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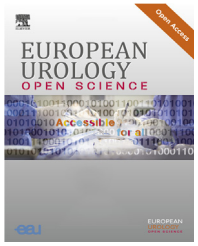

Infections

Comparison Shows that Multiplex Polymerase Chain Reaction Identifies Infection-associated Urinary Biomarker–positive Urinary Tract Infections That Are Missed by Standard Urine Culture

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Abstract

Background: Multiplex polymerase chain reaction (M-PCR) has increased sensitivity for microbial detection compared with standard urine culture (SUC) in cases diagnosed as urinary tract infections (UTIs), leading to questions whether detected microbes are likely causative of UTIs or are incidental findings.
Objective: To compare infection-associated biomarker levels against M-PCR and SUC results in symptomatic cases with a presumptive diagnosis of a UTI by a urologist.
Design, setting, and participants: Participants were ≥60 yr old and presented to urology clinics between January and April 2023 with symptoms of UTIs (n = 583). Urine microbial detection was by M-PCR and SUC. Three infection-associated biomarkers (neutrophil gelatinase-associated lipocalin, interleukin-8, and interleukin-1β) were measured by enzyme-linked immunosorbent assay. Symptomatic cases with elevated biomarkers, detection of uropathogens, and a specialist clinical diagnosis of a UTI were considered definitive UTI cases.
Outcome measurements and statistical analysis: Distributions were compared using two-sample Wilcoxon rank sum test, with two-tailed p values of <0.05 considered statistically significant.
Results and limitations: In cases with M-PCR–positive/SUC-negative results (n = 80), all median biomarker levels were significantly higher (p < 0.0001) than in cases with M-PCR–negative/SUC-negative results (n = 107). Two or more biomarkers were positive in 76% of M-PCR–positive/SUC-negative specimens. Limitation was an inability to examine associations between each individual organism and inflammation.
Conclusions: A significant number of M-PCR–positive/SUC-negative cases had elevated levels of infection-related urinary biomarkers, especially when infection

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was caused by organisms other than *Escherichia coli*. This is a strong indication that microbes detected by M-PCR, which would be missed by SUC, are associated with UTIs.

Patient summary: We compared infection-associated biomarkers in patients diagnosed with urinary tract infections (UTIs) against the detection of microorganisms by standard urine culture (SUC) and multiplex polymerase chain reaction (M-PCR). We found that most patients with microorganisms detected by M-PCR, which were missed by SUC, had elevated markers of inflammation, indicating that these organisms were likely causative of UTIs.

1. Introduction

Urinary tract infections (UTIs) are a global health care burden. In 2018, complicated UTIs (cUTIs) led to >600 000 hospitalizations in the USA, costing an average of $70 063 per hospitalization (excluding catheter-associated UTI cases) [1]. Treating UTIs is a significant reason for outpatient antibiotic prescriptions [2], with most infections treated empirically, increasing the risk of over and ineffective use of antibiotics.

The current UTI diagnostic, standard urine culture (SUC), favors the cultivation of easy-to-grow Gram-negative microbes such as *Escherichia coli*. However, this method is less favorable for the growth of non–*E. coli* microorganisms and is completely inadequate for the growth of fastidious microbes, which are increasingly being recognized as common uropathogens [3,4]. Additionally, the SUC method is significantly less likely to identify polymicrobial infections [5,6].

In previous studies, multiplex polymerase chain reaction (M-PCR) has demonstrated superiority at detecting non–*E. coli* and/or multiple microorganisms present in the urine specimens of patients while delivering a faster turnaround time for results than SUC [5–9]. Further evidence of the clinical utility of M-PCR testing in cUTI management comes from a recent study demonstrating improved patient outcomes [10].

This study correlates the detection of microbes by M-PCR and SUC with cases with elevated levels of infection-associated urine biomarkers in individuals diagnosed to have UTIs in a urology setting. The goal was to determine whether M-PCR is over-reporting microbes in cases that are not UTIs or whether SUC is under-reporting microbes in cases that are clearly UTIs. Recent publications have focused on several promising biomarkers including neutrophil gelatinase-associated lipocalin (NGAL), interleukin (IL)-8, and IL-1β [11,12]. These urinary biomarkers were included here because of their demonstrated positive correlation with active UTIs [13–17].

2. Patients and methods

2.1. Study design

This study compared biomarker levels in symptomatic patients with and without microorganisms detected in their urine by SUC and M-PCR. The purpose was to determine whether organisms detected by M-PCR represent active UTIs as defined by having elevated biomarker levels in the urine of these presumptive UTI cases, in order to address the concern that M-PCR testing may result in overdiagnosis.

The cohort consisted of 583 individuals aged 60 yr and older who presented at urology clinics in 39 US states. All participants were assigned ICD-10-CM codes in the urology specialty setting based on the clinical presentation of the patient. Only specimens sent for UTI diagnostic testing with ICD-10-CM codes for either UTIs or UTI-related conditions were selected for this study. Specimens were included from consecutive eligible patients and collected between January 17, 2023 and April 24, 2023.

Each deidentified urine sample was assigned a repository label associated with a record of the participant's age, sex, and ICD-10-CM code(s), and stored in a biorepository for evaluation at Pathnostics' (Irvine, CA, USA) clinical laboratory. The Western Institutional Review Board deemed this remnant sample study to be exempt under 45 CFR § 46.104(d)(4) as the information was used in a manner that the identity of the participant could not be readily ascertained directly or through identifiers linked to the participants, the participant was not contacted, and the investigator did not reidentify the participants. Urine samples from any previous institutional review board–approved clinical trials where the patient specifically opted out from research use of their remnant samples and corresponding deidentified data were excluded. Upon receipt at the testing laboratory (Pathnostics), each urine specimen was processed for microbial identification by M-PCR and SUC, and for a biomarker analysis by enzyme-linked immunosorbent assay (ELISA).

