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

Bilsen, M.P.; Treep, M.M.; Aantjes, M.J.; Andel, E. van; Stalenhoef, J.E.; Nieuwkoop, C. van; ... ; Lambregts, M.M.C.

Clinical Microbiology and Infection 30 (2024) 216–222

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

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

Manu P. Bilsen [1, *], Maxim M. Treep [2], Margaretha J. Aantjes [1], Esther van Andel [2], Janneke E. Stalenhoef [3], Cees van Nieuwkoop [4, 5], Eliane M.S. Leyten [6], Nathalie M. Delfos [7], Janneke I.M. van Uhm [8], Martijn Sijbom [5], Abimbola A. Akintola [5], Mattijs E. Numans [5], Wilco P. Achterberg [5, 9], Simon P. Mooijaart [9, 10], Martha T. van der Beek [11], Christa M. Cobbaert [2], Simon P. Conroy [12], Leo G. Visser [1], Merel M.C. Lambregts [1]

[1] *Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands*
[2] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands*
[3] *Department of Internal Medicine, OVLG, Amsterdam, The Netherlands*
[4] *Department of Internal Medicine, Haga Teaching Hospital, The Hague, The Netherlands*
[5] *Department of Public Health and Primary Care, Leiden University Medical Center, Health Campus The Hague, The Netherlands*
[6] *Department of Internal Medicine, Haaglanden Medisch Centrum, The Hague, The Netherlands*
[7] *Department of Internal Medicine, Alrijne Hospital, Leiderdorp, The Netherlands*
[8] *Department of Urology, Leiden University Medical Center, Leiden, The Netherlands*
[9] *LUMC Center for Medicine for Older People, Leiden University Medical Center, Leiden, The Netherlands*
[10] *Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands*
[11] *Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands*
[12] *Medical Research Council Unit for Lifelong Health and Ageing at University College London, University College London, London, United Kingdom*

ARTICLE INFO

Article history:
Received 25 July 2023
Received in revised form
27 September 2023
Accepted 30 September 2023
Available online 5 October 2023

Editor: J. Rodríguez-Baño

Keywords:
Antimicrobial stewardship
Asymptomatic bacteriuria
Biomarkers
Diagnostic accuracy
Older patients
Urinary tract infection

ABSTRACT

Objectives: Urinary tract infection (UTI) is common among older women. However, diagnosis is challenging because of frequent chronic lower urinary tract symptoms, 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 12 urine biomarkers for diagnosing UTI in older women.

Methods: In this case-control study, cases were women ≥65 years with ≥2 new-onset lower urinary tract symptoms, pyuria, and one uropathogen $\geq 10^4$ CFU/mL. Controls were asymptomatic and classified as ASB (one uropathogen $\geq 10^5$ 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 receiver operating characteristic curves.

Results: We included 162 community-dwelling and institutionalized older women. Five urine inflammatory biomarkers demonstrated high discriminative ability (area under the curve ≥0.80): interleukin 6, azurocidin, neutrophil gelatinase-associated lipocalin, tissue inhibitor of metalloproteinases 2, and C-X-C motif chemokine 9. Azurocidin exhibited the highest diagnostic accuracy (sensitivity 86% [95% 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 patients with UTI and ASB, compared with pyuria alone.

Discussion: We identified several urine biomarkers that accurately differentiated older women with UTI from asymptomatic women, including ASB. These findings represent a potential advancement towards

* Corresponding author. Manu P. Bilsen, Department of Infectious Diseases, Leiden University Medical Center, Albinusdreef 2, 2333ZA, Leiden, The Netherlands.
E-mail address: m.p.bilsen@lumc.nl (M.P. Bilsen).

https://doi.org/10.1016/j.cmi.2023.09.023

improved diagnostics for UTI in older women and warrant validation in a diverse population. **Manu P. Bilsen, Clin Microbiol Infect 2024;30:216**

