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

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Part II

Diagnostic challenges

Chapter 4

Current pyuria cut-offs promote inappropriate urinary tract infection diagnosis in older women

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Abstract

Background

Pre-existing lower urinary tract symptoms (LUTS), cognitive impairment and the high prevalence of asymptomatic bacteriuria (ASB) complicate the diagnosis of urinary tract infection (UTI) in older women. The presence of pyuria remains the cornerstone of UTI diagnosis. However, over 90% of ASB patients have pyuria, prompting unnecessary treatment. We quantified pyuria by automated microscopy and flowcytometry to determine the diagnostic accuracy for UTI and to derive pyuria thresholds for UTI in older women.

Methods

Women ≥ 65 years with ≥ 2 new-onset LUTS and one uropathogen ≥ 10^4 colony-forming units/mL (CFU/mL) were included in the UTI-group. Controls were asymptomatic and classified as ASB (one uropathogen ≥ 10^5 CFU/mL), negative culture or mixed flora. Patients with an indwelling catheter or antimicrobial pretreatment were excluded. Leukocyte medians were compared and sensitivity-specificity pairs were derived from a receiver operating characteristic-curve.

Results

We included 164 participants. UTI patients had higher median urinary leukocytes compared to control patients (microscopy: 900 versus 26 leukocytes/µl; flowcytometry 1575 versus 23 leukocytes/µl, $p < 0.001$). Area under the curve was 0.93 for both methods. At a cut-off of 264 leukocytes/µl, sensitivity and specificity of microscopy were 88% (positive and negative likelihood ratio 7.2 and 0.1, respectively). The commonly used cut-off of 10 leukocytes/µl had a poor specificity (36%) and a sensitivity of 100%.

Conclusion

The degree of pyuria can help to distinguish UTI in older women from ASB and asymptomatic controls with pyuria. Current pyuria cut-offs are too low and promote inappropriate UTI diagnosis in older women.

Introduction

Urinary tract infection (UTI) incidence increases with age and is higher in women than in men. [1] In older women, diagnosing UTI is complicated for several reasons. Firstly, symptom communication may be affected by cognitive impairment. Secondly, pre-existing lower urinary tract symptoms (LUTS), such as urinary incontinence and urgency, are common and distinguishing acute from chronic LUTS can be challenging. [2] Finally, 20% of community-dwelling and 50% of institutionalised older women have asymptomatic bacteriuria (ASB), defined as the presence of one or more uropathogens ≥ 10^5 colony-forming units per millilitre (CFU/mL) in the absence of signs or symptoms attributable to UTI. [3-5] As a result, inappropriate antimicrobial treatment is common, leading to unnecessary side effects, drug interactions, *Clostridioides difficile* infection and the selection of antimicrobial resistant pathogens. [6, 7] Distinguishing ASB from UTI is further complicated by the fact that over 90% of older women with ASB have concomitant pyuria. [8, 9] Consequently, the positive predictive value of the presence of pyuria for UTI is low in older women. However, it is unclear whether the degree of pyuria differs between older women with UTI and ASB, partly because urine dipstick is the most ordered screening test, providing only semi-quantitative results of leukocyte esterase activity. Pyuria can be quantified in different ways. Initially, Mabeck et al. [10] found that a leukocyte excretion rate of 400,000 per hour could distinguish UTI from asymptomatic women. This rate corresponds with a cut-off value of 10 leukocytes/mm^3 in unspun urine. [11] In clinical practice and research, pyuria is most often quantified by direct or automated microscopy of (un)spun urine, usually after initial dipstick screening. Automated microscopy reduces variability in centrifugation and resuspension of urine and is more efficient than direct microscopy. [12] In recent years, an increasing amount of laboratories are adopting urine flowcytometry for quantification of pyuria. Although cut-off values for 'significant' pyuria vary in the literature and depend on quantification methods, commonly accepted cut-offs include 10 leukocytes/µl and 5-10 leukocytes per high-powered field (hpf). These cut-off values are largely derived from studies involving non-pregnant premenopausal women, in whom ASB is uncommon. [13] The objective of this study was to determine sensitivity and specificity of automated microscopy and urine flowcytometry for diagnosing UTI in older women, with the ultimate goal to derive optimal cut-off values for pyuria for UTI in this population, taking ASB into account.

