Beyond the cloudiness in urinary tract infection: definitions, diagnostics, and strategies for prevention

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Chapter 5

Diagnostic accuracy of urine biomarkers for urinary tract infection in older women: a case-control study

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Abstract

Objectives

Urinary tract infection (UTI) is common among older women. However, diagnosis is challenging due to frequent chronic lower urinary tract symptoms (LUTS), cognitive impairment, and a high prevalence of asymptomatic bacteriuria (ASB). Current urine diagnostics lack specificity, leading to unnecessary treatment and antimicrobial resistance. This study aimed to evaluate the diagnostic accuracy of twelve urine biomarkers for diagnosing UTI in older women.

Methods

In this case-control study, cases were women ≥ 65 years with ≥ 2 new-onset LUTS, pyuria and one uropathogen ≥ 10^4 CFU/mL. Controls were asymptomatic and classified as ASB (one uropathogen ≥ 10^5 CFU/mL), negative culture or mixed flora. Urine biomarker concentrations were measured through liquid chromatography-mass spectrometry and ELISA. Diagnostic accuracy parameters of individual biomarkers and a biomarker model were derived from ROC curves.

Results

We included 162 community-dwelling and institutionalised older women. Five urine inflammatory biomarkers demonstrated high discriminative ability (AUC ≥ 0.80): interleukin 6 (IL-6), azurocidin, neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor of metalloproteinases 2 (TIMP-2), and C-X-C motif chemokine 9 (CXCL-9). Azurocidin exhibited the highest diagnostic accuracy (sensitivity 86% (95% confidence interval (CI) 75-93%) and specificity 89% (95% CI 82-94%) at 16.7 ng/mmol creatinine). A combined biomarker and pyuria model showed improved diagnostic accuracy in UTI and ASB patients, compared to pyuria alone.

Conclusions

We identified several urine biomarkers that accurately differentiated older women with UTI from asymptomatic women, including ASB. These findings represent a potential advancement towards improved diagnostics for UTI in older women and warrant validation in a diverse population.

Introduction

Urinary tract infection (UTI) is the second most common infection requiring hospitalisation among older adults and the most common infection in long-term care facility (LTCF) residents. [1, 2] In older women particularly, diagnosing UTI is challenging for various reasons. Firstly, symptom assessment is hampered by a higher prevalence of cognitive impairment and indwelling catheters. Secondly, chronic lower urinary tract symptoms (LUTS), e.g. urgency, frequency and urinary incontinence, are common and are difficult to distinguish from non-infectious causes, such as genitourinary syndrome of menopause, and overactive bladder. [3] Furthermore, up to 50% of non-catheterised older women have asymptomatic bacteriuria (ASB), of which 90% have concomitant pyuria. [4-8] Hence, the specificity of the most commonly used diagnostics for UTI (leukocyte esterase or nitrite on dipstick and urine cultures) is low in this population. [9] Especially in patients with non-specific symptoms, clinicians are inclined to test for and treat bacteriuria and pyuria, which are easily misclassified as UTI. [10] This potentially inappropriate treatment can contribute to antimicrobial resistance, unnecessary side effects and drug interactions in a population with already high rates of polypharmacy. Moreover, it may promote gut dysbiosis and *Clostridioides difficile* infections. [10-14]

As highlighted by the Infectious Diseases Society of America (IDSA), antimicrobial stewardship begins with diagnostic stewardship, and novel biomarkers with high specificity for UTI are urgently needed to endorse prudent use of antibiotics for UTI in older women. [4] Beyond improving individual patient management, an accurate urine biomarker or biomarker panel would also have implications for clinical trial design, drug development, infection surveillance and infection control efforts. A number of studies have evaluated the diagnostic accuracy of several urine inflammatory markers in patients with UTI and ASB, as summarised in a recent systematic review. [15] However, the majority of the included studies either involved younger patients or defined UTI based on dipstick or urine culture results, and are likely affected by misclassification bias. The primary aim of this study was to assess the diagnostic accuracy of twelve urine biomarkers associated with inflammation and tissue injury, for diagnosing UTI in older women. The selection of these biomarkers was based on a review of the available literature and their theoretical potential if no prior evidence was available. [15-21]

Methods

Study design

This multicentre, prospective, case-control study was conducted across four primary care offices, five emergency departments (one academic and four regional hospitals), four LTCFs, and 14 independent and assisted living facilities in the Leiden and The Hague area in the Netherlands. Details of the study design have been published previously. [8] The study protocol was approved by the regional medical ethics committee and written informed consent was obtained from all participants. This study was registered at the International Clinical Trials Registry Platform (trial ID: NL9477) and is reported in accordance with STARD guidelines. [22]

Participants

Cases consisted of women ≥ 65 years meeting all of the following criteria: ≥ 2 new-onset LUTS (dysuria, frequency, urgency, or suprapubic pain), and pyuria (either ≥ 10 leukocytes/µl or the presence of leukocyte esterase on dipstick), and a urine culture with growth of one uropathogen ≥ 10^4 colony-forming units per millilitre (CFU/mL). Uropathogens included Enterobacterales, enterococci, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, and streptococci. Cases with growth of two or more pathogens were excluded. If fever was present (temperature ≥ 38.0 °C), cases were categorised as having an upper UTI. Controls were women ≥ 65 years without new-onset LUTS or fever. Based on urine culture results, they were subdivided into an ASB group (two consecutive urine cultures, obtained 2-4 weeks apart, with identical uropathogens ≥ 10^5 CFU/mL [4]), a 'negative culture' group (no growth or growth of non-pathogenic micro-organisms < 10^3 CFU/mL), or a 'mixed flora' group (≥ 2 pathogens ≥ 10^3 CFU/mL). Exclusion criteria for both cases and controls included: inability to express symptoms (e.g. due to advanced cognitive impairment), the presence of an indwelling catheter, immunosuppressive drug use, antimicrobial use within 48 hours prior to inclusion, current urolithiasis, and a UTI in the previous month.