Introduction

Urinary tract infection (UTI) is the second most common infection requiring hospitalization 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. First, symptom assessment is hampered by a higher prevalence of cognitive impairment and indwelling catheters. Second, 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-catheterized 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, 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 summarized 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 12 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 4 primary care offices, 5 emergency departments (1 academic and 4 regional hospitals), 4 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 Standards for Reporting of Diagnostic Accuracy Studies (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), pyuria (either ≥10 leukocytes/μL or the presence of leukocyte esterase on dipstick), and a urine culture with growth of one uropathogen $\geq 10^4$ 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 categorized as having an upper UTI. Controls were women ≥65 years without new-onset LUTS or fever. On the basis of the urine culture results, they were subdivided into an ASB group (two consecutive urine cultures, obtained 2–4 weeks apart, with identical uropathogens $\geq 10^5$ 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 $\geq 10^3$ CFU/mL). Exclusion criteria for both cases and controls included: inability to express symptoms (e.g. because of advanced cognitive impairment), the presence of an indwelling catheter, immunosuppressive drug use, antimicrobial use within 48 hours before 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, whereas flyers were used to recruit community-dwelling controls. Eligible cases were visited by the research team within 1 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 catheterization) 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 ELISA. The following biomarkers were measured using LC-MS: neutrophil gelatinase-associated lipocalin (NGAL), insulin-like growth factor-binding protein 7, tissue inhibitor of metalloproteinases 2 (TIMP-2), kidney injury molecule 1, C-X-C motif chemokine 9 (CXCL-9),

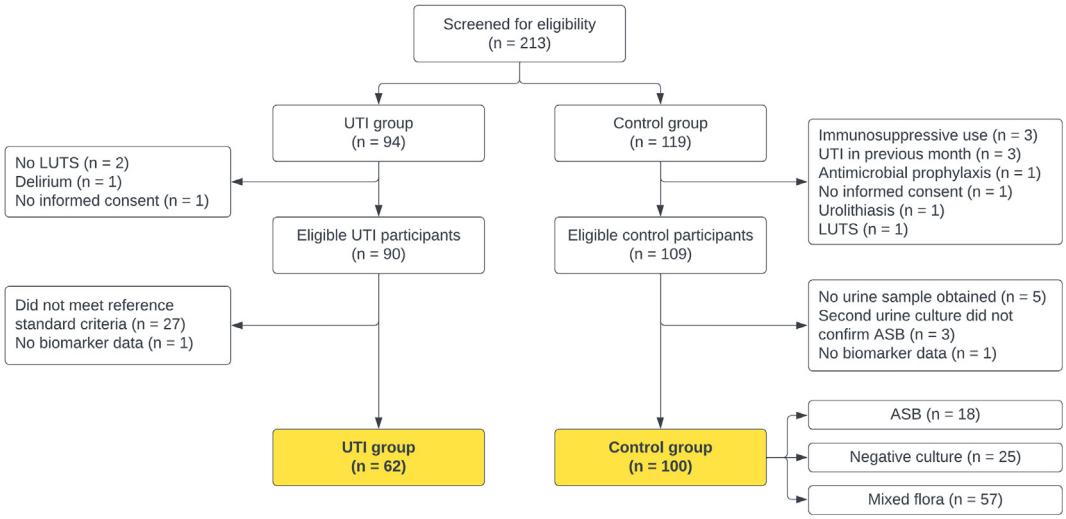

Fig. 1. Overview of screening and selection process. The 27 participants that did not meet reference standard criteria were symptomatic patients who did not have pyuria or urine cultures with growth of 1 uropathogen. For 2 participants, biomarker data were missing. ASB, asymptomatic bacteriuria; LUTS,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

nephrin, solute carrier family 22 member 2, calbindin, and transforming growth factor beta-1. ELISA was used to measure interleukin 6 (IL-6), xanthine oxidase, 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 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

Table 1
Baseline characteristics of cases and controls

Baseline characteristics	UTI (n = 62)	Controls (n = 100)
Age, y, 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	8 (12.9)	8 (8.0)
Urological comorbidity	14 (22.6)	14 (14.0)
Diabetes mellitus	12 (19.4)	11 (11.0)
History of CKD (self-reported)	14 (22.6)	24 (24.0)
ADL-dependency ≥2 Katz-items		
UTI history		
Ever had UTI	56 (90.3)	76 (76.0)
Ever hospitalized for UTI	2 (3.2)	1 (1.0)
Number 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 before 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.
ADL, activities of daily living; CKD, chronic kidney disease; IQR, interquartile range; LTCF, long-term care facility; UTI, urinary tract infection; SD, standard deviation.

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 CXCL-9 was dichotomized 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 summarized in Fig. 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 per cent (13/62) of cases had an upper UTI and 18% (18/100) of controls had ASB. Causative pathogens are summarized in Table S1; E. coli was the most common pathogen in both cases (50/62, 81%) and controls with ASB (14/18, 78%).