Methods

This study is an exploratory analysis of an overarching, case-control study registered at the International Clinical Trials Registry Platform (NL9477). The study was conducted across five hospitals (four regional and one academic), four long-term care facilities (LTCF), three primary care centres, one after-hours primary care clinic, and fourteen senior housing facilities. This study was approved by the regional medical ethics committee (METC-LDD) and was conducted in accordance with the declaration of Helsinki. [14] Written informed consent was obtained from all participants.

Study population

Women aged ≥ 65 years were eligible for inclusion. Exclusion criteria included inability to express symptoms (e.g. due to delirium or cognitive impairment), the presence of an indwelling catheter, immunosuppressive use, antimicrobial use (< 48 hours prior to inclusion), current urolithiasis, and a UTI in the previous month. Stringent criteria were applied to both UTI and control patients, as a consensus-based reference standard for UTI is currently missing. To be eligible for the UTI group, patients were required to have at least two new-onset LUTS (dysuria, frequency, urgency, or suprapubic pain). Furthermore, patients were required to have pyuria, defined as ≥ 10 leukocytes/µl or ≥ 5 leukocytes/hpf or presence of leukocyte esterase, and a monoculture, i.e. one uropathogen ≥ 10^4 CFU/mL for the primary analysis. Enterobacterales, enterococci, *Pseudomonas aeruginosa, Staphylococcus saprophyticus,* and group B streptococci were considered uropathogens. In case of a temperature ≥ 38.0 °C, patients were classified as having an upper UTI. Community-dwelling women and LTCF residents who did not have any LUTS or fever were eligible as controls. Patients were eligible regardless of urine culture results and they were subdivided into three subgroups: ASB, negative culture and mixed flora. ASB was defined as at least two consecutive urine cultures (2 – 4 weeks apart) with the same uropathogen ≥ 10^5 CFU/mL, and a negative culture was defined as no growth or growth of non-pathogenic micro-organisms ≤ 10^3 CFU/mL. Cases and controls were not matched for age or comorbidities.

Study procedures and methods of measurement

The study team was contacted by the treating physician in case of a potential participant at the emergency department, LTCF or primary care office. Asymptomatic LTCF residents were asked to participate by their elderly-care

physician; community-dwelling older women were recruited through flyers. If eligibility criteria were met, participants were visited by the study team within 1 hour. Baseline data included: age, prior medical history (hypertension, chronic kidney disease, diabetes mellitus and urological history), new-onset LUTS and fever. All patients underwent a delirium screening and assessment of dependency in activities of daily living (ADL) through 4AT and Katz questionnaires respectively, and measurement of vital signs. [15, 16]

Urinalysis

Midstream urine was collected in a 100 mL sterile urine container. Urine obtained via single catheterisation was accepted, urine collected from a bedpan was not. After collection, the urine was divided into two V-monovette 10 mL urine tubes (Sarstedt, Nümbrecht, Germany), one for automated microscopy and one for urine flowcytometry. Automated microscopy was performed using the Cobas U701 (Roche, Rotkreuz, Switzerland). [17] After mixing by the analyser, 170 µl of urine was injected into a polycarbonate cuvette. Next, a monolayer of cells was created by centrifuging the cuvette for 10 seconds at 260 g. Cobas U701 output included quantitative measures of leukocytes in cells/µl with a lower limit of detection (LLD) of 1 cell/µl and an upper limit of detection (ULD) of 900 cells/µl. Urine flowcytometry was carried out with the Sysmex UF-4000 (Sysmex, Kobe, Japan). Within the analyser, fluorescent dyes were added to 450 µl of urine, after which urine particles were quantified and classified by analysis of scattered light patterns. LLD was 1 leukocyte/µl and ULD was 10,000 leukocytes/µl. All urine samples were analysed in the Leiden University Medical Center, except for urine samples of the participants who were included in regional hospitals. In the latter case, urine was analysed in the corresponding regional hospital by automated microscopy, as urine flowcytometry was not available. All urine samples were kept at room temperature and analysed within 4 hours of micturition to ensure stability of all urine components.