Procedures

The research team was notified by the attending physician upon identifying a prospective participant. Asymptomatic LTCF residents were invited to participate by their attending physician, while flyers were used to recruit community-dwelling controls. Eligible cases were visited by the research team within one

hour of identification. During the baseline assessment, data on age, previous medical history, new-onset symptoms, and fever were collected. All participants underwent delirium screening and activities of daily living (ADL) assessment using 4AT and Katz questionnaires, and measurement of vital signs.

Midstream urine (or urine obtained through single in-out catheterisation) was collected in a sterile urine container and transported to the laboratory of the Leiden University Medical Center. Samples were transported at room temperature and processed within 4 hours of micturition. (Pre)analytical procedures of urinalysis and microbiological assessments are described elsewhere. [8] In preparation of biomarker analysis, urine was transferred into a 15 mL collection tube and centrifuged (3000 *g* for 8 minutes). The supernatant was transferred into another collection tube and vortexed. Finally, the urine was divided into six aliquots (300 µl per aliquot) and stored at -80 °C until in-batch analysis. Samples underwent no more than a single freeze-thaw cycle.

Biomarker measurements

Biomarker measurements were performed by our in-house developed and validated multiplex liquid chromatography mass spectrometry (LC-MS) with modifications [23] and enzyme-linked immunosorbent assay (ELISA). The following biomarkers were measured using LC-MS: neutrophil gelatinase-associated lipocalin (NGAL), insulin-like growth factor-binding protein 7 (IGFBP-7), tissue inhibitor of metalloproteinases 2 (TIMP-2), kidney injury molecule 1 (KIM-1), C-X-C motif chemokine 9 (CXCL-9), nephrin, solute carrier family 22 member 2 (SLC22A2), calbindin, and transforming growth factor beta-1 (TGF-β1). ELISA was used to measure interleukin 6 (IL-6), xanthine oxidase (XO), and azurocidin (also known as heparin-binding protein). Details on the LC-MS and ELISA analyses are described in the **Supplementary Material**.

Sample size calculation

As sensitivity and specificity values of urine biomarkers were either conflicting or unknown for our population, we assumed sensitivity and specificity values for our sample size calculation. To assess specificity, with an α of 0.05, and with maximum marginal error of estimate of 0.10 (δ) for constructing the confidence interval (CI) of the true value of specificity, assuming a value of 80% and using the normal approximation, the control group needed to consist of 62 participants.

Using the same sample size for the case group resulted in a marginal error (δ) of sensitivity, assuming a true value of 70%, of 0.12.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM, Armonk, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A Mann-Whitney U test was performed to compare median biomarker concentrations between cases and controls, and a Bonferroni-corrected significance level (α) of 0.005 was applied. Sensitivity-specificity pairs were computed for all possible thresholds and plotted in a receiver operating characteristic (ROC) curve using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California). To determine the discriminative ability of each urine biomarker, we calculated the area under the curve (AUC) for the individual biomarkers. The continuous variable CXCL9 was dichotomised as it was undetectable in many participants. 'Optimal' cut-offs for each biomarker were based on Youden's J statistic, and two additional cut-offs were calculated for scenarios in which either a sensitivity of 90% or a specificity of 90% was desired. To investigate whether these biomarkers performed better in combination, we fitted a logistic regression model using backward selection which included all (logarithmically transformed) biomarkers, selected on Akaike's Information Criterion. The AUC of this regression model was compared with the AUC of the best performing individual biomarker using DeLong's test.

We recently published data demonstrating that the degree of pyuria can be helpful in distinguishing UTI in older women from asymptomatic controls, including those with ASB. [8] To investigate the additional value of the biomarkers, we conducted a post hoc analysis comparing the discriminative ability of a model containing both urinary leukocytes and the biomarker panel with urinary leukocytes alone, using DeLong's test. Given that controls in the ASB subgroup showed intermediate levels of pyuria in our previous study (interquartile ranges overlapped with UTI cases) [8], the same comparison was made in a subset of patients with either UTI or ASB.

Results

Between June 2021 and July 2022, 162 participants were enrolled (screening process summarised in **Figure 1**). Participant characteristics are outlined in **Table 1**. Cases and controls were similar in age, comorbidities and ADL-dependency (38/162

participants (23%) were dependent for ≥ 2 Katz-items). Controls were recruited more often in a LTCF (43/100, 43%) compared with cases (7/62, 11%). Twenty-one percent (13/62) of cases had an upper UTI and 18% (18/100) of controls had ASB. Causative pathogens are summarised in **Supplementary Table 1**; *E. coli* was the most common pathogen in both cases (50/62, 81%) and controls with ASB (14/18, 78%).

Table 1: Baseline characteristics of cases and controls.

Baseline characteristics	UTI (n = 62)	Controls (n = 100)
Age in years, mean (SD)	77.2 (8.0)	79.0 (8.1)
Setting		
Emergency department	18 (29.0)	0
LTCF	7 (11.3)	43 (43.0)
Primary care office	37 (60.0)	0
At home	0	57 (57.0)
Comorbidity		
Urological comorbidity	8 (12.9)	8 (8.0)
Diabetes mellitus	14 (22.6)	14 (14.0)
History of CKD (self-reported)	12 (19.4)	11 (11.0)
ADL-dependency ≥ 2 Katz-items	14 (22.6)	24 (24.0)
UTI history		
Ever had UTI	56 (90.3)	76 (76.0)
Ever hospitalised for UTI	2 (3.2)	1 (1.0)
No. of UTI in past year, median (IQR)	1 (0 – 2)	0 (0 – 0)
Antibiotics in previous month	16 (25.8)	20 (20.0)
Catheter in week prior to inclusion	2 (3.2)	2 (2.0)
New-onset symptoms	62 (100)	0
Dysuria	48 (77.4)	-
Frequency	56 (90.3)	-
Urgency	52 (83.9)	-
Suprapubic pain	42 (67.7)	-
Fever (≥ 38.0)	13 (21.0)	-
4AT score ≥ 2	4 (6.5)	1 (1.0)

