High agreement with these labels characterizes technological classification and does not establish subsequent recurrence rates in patients [8]. A separate

discrepancy was identified between IL-8 in the title and IL-18 in the methods and tables of the postoperative urosepsis study; the corresponding numerical findings were not transferred to the main analysis [16].
The assessed Akhlaghpour tables allowed recalculation of the accuracy of five rules in the same selected sample of 579 participants. For the ≥2-marker rule, sensitivity was 84.0% (95% CI 79.8–87.7), specificity 91.2% (86.8–94.6), LR+ 9.58 (6.29–14.60), LR− 0.175 (0.137–0.223), and the DOR 54.79 (31.91–94.06). The DOR does not characterize the risk of subsequent recurrence [2].
Compared with requiring all three markers, the ≥2-marker rule increased sensitivity by 17.9 percentage points (95% CI 14.1–22.4), whereas the stricter rule increased specificity by 6.1 points (3.4–10.1). Exact Holm-adjusted p values were 4.34×10^{-19} and 0.000122, respectively. These exploratory comparisons reflect the expected trade-off between nested thresholds and do not establish clinical superiority. A sensitivity analysis of the two unknown outcomes in Liu et al. gave a recurrence proportion in the original group ranging from 23.8% to 28.6%. These are scenario bounds rather than observed events among participants lost to follow-up [12].
Tables from another publication were presented separately with conditional binomial CIs because individual data needed to adjust for repeated episodes were unavailable. Repeated tests of one sample and overlapping subgroups were not treated as independent studies. Detailed formulas, input counts, and a plot of individual estimates are provided in the statistical supplement [13].

CONCLUSION

The assessed full texts indicate that urinary NGAL, IL-8, IL-1β, and HBP may reflect infectious and inflammatory host responses. Their clinical value nevertheless depends on whether the aim is to detect bacteriuria, establish symptomatic infection, or predict a future outcome. These tasks cannot be combined into a single accuracy measure. An association with culture does not make a biomarker a standalone basis for treatment, and persistently elevated concentrations after therapy do not establish baseline predictive ability. Neither a universal threshold nor reliable prediction of treatment failure was established in the assessed set. Definitive systematic review conclusions require completion of searching, selection, and independent verification.

ADDITIONAL INFORMATION

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