Microbiological assessments

The remaining urine in the sterile container was used for bacteriological culture at the microbiology department. For all included UTI and control patients, 10 µL of non-centrifuged urine was placed on routine culture media and incubated for one day. A culture result was deemed positive in case of growth ≥ 10^4 CFU/

mL and defined as a monoculture if ≥ 90% of the cultured colonies were of one micro-organism.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM, Armonk, USA). Data are presented as percentages, means with standard deviations, or medians with interquartile ranges as appropriate. A Mann-Whitney U test was performed to compare leukocyte medians between UTI patients and controls. As a pyuria threshold for UTI in older women is not known, sensitivity-specificity pairs with associated 95% confidence intervals (95% CI) were calculated for all possible cut-offs and plotted in a receiver operating characteristic (ROC) curve using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California USA). The area under the curve (AUC) was calculated to determine the discriminative ability of the index tests (automated microscopy and urine flowcytometry). Youden's index was used to determine the cut-off value with the optimal trade-off between sensitivity and specificity. In a fraction of UTI cases automated microscopy results were missing, e.g. only semi-quantitative results were available (leukocyte esterase or leukocytes/hpf). The impact of missing automated microscopy results on estimates of accuracy was evaluated by a sensitivity analysis consisting of best- and worst-case scenarios (all missing pyuria results were either considered true positive and negative or false positive and negative respectively). Twenty-seven patients presenting with LUTS were not included in the primary analysis because they did not meet the urine culture criteria for the UTI group. Their urine leukocyte counts were evaluated separately, in the secondary analysis.

Results

Of the 213 screened participants, 199 were eligible for inclusion, of which 164 were included in the primary analysis (**Figure 1**). Baseline characteristics are summarised in **Table 1**. UTI and control groups were comparable in terms of age (overall mean 78.3 years) and comorbidities. Inclusion sites differed between UTI and control groups, e.g. 11% of UTI patients versus 43% of controls were included in a LTCF. ADL dependency scores were comparable. Within the UTI group, the most common new-onset symptom was frequency, followed by urgency and dysuria; 13/63 patients (21%) had an upper UTI.