2.2. Specimen testing

2.2.1. Enzyme-linked immunosorbent assay

The biomarker analysis was performed using ELISA kits purchased from R&D Systems/Bio-Techne (Minneapolis, MN, USA), including human Lipocalin-2/NGAL Quantikine ELISA Kit (Catalog number SLCN20), human IL-8/CXCL8 Quantikine ELISA Kit (Catalog number S8000C), and human IL-1β/IL-1F2 Quantikine ELISA kit (Catalog number SLB50), as per the manufacturer's instructions. Biomarker "positivity" was determined using thresholds presented previously in the literature (NGAL ≥38.0 ng/ml, IL-8 ≥20.6 pg/ml, and IL-1β ≥12.4 pg/ml) [18,19]. For this analysis, "consensus" was defined as two or more biomarkers meeting the positivity threshold.

2.2.2. Standard urine culture

The testing was performed as described previously [5].

2.2.3. M-PCR and pooled antibiotic susceptibility testing

The M-PCR/pooled antibiotic susceptibility testing (P-AST) assay (Guidance UTI; Pathnostics) was performed as described previously [5,6,8]. This study did not analyze results from the P-AST portion of the test.

2.3. Statistical analysis

Analyses were performed according to two microbial density thresholds of positivity (10 000 cells/ml by M-PCR or colony forming units (CFUs)/ml by SUC, and 100 000 cells/ml or CFUs/ml). The 100 000 cells/ml or CFUs/ml threshold is traditionally considered diagnostically significant in the USA; however, clinical reviews and guidelines in addition to our data [20,21] have suggested a lower microbial density threshold of 10 000 cells/ml or CFUs/ml is clinically relevant [22,23]. Participant demographics and ICD-10-CM diagnostic code breakdown were described by summary statistics (eg, mean and standard deviation for continuous variables such as age and count, and percentage for categorical variables such as sex and ICD-10-CM codes). The distribution of all organisms detected by M-PCR and SUC was provided with count and percentage listed. Summary statistics (n, mean, and median) of biomarker results for M-PCR and SUC were compared between different groups. Summary statistics (n, mean, and median) of the polymicrobial infection status as detected by M-PCR (Guidance® UTI) were also provided. All hypothesis tests were two sided, and $p < 0.05$ was considered statistically significant. All data analyses were performed using R version 4.2.2 (R Foundation for Statistical Analysis, Vienna, Austria; https://www.r-project.org/). For the microbial density ≥100 000 CFUs/ml detected by the SUC category, the n was too small for statistical comparison.

3. Results

3.1. Participant demographics and ICD-10-CM codes

The cohort consisted of 583 unique patients, predominantly female (68.3%, n = 398), whose ages ranged from 60.0 to 99.7 yr, with a mean of 76.6 yr (standard deviation = 8.87) and a median of 76.3 yr of age (Supplementary Table 1). The most prevalent ICD-10-CM code was N39.0 for "UTI, site not specified" (n = 534, 81.8%; Supplementary Table 2).

3.2. Bacterial and yeast identification by M-PCR and SUC

M-PCR identified 883 microorganisms in the 583 specimens, indicating that many specimens (40%, n = 231) contained two or more microorganisms (polymicrobial; Supplementary Table 3). *E. coli* was detected in 188 specimens (32%) and non–*E. coli* microorganisms were detected in 221 specimens (38%). SUC identified 496 microorganisms in the 583 specimens (Supplementary Table 3). It detected *E. coli* in 160 specimens (27%) and non–*E. coli* microorganisms in 171 specimens (29%).

3.3. Biomarker levels correlate with microbial detection

Results of microbial detection were categorized into four groups: M-PCR-positive/SUC-negative (n = 86), M-PCR-negative/SUC-positive (n = 26), M-PCR-positive/SUC-positive (n = 351), and M-PCR-negative/SUC-negative (n = 120). Specimens with M-PCR negative/SUC negative results were considered negative for UTIs. NGAL, IL-8, and IL-1β biomarker levels in specimens with microbial densities of ≥10 000 cells/ml and ≥100 000 cells/ml were compared with microbe-negative specimens (Fig. 1). Biomarker results were further stratified into groups by microbes identified (*E. coli*, non–*E. coli*, and polymicrobial) to evaluate their impact on biomarker levels (Fig. 2–4, respectively).

M-PCR–positive/SUC-positive cases at microbial density thresholds of ≥10 000 cells/ml (n = 351) or ≥100 000 cells/ml (n = 244) had significantly elevated levels of all three biomarkers compared with M-PCR–negative/SUC-negative cases ($p < 0.0001$; Fig. 1 and Supplementary Table 4). At both ≥10 000 cells/ml and ≥100 000 cells/ml, M-PCR–positive/SUC-negative cases (n = 86 and n = 52, respectively) also had significantly elevated levels of all three biomarkers ($p \leq 0.005$). However, biomarker levels in M-PCR–negative/SUC-positive cases (n = 26) at a microbial density of ≥10 000 cells/ml were elevated significantly for IL-8 ($p = 0.006$) and IL-1β ($p = 0.021$), but not for NGAL ($p = 0.15$).

3.3.1. *E. coli* detection

Cases in which *E. coli* was detected by both SUC and M-PCR at ≥10 000 cells/ml (n = 157) or ≥100 000 cells/ml (n = 122; Fig. 2 and Supplementary Table 5) had significantly elevated levels of all three biomarkers ($p < 0.0001$), as well as cases in which M-PCR was positive and SUC was negative (n = 21 and n = 15, at 10 000 and 100 000 density thresholds, respectively, $p \leq 0.005$). Only three cases occurred in which SUC was positive for *E. coli* and M-PCR was negative, which had statistical significance only for the elevation of IL-8 ($p = 0.035$), and only one was observed when using the 100 000 cells/ml microbial density threshold.