Biomarker concentrations and diagnostic accuracy

Median urine biomarker concentrations for cases and controls are shown in Fig. 2 and Table S2. LC-MS biomarkers nephrin, solute

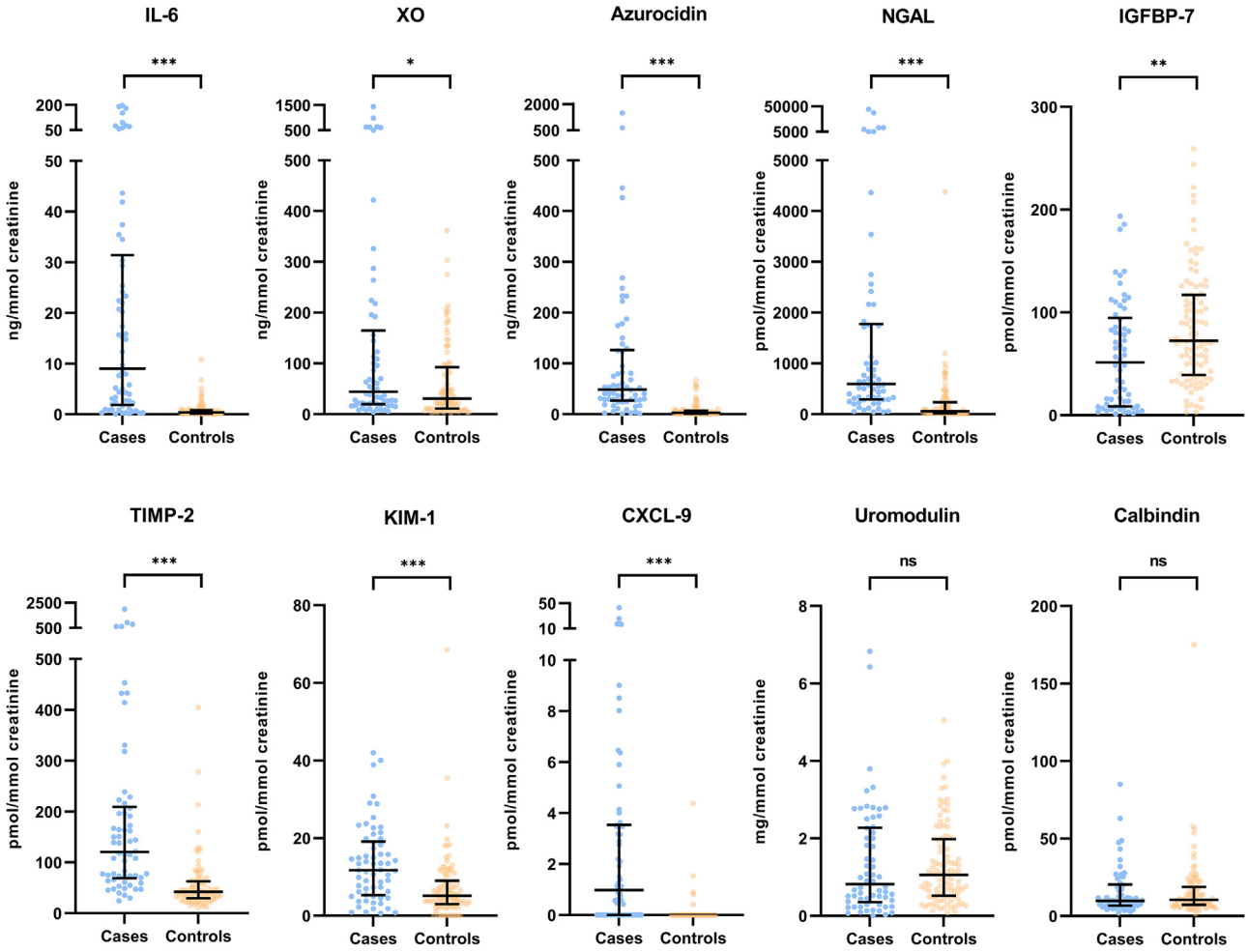

Fig. 2. Scatter dot plots of biomarker concentrations for cases and controls. The horizontal line drawn in the middle denotes the median, and the whiskers represent the interquartile range. Significance levels are indicated by: ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001. CXCL-9, C-X-C motif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

carrier family 22 member 2, and transforming growth factor beta-1 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 of 62 (65%) cases and 5 of 100 (5%) controls ($\chi 2$ 67.6, $p < 0001$).

ROC curves and corresponding AUCs are displayed in Fig. 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), whereas 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 Fig. 3 and model summary in Table S3) contained the following biomarkers: IL-6, xanthine oxidase, 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).