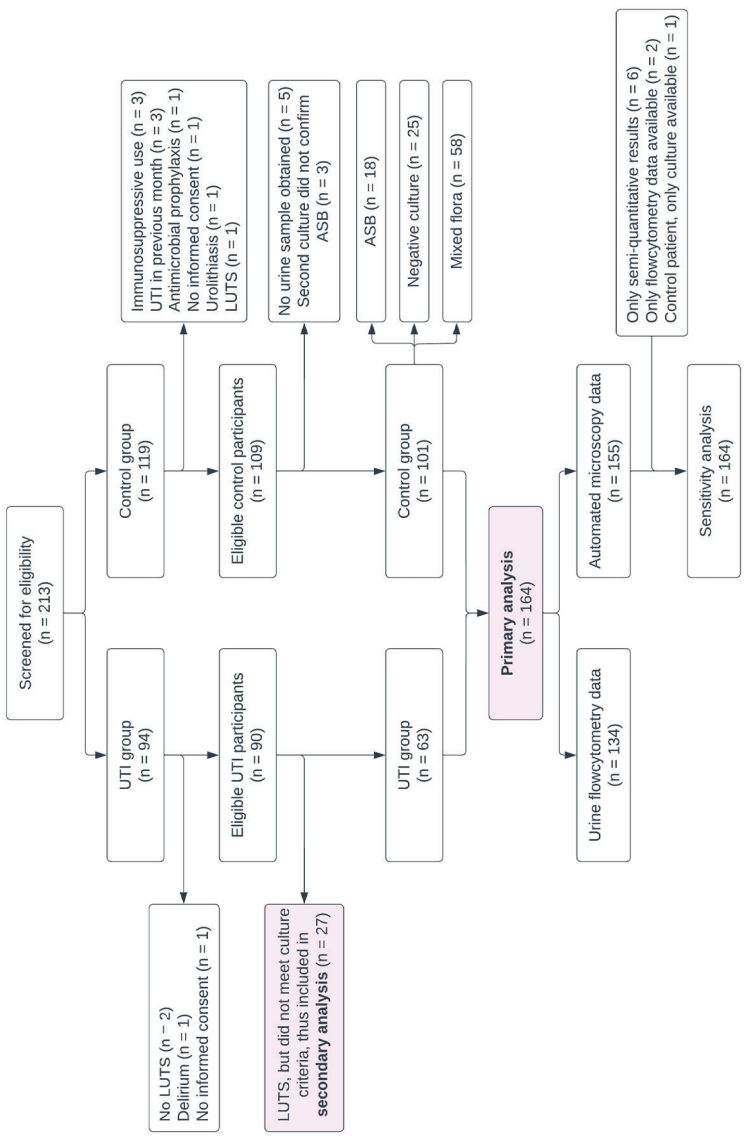

Figure 1: Overview of screening and selection process. All patients in the control group were asymptomatic. Abbreviations: UTI = urinary tract infection, LUTS = lower urinary tract symptoms, ASB = asymptomatic bacteriuria

Table 1: Baseline characteristics of UTI patients and controls.

Baseline characteristics	UTI (n = 63)	Controls (n = 101)
Age in years	77.1 (8.0)	79.0 (8.0)
Setting		
Hospital	18 (28.6)	0
LTCF	7 (11.1)	43 (42.6)
Primary care office	38 (60.3)	0
At home	0	58 (57.4)
Urological history		
Cystocele/rectocele	3 (4.7)	3 (3.0)
Previous urolithiasis	2 (3.2)	1 (1.0)
Previous kidney/bladder malignancy	1 (1.6)	1 (1.0)
Urinary incontinence procedure	1 (1.6)	2 (2.0)
Bladder sphincterotomy	0	1 (1.0)
Other comorbidity		
Diabetes mellitus	14 (22.2)	14 (13.9)
Hypertension	30 (47.6)	47 (46.5)
History of CKD	12 (19.0)	11 (10.9)
UTI history		
Ever had UTI	57 (90.5)	77 (76.2)
Ever hospitalised for UTI	2 (3.2)	1 (1.0)
Number of UTI in past year	1 (0 – 2)	0 (0 – 0)
Antibiotics in previous month	16 (25.4)	20 (19.8)
New-onset symptoms	63 (100)	0
Dysuria	49 (77.8)	-
Frequency	57 (90.5)	-
Urgency	53 (84.1)	-
Suprapubic pain	43 (68.3)	-
Urethral pain	33 (52.4)	-
Flank pain	12 (19.0)	-
New/worsening urinary incontinence	31 (49.2)	-
Recognition of symptoms	46 (73.0)	-
Fever (≥ 38.0)	13 (20.6)	-
ADL-dependency ≥ 2 Katz-items	14 (22.2)	23 (23.8)

Age is expressed as mean (SD), number of UTI in past year as median (IQR), and all other variables are expressed as n (%). The living situation of hospitalised UTI patients was unknown. History of CKD was self-reported. One UTI patient had had renal cell carcinoma twelve years prior, and one control patient had had non-muscle-invasive bladder cancer two years prior. In both patients, there was no evidence of active malignancy. Fever was objectified, 13 patients had an upper UTI. Abbreviations: UTI = urinary tract infection, LTCF = long-term care facility, CKD = chronic kidney disease, ADL = activities of daily living

Nearly all urine samples were midstream samples (162/164 (98.8%)). ASB prevalence in our control group was 18%. Within the UTI group, *E. coli* was the most common causative pathogen (81%), followed by *Klebsiella* spp. (4.8%), and *Proteus mirabilis* (4.8%). Two episodes were caused by extended-spectrum beta-lactamase-producing *E. coli*. In 78% of UTI episodes colony counts were ≥ 10^5 CFU/mL. ASB was caused by *E. coli* in 14 cases (78%), other pathogens included *Klebsiella* spp., *Enterococcus faecalis* and streptococci.