3.3.2. Detection of non–*E. coli* microbes

Cases in which non–*E. coli* microorganisms were detected by both SUC and M-PCR at ≥10 000 cells/ml or CFUs/ml (n = 172) or ≥100 000 cells/ml (n = 104; Fig. 3 and Supplementary Table 6) had significantly elevated levels of all three biomarkers ($p < 0.0001$). Cases in which M-PCR was positive and SUC was negative (n = 65 and n = 37, at ≥10 000 and ≥100 000 cells/ml, respectively) also had significantly elevated levels of all three biomarkers ($p < 0.0001$). However, biomarker levels in M-PCR–negative/SUC-positive cases (n = 23) at a microbial density of ≥10 000 cells/ml were significantly elevated for IL-8 ($p = 0.026$) and IL-1β ($p = 0.018$) only, but not for NGAL ($p = 0.366$).

3.3.3. Detection of polymicrobial cases

Polymicrobial cases, those in which M-PCR detected two or more microorganisms at ≥10 000 cells/ml (n = 231), also had significantly elevated levels of all three biomarkers ($p < 0.0001$) compared with cases in which no microorganisms were detected by either SUC or M-PCR (n = 120; Fig. 4 and Supplementary Table 7).

3.4. M-PCR detects more biomarker-positive UTIs than SUC

In a previous study (paper submitted), we discovered that of the three infection-associated biomarkers, IL-8 had the highest sensitivity (91.2%) and IL-1β had the highest specificity (96.9%) for UTIs. Biomarker consensus, in which two or more biomarkers were positive, provided an ideal balance of sensitivity (84.0%) and specificity (91.2%). We examined biomarker percent positivity rates between M-PCR–positive/SUC-negative and M-PCR–negative/SUC-positive cases at a microbial

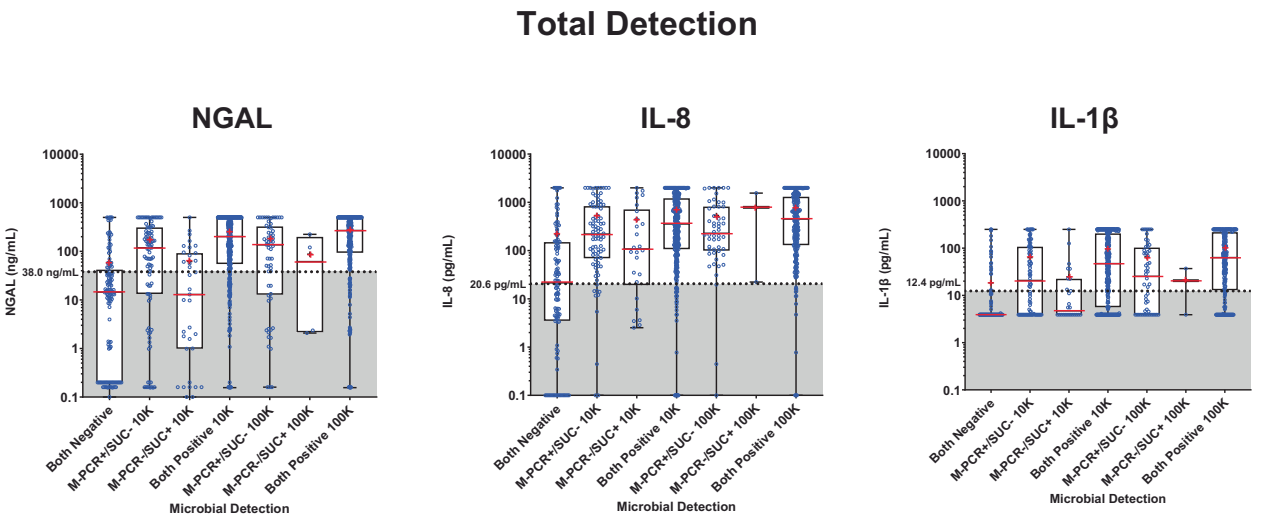

Fig. 1 – Box plots of biomarker levels with microbial detection by standard urine culture (SUC) and multiplex polymerase chain reaction (M-PCR). Detection method: Microbial density categories are along the *x* axis. Individual biomarker values measured by enzyme-linked immunosorbent assay are plotted along the *y* axis as open blue circles. Boxes extend from the first to the third quartiles, with whiskers extending to the minimum and maximum values. Within each plot, a solid red line indicates the median value and a red "+" indicates the mean. A dotted line represents the positivity threshold for each biomarker (neutrophil gelatinase-associated lipocalin [NGAL] ≥38.0 ng/ml, interleukin 8 [IL-8] ≥20.6 pg/ml, and interleukin 1 beta [IL-1β] ≥12.4 pg/ml).