Variables are expressed as n (%) unless otherwise specified. Urological comorbidity included pelvic organ prolapse, previous procedures for urinary incontinence and previous malignancies (n = 1 renal cell carcinoma, n = 1 non-muscle-invasive bladder cancer; no evidence of active malignancy in either patient). All participants with a 4AT score ≥ 2 were able to communicate their symptoms clearly. UTI = urinary tract infection, LTCF = long-term care facility, CKD = chronic kidney disease, ADL = activities of daily living

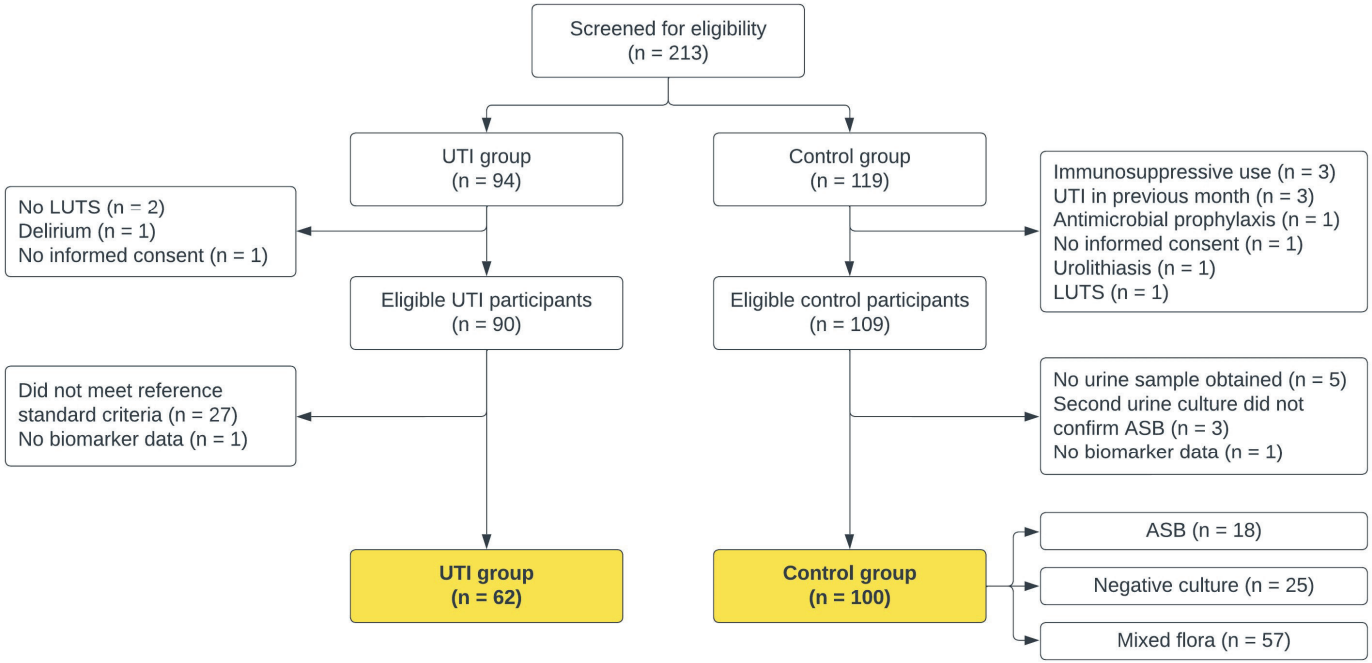

Figure 1: Overview of screening and selection process. The 27 participants that did not meet reference standard criteria were symptomatic patients who did not have pyuria or urine cultures with growth of 1 uropathogen. For 2 participants, biomarker data was missing. UTI = urinary tract infection, LUTS = lower urinary tract symptoms, ASB = asymptomatic bacteriuria

Biomarker concentrations and diagnostic accuracy

Median urine biomarker concentrations for cases and controls are shown in **Figure 2** and **Supplementary Table 2**. LC-MS biomarkers nephrin, SLC22A2, and TGF-β1 were not detected in any participant. Except for uromodulin and calbindin, all biomarkers differed significantly between cases and controls. CXCL-9 was detected in 40/62 (65%) cases and 5/100 (5%) controls (χ^2 67.6, p < 0001).

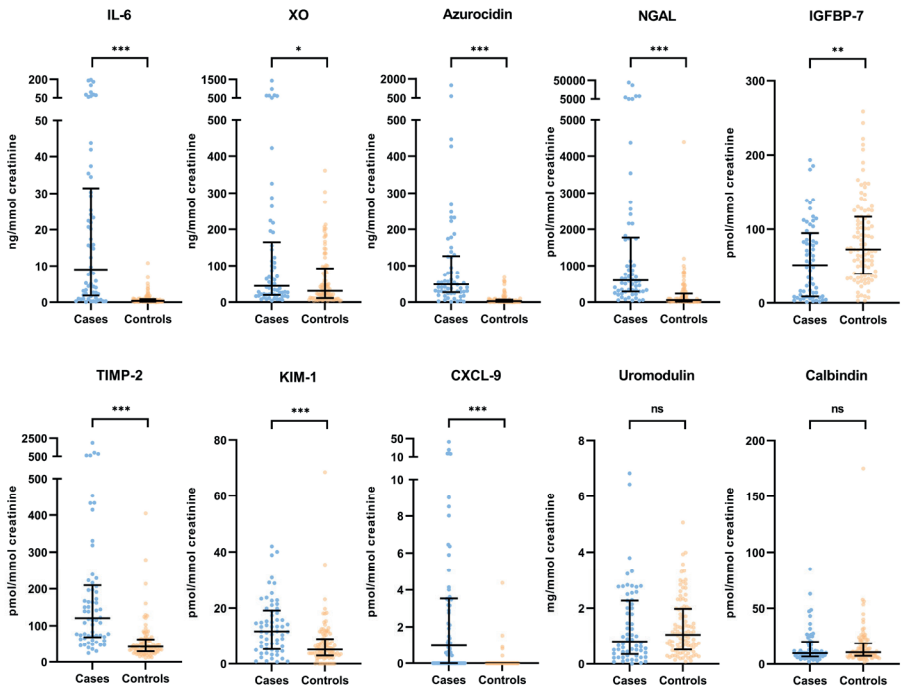

Figure 2: Scatter dot plots of biomarker concentrations for cases and controls. The horizontal line drawn in the middle denotes the median, and the whiskers represent the interquartile range. Significance levels are indicated by: ns = not significant, * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Abbreviations: IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7 (IGFBP-7), TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1 CXCL-9 = C-X-C motif chemokine 9.