Median urine leukocyte values

Median urine leukocyte values in UTI patients and controls are displayed in **Table 2**. UTI patients had higher median leukocyte levels compared to control patients with both quantification methods (automated microscopy: 900 versus 26 leukocytes/µl ($p < 0.001$), and urine flowcytometry 1575 versus 23 leukocytes/µl ($p < 0.001$)). Moreover, median leukocyte values were higher for UTI patients than for ASB patients (automated microscopy: 900 versus 296 leukocytes/µl ($p = 0.002$), urine flowcytometry 1575 versus 197 leukocytes/µl ($p = 0.004$)), although interquartile ranges of these groups overlap.

Table 2: Median urine leukocyte values of UTI patients and controls (with subgroups), measured by automated microscopy and urine flowcytometry.

	UTI group	**Control group**		
	UTI (n = 56)	**ASB** (n = 18)	**Neg. culture** (n = 24)	**Mixed flora** (n = 57)
Automated microscopy in cells/µl, median (IQR)	900 (430 – 900)	296 (49 – 773)	4 (1 – 30)	18 (5 – 57)
	UTI (n = 35)	**ASB** (n = 17)	**Neg. culture** (n = 24)	**Mixed flora** (n = 58)
Urine flowcytometry in cells/µl, median (IQR)	1575 (581 – 4673)	197 (43 – 1368)	6 (1 – 35)	20 (4 – 88)

All values are expressed as median (IQR) as leukocyte values did not follow a normal distribution. The UTI column contains both lower and upper UTI patients. Urine flowcytometry data was missing for 28 UTI patients as they were included in regional hospitals in which urine flowcytometry was not available. For automated microscopy values, 900 cells/µl was the upper limit of detection. Abbreviations: UTI = urinary tract infection, ASB = asymptomatic bacteriuria

Diagnostic accuracy

ROC curves for automated microscopy and urine flowcytometry are displayed in **Figure 2A** and **Figure 2B** and contingency tables for sensitivity and specificity calculations are shown in **Supplementary Tables 1A and 1B**. AUC was 0.93 for both diagnostic methods. At a threshold of 264 leukocytes/µl, sensitivity of automated microscopy was 88% (95% CI 77% - 94%) and specificity was 88% (95% CI 80% - 93%), corresponding with a positive likelihood ratio (LR) of 7.2 and a negative LR of 0.1. For urine flowcytometry, sensitivity was 91% (95% CI 79% - 98%) and specificity was 86% (95% CI 78% - 92%) at a cut-off value of 231 leukocytes/µl, with a positive LR of 6.5 and a negative LR of 0.1. Diagnostic accuracy parameters for several theoretical pyuria thresholds are shown in **Table 3**. Applying the currently used cut-off of 10 leukocytes/µl resulted in a sensitivity of 100% (95% CI 94% - 100%) and specificity of 36% (95% CI 28% - 48%). Diagnostic accuracy remained adequate in the sensitivity analysis (**Supplement 2**). The secondary analysis showed that symptomatic patients with mixed flora or ≥ 2 uropathogens all had urine leukocyte counts above our 'optimal' pyuria threshold (264 leukocytes/µl), and all but two patients with negative cultures had counts below this threshold (**Supplement 2**).