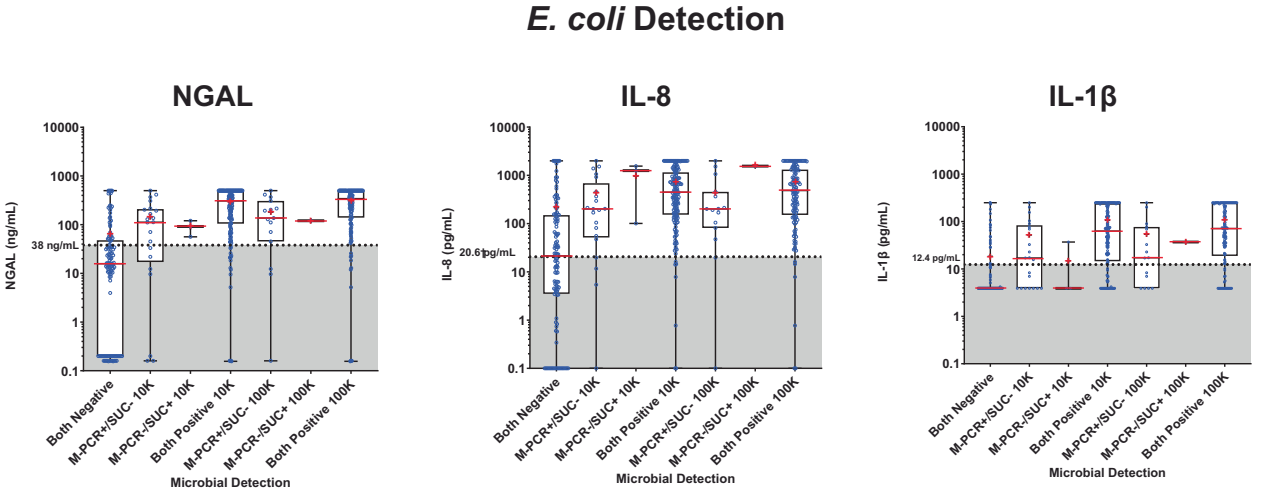

Fig. 2 – Box plots of biomarker levels with microbial detection of *E. coli cases* by SUC and M-PCR. Detection method: Microbial density categories are along the *x* axis. Individual biomarker values measured by ELISA are plotted along the *y* axis as open blue circles. Boxes extend from the first to the third quartiles, with whiskers extending to the minimum and maximum values. Within each plot, a solid red line indicates the median value and a red "+" indicates the mean. A dotted line represents the positivity threshold for each biomarker (NGAL ≥38.0 ng/ml, IL-8 ≥20.6 pg/ml, and IL-1β ≥12.4 pg/ml). ELISA = enzyme-linked immunosorbent assay; IL-1β = interleukin 1 beta; IL-8 = interleukin 8; M-PCR = multiplex polymerase chain reaction; NGAL = neutrophil gelatinase-associated lipocalin; SUC = standard urine culture.

density threshold of ≥10 000 cells/ml in the symptomatic patient study group of 583 individuals, and stratified biomarker results by the presence or absence of detectable *E. coli* and by the presence of polymicrobial infection, regardless of microbial species (Fig. 5).

Of all 86 M-PCR–positive/SUC-negative specimens, 76% overall and 77% with non–*E. coli* microorganisms had two or more positive biomarkers. In contrast, for all 23 M-PC R–negative/SUC-positive specimens, 62% overall and 57% with non–*E. coli* microorganisms had two or more positive biomarkers (Fig. 5). For cases in which *E. coli* was detected, 71% (15/21) of M-PCR–positive/SUC-negative specimens had two or more positive biomarkers. Although there were only three M-PCR–negative/SUC-positive specimens with *E. coli* identified, these all met the criteria for consensus biomarker positivity (Fig. 5).

4. Discussion

The development of accurate and rapid diagnostic testing presents an opportunity to improve antibiotic stewardship and reduce health care costs by optimizing directed treatment and reducing empiric antibiotic use [24,25]. Previous

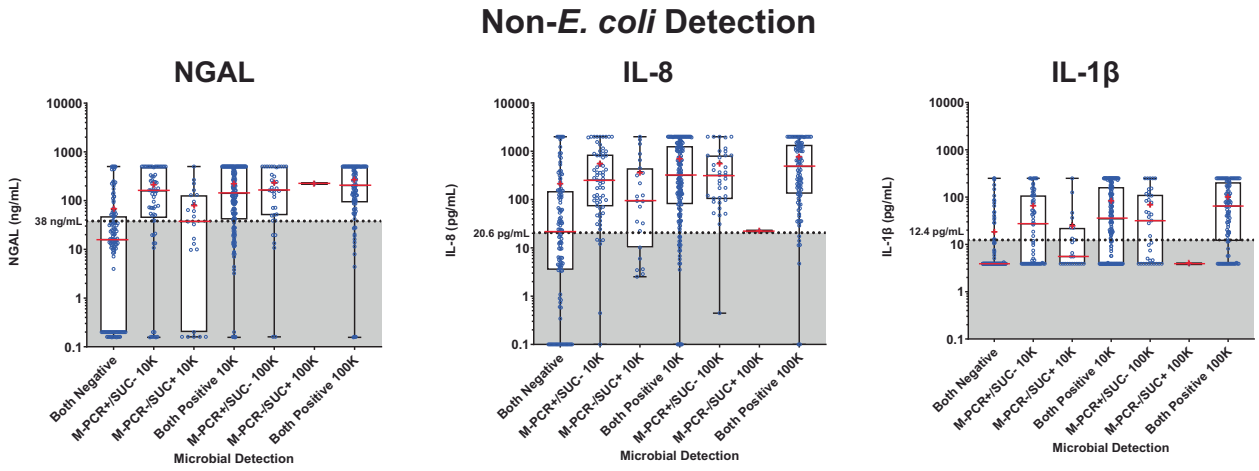

Fig. 3 – Box plots of biomarker levels with detection of non–*E. coli* microorganisms by SUC and M-PCR. Detection method: Microbial density categories are along the *x* axis. Individual biomarker values measured by ELISA are plotted along the *y* axis as open blue circles. Boxes extend from the first to the third quartiles, with whiskers extending to the minimum and maximum values. Within each plot, a solid red line indicates the median value and a red "+" indicates the mean. A dotted line represents the positivity threshold for each biomarker (NGAL ≥38.0 ng/ml, IL-8 ≥20.6 pg/ml; and IL-1β ≥12.4 pg/ml). ELISA = enzyme-linked immunosorbent assay; IL-1β = interleukin 1 beta; IL-8 = interleukin 8; M-PCR = multiplex polymerase chain reaction; NGAL = neutrophil gelatinase-associated lipocalin; SUC = standard urine culture.