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 CXCL-9 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 Tables S4 and S5.

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 after 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 institutionalized patients with UTI compared with LTCF residents with ASB. Median urinary IL-6 and azurocidin concentrations were similar to concentrations found in

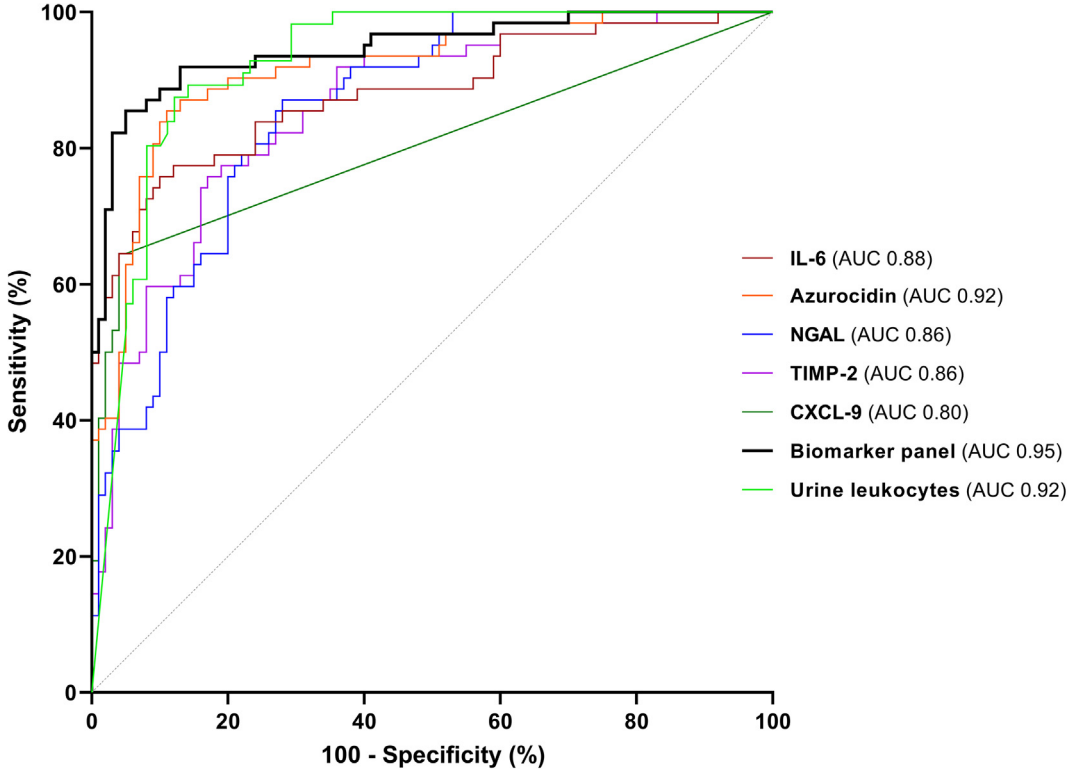

Fig. 3. Receiver operating characteristic curves for IL-6, azurocidin, NGAL, TIMP-2, and CXCL-9, a combined biomarker model and urine leukocytes. Biomarker concentrations were used as test variables, and our UTI definition was used for determining disease status. The true positive rate (sensitivity) 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. The ROC curve of CXCL-9 is diagonal because of 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. CXCL-9, C-X-C motif chemokine 9; IL-6, interleukin 6; NGAL, neutrophil gelatinase-associated lipocalin; ROC, receiver operating characteristic; TIMP-2, tissue inhibitor of metalloproteinases 2; UTI, urinary tract infection; XO, xanthine oxidase.

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

Biomarker	Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	LRpos (95% CI)	LRneg (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. CXCL-9 was dichotomized as it was undetectable in a large number of patients.
CXCL-9, C-X-C motif chemokine 9; IL-6, interleukin 6; LR, likelihood ratio; NGAL, neutrophil gelatinase-associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinases 2.

our study, although IL-6 concentrations were even higher in their UTI group, possibly because of a higher proportion of patients with upper UTI. Rodhe et al. [19] also found significantly higher urinary IL-6 levels in older patients with UTI compared with those with ASB. Both studies only compared UTI and ASB. We deliberately compared patients with UTI with 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 because of 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 patients with UTI 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 standardized (pre)analytical procedures, ensuring reliable biomarker results. In addition, we employed strict criteria to define UTI, included three control subgroups, and recruited older women from diverse health care settings. However, there are certain limitations to acknowledge. First, the study primarily involved a relatively healthy older population, which may restrict the generalizability 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. Second, 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. Finally, 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.