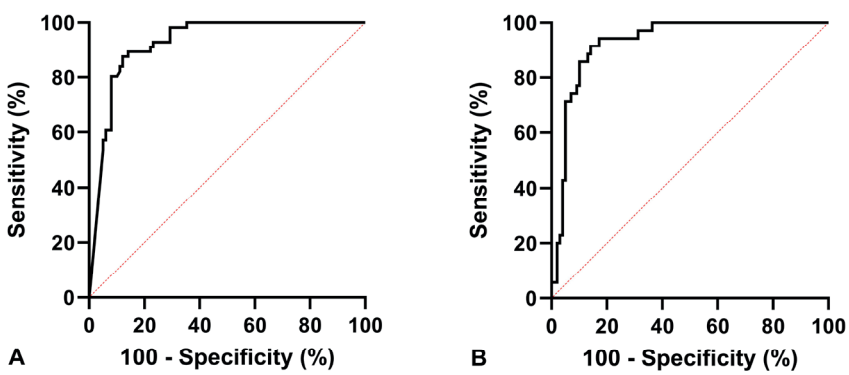

Figure 2: Receiver operating characteristic curves for automated microscopy (A) and urine flowcytometry (B). For both diagnostic methods, the number of leukocytes (per µl) was used as the test variable, and our stringent UTI definition was used for determining disease status. The true positive rate (sensitivity) was plotted against the false positive rate (1 – specificity) for different pyuria cut-offs. The area under the curve was 0.93 for both methods. The reference line is represented by the dotted line.

Table 3: Sensitivity, specificity, and positive and negative likelihood ratios of theoretical pyuria thresholds for diagnosing UTI in older women.

	10 cells/µl	50 cells/µl	100 cells/µl	200 cells/µl	300 cells/µl	400 cells/µl
Sensitivity % (95% CI)	100 (94 – 100)	98 (92 – 100)	93 (84 – 98)	89 (80 – 96)	84 (73 – 92)	77 (65 – 87)
Specificity % (95% CI)	36 (28 – 48)	66 (56 – 75)	71 (61 – 79)	86 (78 – 92)	88 (81 – 93)	92 (86 – 96)
LR_{pos} (95% CI)	1.6 (1.4 – 1.9)	2.9 (2.2 – 3.8)	3.2 (2.3 – 4.3)	6.3 (3.9 – 10.3)	6.9 (4.0 – 11.9)	9.5 (4.8 – 18.7)
LR_{neg} (95% CI)	0.0 (0.0 – 0.1)	0.03 (0.004 – 0.2)	0.1 (0.04 – 0.3)	0.1 (0.06 – 0.3)	0.2 (0.1 – 0.3)	0.3 (0.2 – 0.4)

Diagnostic accuracy parameters are based on automated microscopy results. The currently used cut-off value for pyuria is 10 leukocytes/µl. Abbreviations: LR_{pos} = positive likelihood ratio, LR_{neg} = negative likelihood ratio, CI = confidence interval

Discussion

This explorative study has two important findings. Firstly, we show that the degree of pyuria – quantified by automated microscopy or urine flowcytometry – can help to distinguish UTI in older women from asymptomatic controls, including ASB. Secondly, we demonstrate that the currently used cut-off for pyuria (10 leukocytes/µl) has a very low specificity for UTI in older women, and therefore should not be applied to this population.

Leukocyte counts in UTI

Thus far, the degree of pyuria in UTI and ASB has not been assessed specifically in women aged 65 and over, while a discriminative biomarker is arguably most needed in this population, due to the high prevalence of ASB. In our study, older women with symptomatic UTI had high median urine leukocyte counts (900 and 1575 leukocytes/µl with automated microscopy and urine flowcytometry, respectively). Both Pieretti et al. [18] and Kim et al. [19] quantified pyuria with urine flowcytometry in men and women of all ages, although no separate leukocyte values were given for older patients. Among patients with positive urine cultures, they found median urine leukocyte values of 117 leukocytes/µl and 189 leukocytes/µl, respectively. However, neither of these studies collected clinical data, so misclassification is likely. The discrepancy between urine leukocyte values between these studies and our cohort is likely explained by the fact that we only included cases that met our strict UTI criteria.