ROC curves and corresponding AUCs are displayed in **Figure 3**. IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 all had excellent discriminative ability (AUC ≥ 0.80). Sensitivity, specificity and likelihood ratios for various cut-offs are shown in

Table 2. IL-6 (cut-off 1.88 ng/mmol creatinine) and azurocidin (cut-off 16.7 ng/mmol creatinine) had high specificity (90% (95% CI 83-95%) and 89% (95% CI 82-94%), respectively), while maintaining fair sensitivity (76% (95% CI 64-85%) and 86% (95% CI 75-93%), respectively). After backward selection, our logistic regression model (ROC curve in **Figure 3** and model summary in **Supplementary Table 5**) contained the following biomarkers: IL-6, XO, azurocidin, NGAL, TIMP-2, CXCL-9 and uromodulin. This model had better discriminative ability (AUC 0.95) than the biomarker with the highest AUC in the univariate analysis (azurocidin, AUC 0.92), albeit not statistically significant (p = 0.06).

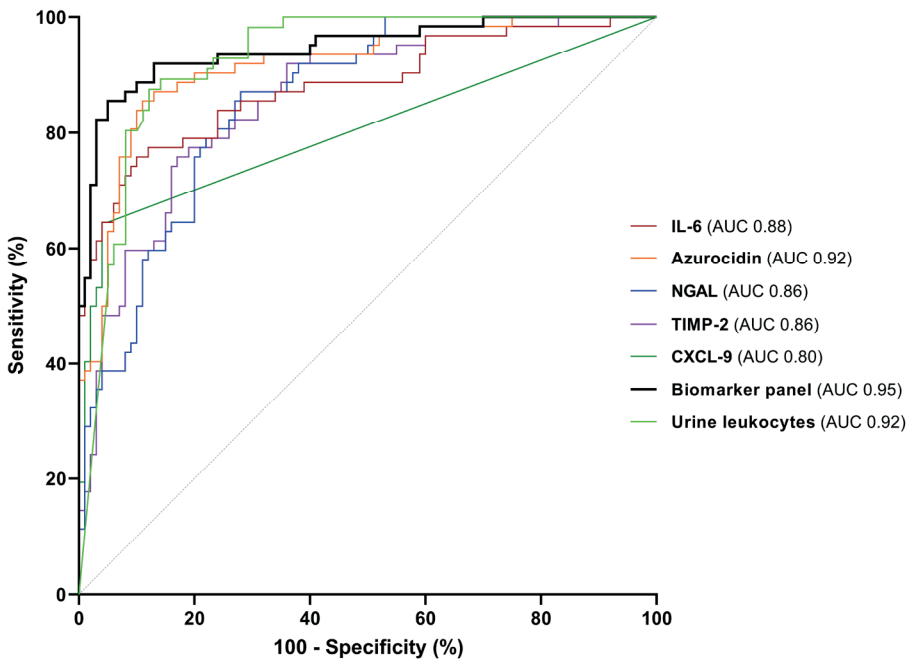

Figure 3: Receiver operating characteristic curves for IL-6, azurocidin, NGAL, TIMP-2, CXCL-9 and a combined biomarker model. Biomarker concentrations were used as test variables, and our UTI definition was used for determining disease status. The true positive rate (sensitivity) was plotted against the false positive rate (1 – specificity) for different biomarker cut-offs. Our combined logistic regression model contained the following logarithmically transformed biomarkers: IL-6, XO, azurocidin, NGAL, TIMP-2, CXCL-9 and uromodulin. Areas under the curve were: IL-6 (0.88), azurocidin (0.92), NGAL (0.86), TIMP-2 (0.86), CXCL-9 (0.80), combined biomarker model (0.95). The ROC curve of CXCL-9 is diagonal due to ties between cases and controls, i.e. CXCL-9 concentration was 0 in some of cases and controls. The reference line is represented by the dotted line. Abbreviations: IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, CXCL-9 = C-X-C motif chemokine 9.

Table 2: Diagnostic accuracy parameters of IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 for various cut-offs.

	Cut-off	Sensitivity % (95%CI)	Specificity % (95%CI)	LR_{pos} (95% CI)	LR_{neg} (95% CI)
IL-6 (ng/mmol creatinine) optimal	1.88	76 (64 – 85)	90 (83 – 95)	7.6 (4.1 – 13.9)	0.3 (0.2 – 0.4)
High sensitivity preferred	0.28	90 (81 – 96)	43 (34 – 53)	1.6 (1.3 – 1.9)	0.2 (0.1 – 0.5)
High specificity preferred	1.88	76 (64 – 85)	90 (83 – 95)	7.6 (4.1 – 13.9)	0.3 (0.2 – 0.4)
Azurocidin (ng/mmol creatinine) optimal	16.7	86 (75 – 93)	89 (82 – 94)	7.8 (4.4 – 13.7)	0.2 (0.09 – 0.3)
High sensitivity preferred	8.7	90 (81 – 96)	80 (72 – 97)	4.5 (3.0 – 6.7)	0.1 (0.05 – 0.3)
High specificity preferred	17.0	84 (73 – 92)	90 (83 – 95)	8.4 (4.6 – 15.3)	0.2 (0.1 – 0.3)
NGAL (pmol/mmol creatinine) optimal	201	87 (77 – 94)	72 (63 – 80)	3.1 (2.2 – 4.3)	0.2 (0.09 – 0.3)
High sensitivity preferred	115	90 (81 – 96)	63 (53 – 72)	2.4 (1.9 – 3.2)	0.2 (0.07 – 0.3)
High specificity preferred	598	50 (38 – 62)	90 (83 – 95)	5.0 (2.6 – 9.5)	0.6 (0.4 – 0.7)
TIMP-2 (pmol/mmol creatinine) optimal	69.7	76 (64 – 85)	83 (75 – 89)	4.4 (2.8 – 7.0)	0.3 (0.2 – 0.5)
High sensitivity preferred	47.1	90 (81 – 96)	64 (54 – 73)	2.5 (1.9 – 3.3)	0.2 (0.07 – 0.3)
High specificity preferred	89.4	60 (47 – 71)	90 (83 – 95)	6.0 (3.2 – 11.1)	0.4 (0.3 – 0.6)
CXCL-9 (pmol/mmol creatinine)	Present or absent	65 (52 – 75)	95 (90 – 98)	12.9 (5.4 – 30.9)	0.4 (0.3 – 0.5)