Author contributions

Conceptualisation and methodology: MPB, JES, CvN, JIMvU, AAA, MEN, WPA, MTvdB, CMC, SPC, LGV, and MMCL; recruitment: MPB, MJA, JIMvU, and MMCL; laboratory analysis: MMT, EvA, and CMC; writing—original draft preparation: MPB; data interpretation: MPB, MMCL, and LGV; writing—review and editing: MPB, MMT, MJA, EvA, JES, CvN, EMSL, NMD, JIMvU, MS, AAA, MEN, WPA, SPM, MTvdB, CMC, SPC, LGV, and MMCL; supervision: MMCL and LGV. All authors have read and agreed to the final version of the manuscript.

Transparency declaration

MMCL reports grants or contracts as the principal investigator on the Embrace Study. LGV reports grants or contracts as the co-investigator on the Embrace Study. JES reports consulting fees from Viiv Expert Board HIV, unrelated to this manuscript; payment or honoraria from Nederlandse Internisten Vereniging: Centraal Onderwijs Interne Geneeskunde Infection and Immunity (a course for internists in training); and participation as the Chair of Dutch

Infection Prevention Guideline Committee 'Urinary Catheterization'. MEN reports payment for expert testimony for the development of guidelines for primary and secondary care, focusing on GERD and Dyspepsia, Ondansetron, and Non-Alcoholic Fatty Liver Disease, and is a committee member for these guidelines (paid to author); unpaid membership to Network Academic Primary Care, the Netherlands; the Nederlands Huisartsen Genootschap Primary Care Practice Accreditation Board; and the Advisory Board of SIR Institute for Pharmacy Practice and Policy. SPC reports royalties for textbook editing on geriatric emergency medicine, including urinary tract infections; consulting fees as clinical lead of the UK Frailty Improvement Network; and travel support for teaching or speaking on geriatric care across Europe. CMC serves as Chair of the International Federation of Clinical Chemistry and Laboratory Medicine Scientific Division (independent).

This work was supported by the Netherlands Organisation for Health Research and Development (ZonMw) [grant number 10150511910054].

Acknowledgements

The authors would like to acknowledge Renee Ruhaak and Fred Romijn for coordinating and carrying out the laboratory analysis in the Leiden University Medical Center. Moreover, we thank Hanneke Engel (Alrijne ziekenhuis), Piet Sturm (Haaglanden Medisch Centrum), John Hagenaar (Haga ziekenhuis), Margret de Koning (Onze Lieve Vrouwe Gasthuis) for coordinating the laboratory analysis in the peripheral hospitals. Furthermore, the authors thank Rolf Groenwold and Jelle Goeman for their contribution to the statistical analysis. Finally, we would like to thank the following collaborators for their contribution to participant recruitment: Brenda Elzer (Leiden University Medical Center), Lenneke Vonk (Haaglanden Medisch Centrum), Barbara de Doelder (Huisartsenpraktijk Hoekstra en de Doelder), Angelique Meskers-van Geel (Huisarstenpraktijk Meskers), Maaike Lunstroot and Marja Koppejan (Huisartsenpraktijk Hubertusduin), Johan Verloop and Els van Dijk (Marente van Wijckerslooth), Jens van Leeuwen (WZH Sammersbrug), Sander van de Haselkamp (WZH Het Anker), Fleur van Zuylen-Jongejan (Topaz Overrhyn), Sylvia van der Drif-Verbree (Topaz Revitel).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.09.023.

References

[1] Tsan L, Langberg R, Davis C, Phillips Y, Pierce J, Hojlo C, et al. Nursing home-associated infections in Department of Veterans Affairs community living centers. Am J Infect Control 2010;38:461–6. https://doi.org/10.1016/j.ajic.2009.12.009.

[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;165:2514–20. https://doi.org/10.1001/archinte.165.21.2514.

[3] Irwin DE, Milsom I, Hunskaar S, Reilly K, Kopp Z, Herschorn 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;50:1306–15. https://doi.org/10.1016/j.eururo.2006.09.019.

[4] Nicolle LE, Gupta K, Bradley SF, Colgan R, DeMuri GP, Drekonja D, et al. Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. Clin Infect Dis 2019;68:1611–5. https://doi.org/10.1093/cid/ciz021.