Leukocyte counts in ASB

In our study, women with ASB had median counts of 296 leukocytes/µl. Cai et al. [20] included premenopausal women with ASB and a history of recurrent UTI and quantified pyuria with direct microscopy. At baseline, these patients had median urine leukocyte values of 19 per hpf, which corresponds to approximately 100 leukocytes/µl. [21, 22]. This study suggests that higher degrees of pyuria, well above 10 leukocytes/µl, do not necessarily mean that a patient has a UTI, even in premenopausal women. Moreover, urine leukocyte values increased to 54 per hpf (approximately 250 leukocytes/µl) if women developed LUTS during the study and had a positive urine culture. This is in line with our findings that the degree of pyuria is higher in symptomatic patients with positive urine cultures.

Diagnostic accuracy of microscopy and flowcytometry

The majority of UTI studies investigating the discriminative ability of automated microscopy and urine flowcytometry are limited by the absence of a reference standard for UTI. As a consequence, these studies choose a positive urine culture as the reference test, while this does not discriminate between UTI and ASB. Instead, Foudraine et al. [23] defined UTI with an expert panel, taking symptoms and urine culture results into account. They found that automated microscopy had a sensitivity of 86% and a specificity of 82% at a cut-off value of 74 leukocytes/µl. As their study population was younger and antibiotic pretreatment was common, possibly explaining lower pyuria levels, results may not be directly comparable to our study. Diagnostic accuracy parameters are influenced by the studied population, more specifically, how cases and controls are defined. Our control group did not only consist of asymptomatic women with negative urine cultures but rather represents the distribution of urine culture results in asymptomatic older women. For example, the prevalence of ASB in our control group (18%) is very similar to the prevalence of ASB in community-dwelling older women. [4]

Leukocyte counts in symptomatic patients with mixed flora

Our case group only consisted of clear-cut UTI patients fulfilling our stringent criteria. However, urine leukocyte levels were also determined in the ‘suspected UTI’ patients that had new-onset LUTS, but were excluded from the primary analysis because they did not meet our culture criteria. Intriguingly, all excluded patients with either mixed flora or two uropathogens had leukocyte levels above our ‘optimal’ pyuria threshold and all but two patients with negative urine

cultures had levels below that threshold. The finding that all symptomatic patients with mixed flora had high degrees of pyuria, suggests that these patients might have had a true UTI. This is supported by a study showing that over 90% of symptomatic women with *E. coli* as part of mixed flora in their midstream urine cultures actually had *E. coli* bladder bacteriuria as demonstrated by single catheterisation. [9]

Clinical implications

In asymptomatic controls, median urine leukocyte values were higher than the most commonly used cut-off value of 10 leukocytes/µl. Therefore, applying the current pyuria threshold to older women leads to misclassification of many of these women, both with and without ASB. This has several consequences. Firstly, the true cause of the symptoms (e.g. vaginal atrophy, Candida vulvovaginitis, and overactive bladder) remains unidentified and thus untreated if symptoms are wrongfully attributed to UTI. Secondly, it leads to overprescription of antimicrobials, contributing to gut dysbiosis, side effects and selection of resistant pathogens. Gupta et al. [24] show that 25% of asymptomatic patients with pyuria on routine preoperative urinalysis (without urine cultures) were treated with antimicrobials, and that the degree of pyuria predicted prescribing of antimicrobials. These findings, combined with our own data, imply that separate, higher reference values are needed for older women with regards to pyuria. For instance, a threshold of 300 leukocytes/µl would be a considerable improvement, increasing specificity to avoid overtreatment, while still maintaining a fair sensitivity. As in any diagnostic test, pyuria levels should be interpreted within the clinical context of individual patients and should not be the only deciding factor when diagnosing UTI. Since both older women with UTI and asymptomatic older women have a high pretest probability of pyuria, and leukocyte esterase activity is a very rough estimate of the absolute number of leukocytes in the urine [21], the role of urine dipsticks in older patients should, at best, be limited to ruling out UTI. Besides clinical implications, there are also implications for research, as misclassification influences the validity of UTI studies.