The optimal cut-off value was based on Youden's J statistic, and two additional cut-offs were calculated for scenarios in which either a sensitivity of 90% or a specificity of 90% was desired. CXCL9 was dichotomised as it was undetectable in a large number of patients. IL-6 = interleukin 6, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, CXCL-9 = C-X-C motif chemokine 9.

Post hoc and subgroup analyses

Overall, the model combining the biomarker panel and urinary leukocytes did not perform significantly better than urinary leukocytes alone; both showed high diagnostic accuracy (AUC 0.95 vs. 0.92). In the subset of patients with either UTI or ASB, the combined biomarker and leukocyte model demonstrated higher diagnostic accuracy (AUC 0.89) compared with urinary leukocytes alone (AUC 0.73), p = 0.01. This effect was also observed for the combination of CXCL9 and leukocytes (AUC 0.86, p = 0.04), but not for other biomarker-leukocyte combinations. Median urine biomarker concentrations for case and control subgroups are detailed in **Supplementary Tables 3 and 4**.

Discussion

In this study, we identified five urine biomarkers with high diagnostic accuracy for UTI in older women. Urinary IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9 accurately differentiated older women with UTI from asymptomatic women, including those with ASB. These findings advance the development of better diagnostics for UTI in older women.

Comparison with previous studies

Most urine biomarker research has been performed in children. [16, 24] A few studies have investigated the diagnostic performance of IL-6, azurocidin and NGAL in (older) adults. IL-6 is secreted by urothelial cells following pathogen exposure, and induces an acute phase response. [25] Azurocidin and NGAL are neutrophil-granule derived proteins that exhibit their antibacterial effect through monocyte chemotaxis and sequestration of siderophore-bound iron, respectively. [26, 27] Our findings regarding IL-6 and azurocidin are consistent with findings from previous studies. Kjölvmark et al. [18] observed significantly higher levels of IL-6 and azurocidin in community-dwelling and institutionalised patients with UTI compared with LTCF residents with ASB. Median urinary IL-6 and azurocidin concentrations were similar to concentrations found in our study, although IL-6 concentrations were even higher in their UTI group, possibly due to a higher proportion of upper UTI patients. Rodhe et al. [19] also found significantly higher urinary IL-6 levels in older patients with UTI compared to those with ASB. Both studies only compared UTI and ASB. We deliberately compared patients with UTI to asymptomatic controls (including ASB), as this is the primary distinction to be made in clinical practice, given that urine culture results are not available

at the time of presentation. The diagnostic accuracy of NGAL was previously demonstrated by Price et al. [20], who reported an even higher AUC, likely due to their control group being younger and lacking patients with ASB. CXCL-9, a chemokine that differentiates pyelonephritis from cystitis in children [21], was detected in the majority of UTI patients but only in 5% of controls. Notably, CXCL-9 was undetectable in all 1443 middle-aged participants in a prior LC-MS reference value study [23], supporting the biomarker's high specificity. We did not find any study evaluating the diagnostic accuracy of TIMP-2 for UTI.

Biomarker panel

In clinical practice, pyuria is often assessed when diagnosing UTI. Our recent study showcased that the degree of pyuria can aid in differentiating UTI from asymptomatic controls. [8] The biomarkers evaluated in our current study displayed comparably high diagnostic accuracy. An additional value of the biomarker panel lies in the distinction between UTI and ASB, as urinary leukocyte counts showed some overlap in our previous study. [8] Our post hoc analysis showed that a combination of urine biomarkers and leukocytes had a significantly higher diagnostic accuracy in this subgroup than urine leukocytes alone. Particularly in cases with intermediate degrees of pyuria, this panel could assist the clinician in deciding whether to initiate empirical treatment or not.

Strengths and limitations

The strengths of this study include the implementation of robust and standardised (pre)analytical procedures, ensuring reliable biomarker results. Additionally, we employed strict criteria to define UTI, included three control subgroups, and recruited older women from diverse healthcare settings. However, there are certain limitations to acknowledge. Firstly, the study primarily involved a relatively healthy older population, which may restrict the generalisability of our findings to a more frail population. However, given the absence of an agreed-upon reference standard for UTI, the selection of distinct cases and controls was necessary to identify promising biomarkers warranting further validation. Secondly, we did not measure serum creatinine levels, which prevented us from exploring this potential relationship in our study. [17] As with any case-control study, there is a possibility of overestimated diagnostic accuracy parameters and unmeasured confounding. Lastly, we acknowledge minor differences between cases and controls regarding baseline characteristics. However, additional regression analysis (not shown) did not

demonstrate an effect of age, diabetes mellitus or ADL-dependency on biomarker concentrations.

Conclusions

In conclusion, we have identified five urine biomarkers that exhibit high diagnostic accuracy for UTI in older women: IL-6, azurocidin, NGAL, TIMP-2 and CXCL-9. Moreover, a biomarker panel showed additional value, on top of pyuria, for discriminating UTI from ASB. The performance of these biomarkers needs to be prospectively validated in a broader population with various clinical presentations (including non-specific symptoms), comorbidities and levels of frailty. Future research should then focus on whether the implementation of this diagnostic tool, for instance as a point-of-care test, improves individual patient management, infection surveillance and control efforts, combats antimicrobial resistance, and reduces misclassification bias in UTI studies.