[5] Rodhe N, Lofgren S, Matussek A, Andre M, Englund L, Kuhn I, et al. Asymptomatic bacteriuria in the elderly: high prevalence and high turnover of strains. Scand J Infect Dis 2008;40:804–10. https://doi.org/10.1080/00365540802195242.

[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;43:772–5. https://doi.org/10.1111/j.1532-5415.1995.tb07048.x.

[7] Boscia JA, Abrutyn E, Levison ME, Pitsakis PG, Kaye D. Pyuria and asymptomatic bacteriuria in elderly ambulatory women. Ann Intern Med 1989;110:404–5. https://doi.org/10.7326/0003-4819-110-5-404.

[8] Bilsen MP, Aantjes MJ, van Andel E, Stalenhoef JE, van Nieuwkoop C, Leyten EMS, et al. Current pyuria cutoffs promote inappropriate urinary tract infection diagnosis in older women. Clin Infect Dis 2023;76:2070–6. https://doi.org/10.1093/cid/ciad099.

[9] Joseph A. The diagnosis and management of UTI in >65s: to dipstick or not? The argument against dipsticks. Infect Prev Pract 2020;2:100063. https://doi.org/10.1016/j.infpip.2020.100063.

[10] Mitchell SL, Shaffer ML, Loeb MB, Givens JL, Habtemariam D, Kiely DK, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med 2014;174:1660–7. https://doi.org/10.1001/jamainternmed.2014.3918.

[11] Rotjanapan P, Dosa D, Thomas KS. Potentially inappropriate treatment of urinary tract infections in two Rhode Island nursing homes. Arch Intern Med 2011;171:438–43. https://doi.org/10.1001/archinternmed.2011.13.

[12] Worby CJ, Schreiber HLt, Straub TJ, van Dijk LR, Bronson RA, Olson BS, et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. Nat Microbiol 2022;7:630–9. https://doi.org/10.1038/s41564-022-01107-x.

[13] Langford BJ, Brown KA, Diong C, Marchand-Austin A, Adomako K, Saedi A, et al. The benefits and harms of antibiotic prophylaxis for urinary tract infection in older adults. Clin Infect Dis 2021;73:e782—91. https://doi.org/10.1093/cid/ciab116.

[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;32:84–6. https://doi.org/10.1086/657664.

[15] Edwards G, Seeley A, Carter A, Patrick Smith M, Cross E, Hughes K, et al. What is the diagnostic accuracy of novel urine biomarkers for urinary tract infection? Biomark Insights 2023;18:11772719221144459. https://doi.org/10.1177/11772719221144459.

[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. https://doi.org/10.3205/id000068.

[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;18:10. https://doi.org/10.1186/s12014-021-09315-z.

[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;85:243–8. https://doi.org/10.1016/j.diagmicrobio.2016.03.005.

[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;27:74–9. https://doi.org/10.1080/02813430902757634.

[20] Price JR, Guran L, Lim JY, Megli CJ, Clark AL, Edwards SR, et al. Neutrophil gelatinase-associated lipocalin biomarker and urinary tract infections: a diagnostic case-control study (NUTI study). Female Pelvic Med Reconstr Surg 2017;23:101–7. https://doi.org/10.1097/SPV.0000000000000366.

[21] Shaikh N, Martin JM, Hoberman A, Skae M, Milkovich L, Nowalk A, et al. Host and bacterial markers that differ in children with cystitis and pyelonephritis. J Pediatr 2019;209. https://doi.org/10.1016/j.jpeds.2019.01.012. 146—53.

[22] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. Stard 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ 2015;351:h5527. https://doi.org/10.1136/bmj.h5527.

[23] van Duijl TT, Ruhaak LR, Hoogeveen EK, de Mutsert R, Rosendaal FR, le Cessie S, 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;59:420–32. https://doi.org/10.1177/00045632221121780.

[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;22:193. https://doi.org/10.1186/s12875-021-01530-9.

[25] Ching CB, Gupta S, Li B, Cortado H, Mayne N, Jackson AR, et al. Interleukin-6/Stat 3 signaling has an essential role in the host antimicrobial response to urinary tract infection. Kidney Int 2018;93:1320–9. https://doi.org/10.1016/j.kint.2017.12.006.

[26] Linder A, Soehnlein O, Akesson P. Roles of heparin-binding protein in bacterial infections. J Innate Immun 2010;2:431–8. https://doi.org/10.1159/000314853.

[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;291:25901–10. https://doi.org/10.1074/jbc.M116.759183.