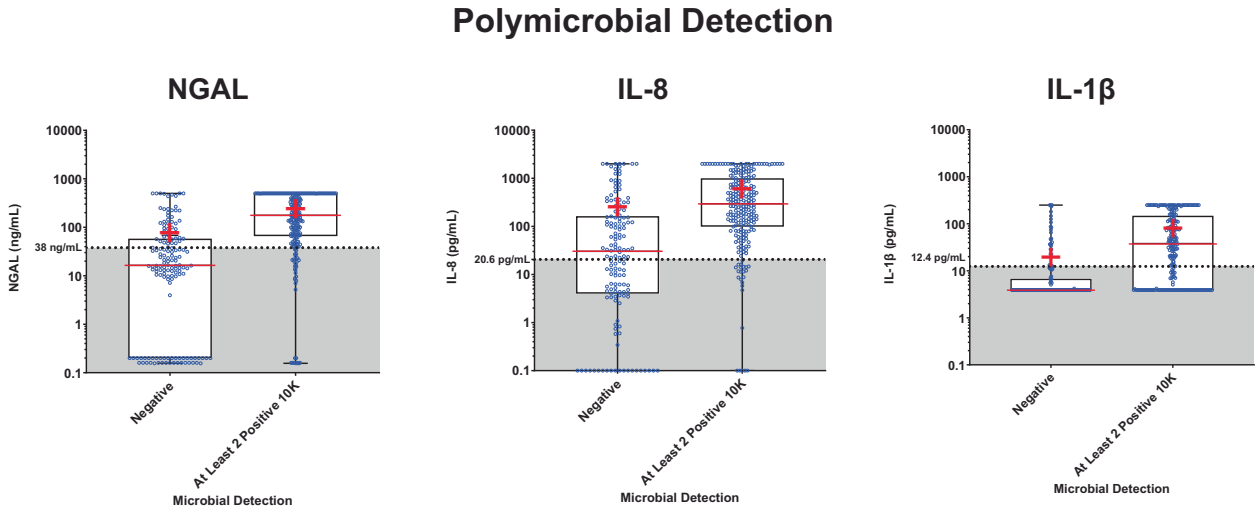

Fig. 4 – Box plots of biomarker levels with polymicrobial detection by M-PCR. Detection method: Microbial density categories are across the *x* axis. Individual biomarker values measured by ELISA are plotted along the *y* axis as open blue circles. Boxes extend from the first to the third quartiles, with whiskers extending to the minimum and maximum values. Within each violin, a solid red line indicates the median value and a red "+" indicates the mean. A dotted line represents the positivity threshold for each biomarker (NGAL ≥38.0 ng/ml, IL-8 ≥20.6 pg/ml, and IL-1β ≥12.4 pg/ml). ELISA = enzyme-linked immunosorbent assay; IL-1β = interleukin 1 beta; IL-8 = interleukin 8; M-PCR = multiplex polymerase chain reaction; NGAL = neutrophil gelatinase-associated lipocalin.

retrospective studies have demonstrated that approximately $64 239 in health care expenditures is averted when a single patient avoids hospitalization and/or emergency department visits for a UTI [26], and that the use of M-PCR/P-AST testing to guide management of UTIs was associated with a 13.7% decrease in hospital admissions and/or emergency department utilization when compared with the use of SUC testing (p = 0.003) [9]. Additionally, among Medicare enrollees, the average total 1-yr UTI-related cost was reportedly $501.85 (95% confidence interval: $79.87, $562.08; p = 0.004) lower per patient managed using M-PCR/P-AST versus SUC ($629.55 vs $1131.39), due to lower utilization of hospital, emergency department care, and urgent care [27]. This conclusion was further supported by a recent prospective study that followed 577 symptomatic adults (n = 207 males and n = 370 females) presenting to urology/urogynecology clinics and diagnosed with a cUTI and/or recurrent UTI using either M-PCR/P-AST or SUC. After patient matching for confounding factors including age and sex, the M-PCR/P-AST arm was shown to have reduced empirical treatment use (p < 0.0001), lower composite negative events (p = 0.018), and fewer individual negative outcomes of UTI-related medical provider visits and UTI-related visits for hospitalization/urgent care center/