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Author contributions

Conceptualisation and methodology M.P.B., J.E.S., C.N., J.I.M.U., A.A.A., M.E.N., W.P.A., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; recruitment M.P.B., M.J.A., J.I.M.U., M.M.C.L.; laboratory analysis M.M.T, E.A., C.M.C; writing – original draft preparation M.P.B.; data interpretation M.P.B., M.M.C.L., L.G.V.; writing – review and editing M.P.B, M.M.T., M.M.J.A., E.A., J.E.S., C.N., E.M.S., N.M.D., J.I.M.U, M.S., A.A.A., M.E.N., W.P.A., S.P.M., M.T.B., C.M.C., S.P.C., L.G.V., M.M.C.L.; supervision M.M.C.L. and L.G.V. All authors have read and agreed to the final version of the manuscript.

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Potential conflicts of interest (also mentioned in ICMJE forms)

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References

1. Tsan L, Langberg R, Davis C, et al. Nursing home-associated infections in Department of Veterans Affairs community living centers. Am J Infect Control **2010**; 38(6): 461-6.
2. Curns AT, Holman RC, Sejvar JJ, Owings MF, Schonberger LB. Infectious disease hospitalizations among older adults in the United States from 1990 through 2002. Arch Intern Med **2005**; 165(21): 2514-20.
3. 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 **2006**; 50(6): 1306-14; discussion 14-5.
4. Nicolle LE, Gupta K, Bradley SF, et al. Clinical Practice Guideline for the Management of Asymptomatic Bacteriuria: 2019 Update by the Infectious Diseases Society of America. Clin Infect Dis **2019**; 68(10): 1611-5.
5. Rodhe N, Lofgren S, Matussek A, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis **2008**; 40(10): 804-10.
6. Ouslander JG, Schapira M, Fingold S, Schnelle J. Accuracy of rapid urine screening tests among incontinent nursing home residents with asymptomatic bacteriuria. J Am Geriatr Soc **1995**; 43(7): 772-5.
7. Boscia JA, Abrutyn E, Levison ME, Pitsakis PG, Kaye D. Pyuria and asymptomatic bacteriuria in elderly ambulatory women. Ann Intern Med **1989**; 110(5): 404-5.
8. Bilsen MP, Aantjes MJ, van Andel E, et al. Current Pyuria Cutoffs Promote Inappropriate Urinary Tract Infection Diagnosis in Older Women. Clin Infect Dis **2023**; 76(12): 2070-6.
9. Joseph A. The Diagnosis and Management of UTI in >65s: To Dipstick or Not? The Argument Against Dipsticks. Infect Prev Pract **2020**; 2(3): 100063.
10. Mitchell SL, Shaffer ML, Loeb MB, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med **2014**; 174(10): 1660-7.
11. Rotjanapan P, Dosa D, Thomas KS. Potentially inappropriate treatment of urinary tract infections in two Rhode Island nursing homes. Arch Intern Med **2011**; 171(5): 438-43.
12. Worby CJ, Schreiber HLt, Straub TJ, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol **2022**; 7(5): 630-9.
13. Langford BJ, Brown KA, Diong C, et al. The Benefits and Harms of Antibiotic Prophylaxis for Urinary Tract Infection in Older Adults. Clin Infect Dis **2021**; 73(3): e782-e91.
14. Das R, Towle V, Van Ness PH, Juthani-Mehta M. Adverse outcomes in nursing home residents with increased episodes of observed bacteriuria. Infect Control Hosp Epidemiol **2011**; 32(1): 84-6.
15. Edwards G, Seeley A, Carter A, et al. What is the Diagnostic Accuracy of Novel Urine Biomarkers for Urinary Tract Infection? Biomark Insights **2023**; 18: 117727192211144459.
16. Horvath J, Wullt B, Naber KG, Koves B. Biomarkers in urinary tract infections - which ones are suitable for diagnostics and follow-up? GMS Infect Dis **2020**; 8: Doc24.

17. van Duijl TT, Soonawala D, de Fijter JW, Ruhaak LR, Cobbaert CM. Rational selection of a biomarker panel targeting unmet clinical needs in kidney injury. Clin Proteomics **2021**; 18(1): 10.

18. Kjolvmark C, Tschernij E, Oberg J, Pahlman LI, Linder A, Akesson P. Distinguishing asymptomatic bacteriuria from urinary tract infection in the elderly - the use of urine levels of heparin-binding protein and interleukin-6. Diagn Microbiol Infect Dis **2016**; 85(2): 243-8.

19. Rodhe N, Lofgren S, Strindhall J, Matussek A, Molstad S. Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria. Scand J Prim Health Care **2009**; 27(2): 74-9.

20. Price JR, Guran L, Lim JY, et al. Neutrophil Gelatinase-Associated Lipocalin Biomarker and Urinary Tract Infections: A Diagnostic Case-Control Study (NUTI Study). Female Pelvic Med Reconstr Surg **2017**; 23(2): 101-7.

21. Shaikh N, Martin JM, Hoberman A, et al. Host and Bacterial Markers that Differ in Children with Cystitis and Pyelonephritis. J Pediatr **2019**; 209: 146-53 e1.

22. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ **2015**; 351: h5527.

23. van Duijl TT, Ruhaak LR, Hoogeveen EK, et al. Reference intervals of urinary kidney injury biomarkers for middle-aged men and women determined by quantitative protein mass spectrometry. Ann Clin Biochem **2022**; 59(6): 420-32.

24. Boon HA, Struyf T, Bullens D, Van den Bruel A, Verbakel JY. Diagnostic value of biomarkers for paediatric urinary tract infections in primary care: systematic review and meta-analysis. BMC Fam Pract **2021**; 22(1): 193.

25. Ching CB, Gupta S, Li B, et al. Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. Kidney Int **2018**; 93(6): 1320-9.

26. Linder A, Soehnlein O, Akesson P. Roles of heparin-binding protein in bacterial infections. J Innate Immun **2010**; 2(5): 431-8.