Strengths and limitations

Strengths of our study include the use of a stringent UTI definition instead of urine culture as a reference standard, the consistency of results across two quantification

methods (identical AUCs), inclusion of participations from multiple settings, and the rapid analysis of urine samples, increasing reliability of results. Our study has several limitations. Results may not be generalisable to institutionalised older people with high frailty and/or advanced dementia. However, our population was chosen to prove a concept for which a clear definition and reliable assessment of UTI and ASB was deemed necessary. Moreover, our control group contained a higher proportion of LTCF residents than our UTI group. Nonetheless, ADL dependency scores were similar between the UTI and control groups, and median leukocyte values within the LTCF subgroup were comparable to the values of the overall group.

Conclusion

In conclusion, the degree of pyuria should be taken into account when evaluating older women for UTI. Current pyuria cut-offs for UTI are too low and promote inappropriate UTI diagnosis in this population, affecting patient care, antimicrobial stewardship efforts and research. The impact of higher cut-off values on prescription behaviour and UTI related outcomes in older women deserves further study.

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Author contributions

Conceptualisation and methodology M.P.B., J.E.S., C.N., 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., M.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.J.A., E.A., J.E.S., C.N., E.M.S., N.M.D., M.S., 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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Conflicts of interest

None of the authors have an association that might pose a conflict of interest.

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Supplement

Supplementary Table 1A: Cross tabulation of automated microscopy results against reference standard.

	UTI	Control	Total
Positive (≥ 264 leukocytes/µl)	49	12	61
Negative (< 264 leukocytes/µl)	7	87	94
Total	56	99	155

Index test results (automated microscopy) are displayed in the left column. For calculation of sensitivity/specificity and likelihood ratios, all values below 264 leukocytes/µl were considered negative, and all values of 264 leukocytes/µl and higher were considered positive

Supplementary Table 1B: Cross tabulation of urine flowcytometry results against reference standard.

	UTI	Control	Total
Positive (≥ 231 leukocytes/µl)	32	14	46
Negative (< 231 leukocytes/µl)	3	85	88
Total	35	99	134

Index test results (urine flowcytometry) are displayed in the left column. For calculation of sensitivity/specificity and likelihood ratios, all values below 231 leukocytes/µl were considered negative, and all values of 231 leukocytes/µl and higher were considered positive.

Supplement 2

Sensitivity analysis

In the best-case scenario, i.e. missing automated microscopy data being either true positive or true negative, sensitivity and specificity were 89% (95% CI 80% – 95%) and 88% (95% CI 81% - 94%) respectively. In the worst-case scenario, sensitivity and specificity were 78% (95% CI 67% - 87%) and 86% (95% CI 79% - 92%).

Secondary analysis of cases not meeting culture criteria

Of the 27 patients who were not included in the primary analysis, four patients did not have pyuria. All four of these patients had negative urine cultures, strongly suggesting that their symptoms were caused by a condition other than UTI. The remaining 23 patients did have pyuria but had either mixed flora or ≥ 2 uropathogens (n = 9), or negative cultures (no growth or growth of non-pathogenic micro-organisms, n = 14). Urine leukocyte levels were available for 20/23 (87%) patients. The remaining three patients either had only dipstick results available (leukocyte esterase positive) or pyuria could not be reliably quantified due to macroscopic haematuria. Median urine leukocyte values were 900 leukocytes/µl (IQR 745 -900) in patients with mixed flora or ≥ 2 uropathogens, and 89 leukocytes/µl (IQR 42 – 187) in patients with negative cultures. All patients with mixed flora or ≥ 2 uropathogens had leukocyte counts above our 'optimal' pyuria threshold of 264 leukocytes/µl, and all but two patients with negative cultures had leukocyte counts below the optimal pyuria threshold.