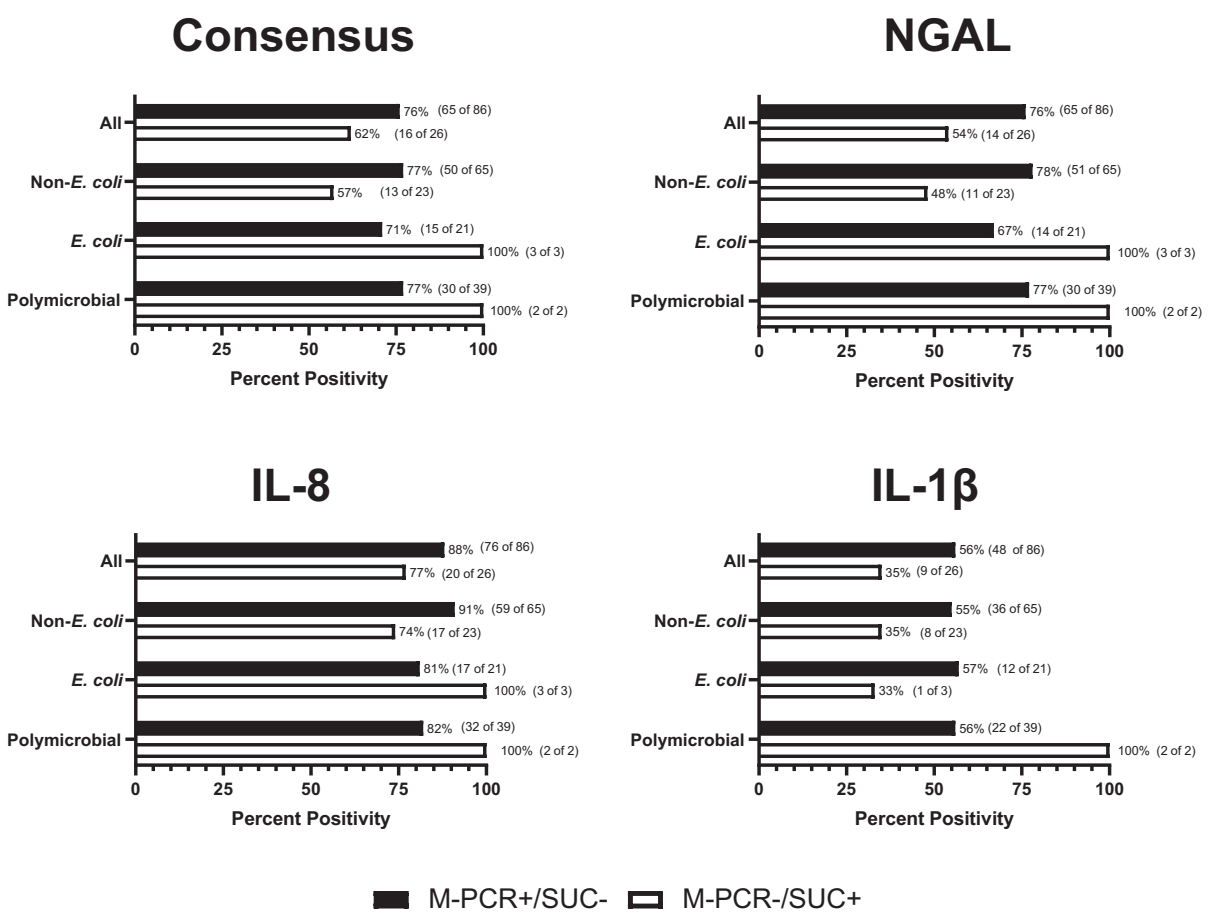

Fig. 5 – Biomarker percent positivity with microbial detection by M-PCR and SUC. Comparison of percent positivity for biomarker consensus, NGAL, IL-8, and IL-1β between specimens in which M-PCR and SUC results were discordant at a microbial density threshold of >10 000 cells/ml or CFUs/ml. Biomarker positivity was defined by previously published thresholds (NGAL ≥38.0 ng/mL, IL-8 ≥20.6 pg/ml, and IL-1β ≥12.4 pg/ml). CFU = colony forming unit; IL-1β = interleukin 1 beta; IL-8 = interleukin 8; M-PCR = multiplex polymerase chain reaction; NGAL = neutrophil gelatinase-associated lipocalin; SUC = standard urine culture.

emergency room ($p < 0.05$) compared with the SUC arm [28].

M-PCR has previously been demonstrated to be more sensitive than SUC, to provide faster results, and to result in better patient outcomes [5,6,10]. To answer the question of whether M-PCR testing results in a large number of false positives or whether SUC was underdiagnosing a significant number of UTIs, we evaluated M-PCR and SUC results with levels of three infection-associated biomarkers (NGAL, IL-8, and IL-1β) in the urine of symptomatic patients with a presumptive diagnosis of a UTI from a urology/urogynecology specialty setting. We compared the biomarker levels among specimens with no microorganisms detected by either M-PCR or SUC, with specimens that were M-PCR-positive/SUC-negative, M-PCR-negative/SUC-positive, and M-PCR-positive/SUC-positive. We evaluated two thresholds of microbe positivity: 100 000 cells/ml or CFUs/ml, which is traditionally considered diagnostically significant for UTIs in the USA, and 10 000 cells/ml or CFUs/ml, which has been suggested as more clinically relevant in recent clinical reviews and guidelines and in our own studies [20,22,23]. Since M-PCR has previously been demonstrated to be superior at detecting non–*E. coli* or polymicrobial infections [5–9], we further examined cases where *E. coli* was detected, non–*E. coli* organisms were detected, and two or more microorganisms were detected in the same specimen (polymicrobial).

We focused our analyses on cases in which the SUC and M-PCR results were discordant. From the 583 samples tested, 86 were found to be M-PCR-positive/SUC-negative. The median biomarker levels were significantly different for all three biomarkers in this group compared with negative (no infectious organisms detected). Only 26 samples were M-PCR-negative/SUC-positive, and biomarker results were not statistically different from negative, although these were somewhat higher than those of cases that were negative by both tests. Interestingly, the M-PCR–negative/SUC-positive scenario almost exclusively occurred when a threshold of ≥10 000 cells/ml by M-PCR or CFUs/ml by SUC was used and may have been reported as "negative" by SUC according to the current standard practices for the USA, which typically use a threshold of ≥100 000 CFUs/ml.

Across all M-PCR–positive/SUC-negative specimens and in those with non–*E. coli* organisms, the median level of

all three biomarkers (NGAL, IL-8, and IL-1β) was significantly higher ($p < 0.0001$) than in cases in which both M-PCR and SUC were negative, with >75% of positive cases achieving biomarker consensus positivity. M-PCR–positive/SUC-negative specimens with *E. coli* identified also exhibited elevated median biomarker levels ($p \leq 0.005$) compared with dual-negative specimens. M-PCR–negative/SUC-positive specimens had median levels of IL-8 and IL-1β significantly elevated ($p < 0.05$), but these did not exhibit elevated median NGAL levels ($p > 0.05$). This is a strong indication that in cases of disagreement, M-PCR is a more reliable indicator of infection, as indicated by universally elevated median biomarker levels and high biomarker percent positivity.

Historically, *E. coli* has been considered the primary cause of UTIs and was the most frequently detected microbial species by both SUC ($n = 160$) and M-PCR ($n = 188$) in our study [3]. However, SUC, which is optimized for the detection of nonfastidious Gram-negative uropathogens such as *E. coli,* still missed many *E. coli* cases (12%, $n = 21$), which were detected by M-PCR and had elevated biomarker levels. SUC also failed to detect a significant number of non–*E. coli* organisms routinely identified by M-PCR. Fastidious organisms, including *Aerococcus urinae* and *Actinotignum schaalii,* are being increasingly recognized as uropathogens that may cause or complicate UTIs, especially in high-risk, hospitalized, or elderly patients [29–33]. In this study, both *A. urinae* and *A. schaalii* were among the top five most prevalent organisms identified by M-PCR ($n = 116$ and $n = 118$, respectively; Supplementary Table 3). Failure to identify these organisms can result in many UTIs going untreated based on negative culture results, potentially prolonging symptoms in patients and resulting in complications, such as urosepsis, in high-risk patients.