27. Shields-Cutler RR, Crowley JR, Miller CD, Stapleton AE, Cui W, Henderson JP. Human Metabolome-derived Cofactors Are Required for the Antibacterial Activity of Siderocalin in Urine. J Biol Chem **2016**; 291(50): 25901-10.

Supplement

Supplementary methods: biomarker analysis and quality control

ELISA

ELISA analyses were carried out using the Quantikine™ human IL-6 kit (R&D Systems, Minneapolis, MN, Art. No. HS600C), the XO kit (Cusabio, Houston, TX, Art. No. CSB-E13124h), and the human azurocidin kit (Cusabio, Houston, TX, Art. No. CSB-E09698h). All analyses were executed in accordance with the provided manuals and quality controls were performed for each kit. The IL-6 ELISA kit performance was tested with low, medium and high concentration quantitative controls purchased from R&D systems (Quantikine™, Immunoassay Control Group 246, Cat. No. QC246). All quantitative controls passed the predefined criteria provided by the manufacturer. In addition, an in-house prepared urine pool of healthy individuals was used as an internal quality control. Finally, two in-house prepared single-donor (kidney transplantation patient) samples were used for quality assurance, as no quality controls were provided by the manufacturer. Average coefficients of variation (CV) were 6.3% (IL-6), 20.1% (XO), and 13.9% (AZU). Lower limits of detection were 0.03 pg/mL (IL-6), 0.04 ng/mL (XO) and 2.0 pg/mL (azurocidin), respectively. If the upper limit of detection was reached, samples were diluted as prescribed by the manual. Final biomarker concentrations were normalised for urinary dilution using creatinine (mmol/L) and reported in ng/mmol creatinine. Creatinine concentrations were determined for each sample with an enzymatic assay using a Cobas C502 (Roche Diagnostics, Rotkreuz, Switzerland).

LC-MS

The other nine biomarkers were analysed with our in-house developed and validated multiplex LC-MS test with modifications. The use of alternative antibodies for calbindin (R&D systems AF3320; Polyclonal Goat IgG) and TGF-β1 (R&D systems BAF240; Polyclonal Chicken IgY) improved the measuring range and increased sensitivity by 10-fold. For KIM-1, TIMP-2, CXCL-9 and TGF-β1 optimised LC-MS settings were used. The optimised method was employed to measure the samples in a total of three batches in 96-well format including five urine-based calibrators and two urine-based internal quality controls in duplicate per batch, for the purpose of quantification and quality assurance, respectively. The performance of the LC-MS instrument passed the criteria of the system suitability test that was run prior to and after each sample batch. Internal quality controls for all three

batches passed the predefined criteria. Specifically, the average CVs for QC1 and QC2 were 13.7% and 15.4%, respectively. LC–MS biomarker concentrations (pmol/L) were normalised for urinary dilution and reported in pmol/mmol creatinine.

Supplementary Table 1: List of causative pathogens in cases and controls with asymptomatic bacteriuria (ASB).

	Cases (n = 62)	**Controls with ASB (n = 18)**
Escherichia coli n (%)	50 (81)	14 (79)
Klebsiella spp. n (%)	3 (5)	2 (11)
Proteus mirabilis n (%)	3 (5)	0
Citrobacter (non) koseri n (%)	2 (3)	0
Enterococcus faecalis/faecium n (%)	2 (3)	1 (6)
Pseudomonas aeruginosa n (%)	2 (3)	0
Group C *Streptococcus* n (%)	0	1 (6)

In two cases, *Escherichia coli* isolates produced extended-spectrum beta-lactamase. In all controls with ASB, we required growth of identical pathogens in two consecutive urine cultures, obtained two to four weeks apart, with at least 10^5 colony-forming units per millilitre. *Klebsiella* spp. included *Klebsiella pneumoniae* (n = 4) and *Klebsiella oxytoca* (n = 1).

Supplementary Table 2: Median urine biomarker concentrations for cases and controls.

	Cases (n = 62)	Controls (n = 100)	Unadjusted P-value	AUC (95%CI)
IL-6 ng/mmol creatinine, median (IQR)	9.0 (1.9 – 31.4)	0.34 (0.16 – 0.83)	< 0.001	0.88 (0.82 – 0.94)
XO ng/mmol creatinine, median (IQR)	44.3 (19.6 – 164.6)	30.8 (11.2 – 92.7)	0.04	0.60 (0.51 – 0.69)
Azurocidin ng/mmol creatinine, median (IQR)	48.4 (27.1 – 126.5)	2.6 (0.90 – 6.9)	< 0.001	0.92 (0.87 – 0.96)
NGAL pmol/mmol creatinine, median (IQR)	594 (289 – 1772)	59 (20 – 234)	< 0.001	0.86 (0.80 – 0.91)
IGFBP-7 pmol/mmol creatinine, median (IQR)	51.3 (8.7 – 94.6)	72.4 (39.2 – 117.0)	0.002	0.65 (0.56 – 0.74)
TIMP-2 pmol/mmol creatinine, median (IQR)	120 (69 – 209)	42 (29 – 63)	< 0.001	0.86 (0.80 – 0.92)
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (5.3 – 19.1)	5.2 (3.0 – 9.0)	< 0.001	0.72 (0.64 – 0.80)
CXCL-9 pmol/mmol creatinine, median (IQR)	0.98 (0 – 3.5)	0 (0 – 0)	< 0.001	0.80 (0.72 – 0.88)
Uromodulin mg/mmol creatinine, median (IQR)	0.82 (0.35 – 2.27)	1.06 (0.52 – 1.98)	0.38	0.54 (0.45 – 0.64)
Calbindin pmol/mmol creatinine, median (IQR)	9.8 (6.7 – 20.3)	10.5 (7.3 – 18.9)	0.65	0.52 (0.43 – 0.62)

All values are normalised for urinary dilution. A Mann-Whitney U test was performed to compare median biomarker concentrations between cases and controls. Uncorrected p-values are shown, we applied a Bonferroni-corrected significance level (α) of 0.005. The area under the curve (AUC) of each individual biomarker was derived from the receiver operating characteristic curve of each biomarker. Abbreviations: IQR = interquartile range, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 3: Summary of the logistic regression model with a combination of biomarkers obtained through backward selection.

	Beta	Odds ratio (95%CI)	P-value
XO	-1.11	0.33 (0.09 – 1.13)	0.09
Azurocidin	1.38	3.96 (1.22 – 15.23)	0.03
NGAL	1.16	3.19 (0.75 – 16.07)	0.13
TIMP-2	-2.38	0.09 (0.004 – 1.88)	0.13
IL-6	1.97	7.19 (1.78 – 35.14)	0.009
CXCL-9	1.66	5.27 (1.61 – 20.53)	0.01
Uromodulin	-1.30	0.27 (0.05 – 1.45)	0.14

The R package MASS was used for backwards variable selection. All variables in this model were logarithmically transformed, due to the large variance observed in some of these biomarkers. Abbreviations: XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, TIMP-2 = tissue inhibitor of metalloproteinases 2, IL-6 = interleukin 6, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 4: Median urine biomarker concentrations for lower versus upper UTI.

	Lower UTI (n = 49)	**Upper UTI (n = 13)**	**Unadjusted P-value**
IL-6 ng/mmol creatinine, median (IQR)	5.2 (1.1 – 27.2)	23.3 (13.6 – 50.1)	0.046
XO ng/mmol creatinine, median (IQR)	32.6 (17.7 – 98.0)	192.0 (35.6 – 560.8)	0.02
Azurocidin ng/mmol creatinine, median (IQR)	47.5 (28.3 – 127.9)	49.3 (16.0 – 163.4)	0.72
NGAL pmol/mmol creatinine, median (IQR)	576 (287 – 1790)	610 (265 – 2990)	0.94
IGFBP-7 pmol/mmol creatinine, median (IQR)	34.3 (8.1 – 84.8)	82.0 (37.8 – 146.6)	0.03
TIMP-2 pmol/mmol creatinine, median (IQR)	115 (62 – 202)	151 (73 – 271)	0.30
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (4.8 – 19.8)	13.4 (6.8 – 16.2)	0.97
CXCL-9 pmol/mmol creatinine, median (IQR)	1.08 (0 – 3.56)	0.82 (0.21 – 4.97)	0.79
Uromodulin mg/mmol creatinine, median (IQR)	0.75 (0.39 – 2.08)	0.91 (0.19 – 2.67)	0.95
Calbindin pmol/mmol creatinine, median (IQR)	8.8 (6.0 – 12.3)	26.0 (9.9 – 45.4)	0.001

All values are normalised for urinary dilution. A Mann-Whitney U test was performed to compare median biomarker concentrations between lower and upper UTI patients. P-values not corrected for multiple testing are shown. Abbreviations: UTI = urinary tract infection, IQR = interquartile range, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.

Supplementary Table 5: Median biomarker concentrations for control subgroups.

	UTI group	Control group				
	UTI (n = 62)	**ASB (n = 18)**	**Neg. culture (n = 25)**	**Mixed flora (n = 57)**	**P-value***	**AUC****
IL-6 ng/mmol creatinine, median (IQR)	9.0 (1.9 – 31.4)	0.65 (0.18 – 2.19)	0.20 (0.15 – 0.49)	0.39 (0.15 – 0.82)	< 0.001	0.82
XO ng/mmol creatinine, median (IQR)	44.3 (19.6 – 164.6)	27.5 (9.2 – 63.4)	39.1 (15.6 – 82.5)	34.9 (11.8 – 99.0)	0.054	0.65
Azurocidin ng/mmol creatinine, median (IQR)	48.4 (27.1 – 126.5)	6.4 (2.3 – 20.2)	1.3 (0.8 – 3.4)	2.8 (0.8 – 6.4)	< 0.001	0.82
NGAL pmol/mmol creatinine, median (IQR)	594 (289 – 1772)	320 (129 – 699)	23 (11 – 73)	55 (21 – 219)	0.03	0.67
IGFBP-7 pmol/mmol creatinine, median (IQR)	51.3 (8.7 – 94.6)	54.1 (28.6 – 72.8)	95.8 (50.2 – 152.4)	79.6 (39.8 – 115.8)	0.92	0.51
TIMP-2 pmol/mmol creatinine, median (IQR)	120 (69 – 209)	44 (35 – 131)	42 (28 – 58)	41 (27 – 59)	0.002	0.74
KIM-1 pmol/mmol creatinine, median (IQR)	11.7 (5.3 – 19.1)	7.0 (3.7 – 12.5)	3.5 (1.6 – 6.7)	5.3 (3.0 – 8.7)	0.13	0.62
CXCL-9 pmol/mmol creatinine, median (IQR)	0.98 (0 – 3.5)	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	< 0.001	0.80
Uromodulin mg/mmol creatinine, median (IQR)	0.82 (0.35 – 2.27)	1.05 (0.32 – 1.39)	0.84 (0.38 – 2.72)	1.13 (0.65 – 2.00)	0.85	0.51
Calbindin pmol/mmol creatinine, median (IQR)	9.8 (6.7 – 20.3)	13.7 (7.0 – 24.6)	9.4 (7.2 – 17.1)	10.8 (7.2 – 19.1)	0.43	0.56

*P-value is shown for comparison UTI versus ASB, using a Mann-Whitney U test. **Area under the receiver operating characteristic curve is shown for discriminating UTI from ASB. All values are normalised for urinary dilution. Abbreviations: UTI = urinary tract infection, ASB = asymptomatic bacteriuria, AUC = area under the curve, IL-6 = interleukin 6, XO = xanthine oxidase, NGAL = neutrophil gelatinase-associated lipocalin, IGFBP-7 = insulin-like growth factor-binding protein 7, TIMP-2 = tissue inhibitor of metalloproteinases 2, KIM-1 = kidney injury molecule 1, CXCL-9 = C-X-C motif chemokine 9.