In addition, polymicrobial infections are typically either misidentified as monomicrobial, when a single organism dominates SUC, or dismissed as "contaminated samples," when multiple organisms grow in SUC. Of the 583 specimens in this study, 40% ($n = 231$) had polymicrobial infections with two or more organisms detected at ≥10 000 cells/ml by M-PCR, and the median biomarker levels in the polymicrobial specimens were significantly elevated ($p < 0.0001$), with 77% achieving biomarker consensus positivity. This indicates that many patients symptomatic for UTIs and tested using SUC may have a polymicrobial infection that is either dismissed as contamination or misdiagnosed as a monomicrobial infection, potentially resulting in suboptimal treatment.

The unique strength of this study was the direct comparison of microbial identity and density results of the same urine specimen using both the current standard of care, SUC, and a novel molecular method, M-PCR, at two microbial density thresholds (10 000 and 100 000 cells/ml or CFUs/ml) combined with the measures of the immune response according to the biomarkers NGAL, IL-8, and IL-1β. This approach allowed us to directly associate the presence and density of microorganisms with infection-associated immune responses in the urinary tract of each patient to make comparisons between detection methods. The use of a large study population recruited through urology offices across 39 states in the continental USA further strengthened the study.

Despite the large study group size, the number of specimens containing specific individual microorganisms precluded the ability to delve into the correlation between specific microorganisms and infection-related biomarkers. Additional future studies in this area will provide further insights for the diagnosis of UTIs. Another limitation inherent to the use of biobanked urine specimens in this study was the unavailability of detailed clinical presentation/symptoms, treatment, and clinical outcome records. We have recently published a study demonstrating improved clinical outcomes with the use of the M-PCR/P-AST assay, although this study did not examine biomarker levels [34]. A future study will compare infection-associated biomarkers in conjunction with symptom, treatment, and clinical outcome data between M-PCR/P-AST and SUC.

The evidence provided by this study that microorganisms detected by M-PCR correlate with the biomarkers of infection counters the concern that M-PCR overdiagnoses UTIs.

5. Conclusions

Significant debate exists regarding the validity of M-PCR–positive/SUC-negative case results. The findings of this study indicate that >75% of M-PCR–positive/SUC-negative cases are true UTIs, as evidenced by elevated levels of urinary biomarkers in a symptomatic population with a presumptive UTI diagnosis from a urology setting. Many of these infections either were caused by organisms other than *E. coli*, especially fastidious organisms, or were polymicrobial in nature. The low sensitivity of SUC for detecting these cases, combined with a slower time to results, makes it important to strongly consider advanced UTI tests that provide improved results. This study indicates that many patients with UTI infections are likely being underdiagnosed by SUC, especially when the infection is non–*E. coli*–based or polymicrobial.

Author contributions: David Baunoch had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Baunoch.
Acquisition of data: Baunoch, Festa.
Analysis and interpretation of data: Haley, Luke, Baunoch, Mathur, Anderson, Festa.
Drafting of the manuscript: Haley.
Critical revision of the manuscript for important intellectual content: Luke, Baunoch, Mathur, Anderson.
Statistical analysis: Wang, Jiang.
Obtaining funding: Baunoch.
Administrative, technical, or material support: Luke.
Supervision: Festa.
Other: None.

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(eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Emery Haley, Natalie Luke, Mohit Mathur, Richard A. Festa, Lori Anderson, and David Baunoch are employees of Pathnostics. Jimin Wang and Yan Jiang are paid consultants of Pathnostics.

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Ethics statement: The Western Institutional Review Board exempted the study from review.

Data sharing: All relevant data are present within the manuscript text, figures, and tables, or are available by request to the authors.

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Appendix A. Supplementary data

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References

[1] Zilberberg MD, Nathanson BH, Sulham K, Shorr AF. Descriptive epidemiology and outcomes of hospitalizations with complicated urinary tract infections in the United States, 2018. Open Forum Infect Dis 2022;9:ofab591.
[2] Grigoryan L, Nash S, Zoorob R, et al. Qualitative analysis of primary care provider prescribing decisions for urinary tract infections. Antibiotics 2019;8:84.
[3] Price TK, Hilt EE, Dune TJ, Mueller ER, Wolfe AJ, Brubaker L. Urine trouble: should we think differently about UTI? Int Urogynecol J 2018;29:205–10.
[4] Price TK, Dune T, Hilt EE, et al. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol 2016;54:1216–22.
[5] Wojno KJ, Baunoch D, Luke N, et al. Multiplex PCR based urinary tract infection (UTI) analysis compared to traditional urine culture in identifying significant pathogens in symptomatic patients. Urology 2019;136:119–26.
[6] Vollstedt A, Baunoch D, Wojno K, et al. Multisite prospective comparison of multiplex polymerase chain reaction testing with urine culture for diagnosis of urinary tract infections in symptomatic patients. J Sur Urol 2020;1:JSU-102.
[7] Vollstedt A, Baunoch D, Wolfe A, et al. Bacterial interactions as detected by pooled antibiotic susceptibility testing (P-AST) in polymicrobial urine specimens. J Surg Urol 2020;1:101.
[8] Baunoch D, Luke N, Wang D, et al. Concordance between antibiotic resistance genes and susceptibility in symptomatic urinary tract infections. Infect Drug Resist 2021;14:3275–86.
[9] Daly A, Baunoch D, Rehling K, et al. Utilization of M-PCR and P-AST for diagnosis and management of urinary tract infections in home-based primary care. JOJ Urol Nephrol 2020;7:555707.
[10] Korman HJ, Baunoch D, Luke N, et al. A diagnostic test combining molecular testing with phenotypic pooled antibiotic susceptibility improved the clinical outcomes of patients with non-*E. coli* or polymicrobial complicated urinary tract infections. Res Rep Urol 2023;15:141–7.
[11] Martino FK, Novara G. Asymptomatic bacteriuria or urinary tract infection? New and old biomarkers. Int J Transl Med 2022;2:52–65.
[12] Horváth J, Wullt B, Naber KG, Köves B. Biomarkers in urinary tract infections—which ones are suitable for diagnostics and follow-up? GMS Infect Dis 2020;8:Doc24.
[13] Rodhe N, Löfgren S, Strindhall J, Matussek A, Mölstad S. Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria. Scand J Prim Health Care 2009;27:74–9.
[14] Zaki MES. Interleukin 8 is a surrogate marker for rapid diagnosis of bacteriuria. Immunol Invest 2008;37:694–703.
[15] Tyagi P, Tyagi V, Qu X, Chuang YC, Kuo HC, Chancellor M. Elevated CXC chemokines in urine noninvasively discriminate OAB from UTI. Am J Physiol Renal Physiol 2016;311:F548–54.
[16] Price JR, Guran L, Lim JY, et al. Neutrophil gelatinase–associated lipocalin biomarker and urinary tract infections. Female Pelvic Med Reconstr Surg 2017;23:101–7.
[17] Urbschat A, Obermüller N, Paulus P, et al. Upper and lower urinary tract infections can be detected early but not be discriminated by urinary NGAL in adults. Int Urol Nephrol 2014;46:2243–9.
[18] Bolignano D, Donato V, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis 2008;52:595–605.
[19] Yuan Q, Huang R, Tang L, et al. Screening biomarkers and constructing a predictive model for symptomatic urinary tract infection and asymptomatic bacteriuria in patients undergoing cutaneous ureterostomy: a metagenomic next-generation sequencing study. Dis Markers 2022;2022:7056517.
[20] Parnell LKS, Luke N, Mathur M, Festa RA, Haley E, Wang J, Jiang Y, Anderson L, Baunoch D. Elevated UTI Biomarkers in Symptomatic Patients with Urine Microbial Densities of 10,000 CFU/mL Indicate a Lower Threshold for Diagnosing UTIs. Diagnostics 2023;13:2688. https://doi.org/10.3390/diagnostics131626882.
[21] Hilt Evann E, Parnell Laura KS, Wang Dakun, Stapleton Ann E, Lukacz Emily S. Microbial Threshold Guidelines for UTI Diagnosis: A Scoping Systematic Review. Pathology and Laboratory Medicine International 2023;15:43–63. https://doi.org/10.2147/PLMI.S409488.
[22] McNulty C. PHE/NHS diagnosis of urinary tract infections. 2010. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/927195/UTI_diagnostic_flowchart_NICE-October_2020-FINAL.pdfov.uk.
[23] Kouri T, Fogazzi G, Gant V, Hallander H, Hofmann W, Guder WG. European urinalysis guidelines. Scand J Clin Lab Invest 2000;60:1–96.
[24] Sabih A, Leslie SW. Complicated urinary tract infections. StatPearls [Internet]. January 1, 2022. https://www.ncbi.nlm.nih.gov/books/NBK436013/.
[25] Balasubramanian S, Wang X, Sahil S, Cheng A, Sutkin G, Shepherd JP. Risk factors for the development of acute pyelonephritis in women with a positive urine culture. Neurourol Urodyn 2022;41:1582–9.
[26] Turner RM, Wu B, Lawrence K, Hackett J, Karve S, Tunceli O. Assessment of outpatient and inpatient antibiotic treatment patterns and health care costs of patients with complicated urinary tract infections. Clin Ther 2015;37:2037–47.
[27] Ko DSC, Lukacz ES, Juster IA, et al. Real-world evidence that a novel diagnostic combining molecular testing with pooled antibiotic susceptibility testing is associated with reduced infection severity and lower cost compared with standard urine culture in patients with complicated or persistently recurrent urinary tract infections. Ju Open Plus 2023;1:e00021.
[28] Haley E, Luke N, Korman H, et al. Improving patient outcomes while reducing empirical treatment with multiplex-polymerase-chain-reaction/pooled-antibiotic-susceptibility-testing assay for complicated and recurrent urinary tract infections. Diagnostics 2023;13:3060.
[29] Higgins A, Garg T. *Aerococcus urinae*: an emerging cause of urinary tract infection in older adults with multimorbidity and urologic cancer. Urol Case Rep 2017;13:24–5.
[30] Senneby E, Petersson AC, Rasmussen M. Clinical and microbiological features of bacteraemia with *Aerococcus urinae*. Clin Microbiol Infect 2012;18:546–50.

[31] Greninger AL, Streithorst J, Chiu CY, Miller S. First complete genome sequence of *Corynebacterium riegelii*. Genome Announc 2017;5: e00084-17.
[32] Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Microbiol Spectr 2016;4.
[33] Lotte R, Lotte L, Ruimy R. Actinotignum schaalii (formerly Actinobaculum schaalii): a newly recognized pathogen—review of the literature. Clin Microbiol Infect 2016;22:28–36.
[34] Haley E, Luke N, Korman H, Baunoch D, Wang D, Zhao X, Mathur M. Improving Patient Outcomes While Reducing Empirical Treatment with Multiplex-Polymerase-Chain-Reaction/Pooled-Antibiotic-Susceptibility-Testing Assay for Complicated and Recurrent Urinary Tract Infections. Diagnostics 2023;13:3060. https://doi.org/10.3390/diagnostics13193060.