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Cut-off values to rule out urinary tract infection should be gender-specific

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ABSTRACT

The diagnosis of urinary tract infection (UTI) by urine culture is an expensive and time-consuming procedure. Using a screening method, to identify negative samples, would improve the procedure and reduce costs. In this study, urine flow cytometry, of over 7000 urine samples, was assessed by retrospective analysis. With a cut-off value of >200 bacteria/μl, we obtained a sensitivity of 93.0%, a specificity of 63.5%, and a negative predictive value (NPV) of 96.2%. As a result the culturing of 49% of all samples could be avoided. In addition, the data was retrospectively analyzed to determine if the introduction of gender-specific cut-off values could improve screening results. The obtained receiver operator curves are indeed significantly different when gender specific cut-offs were used. When a NPV of 95% is considered acceptable the unisex cut-off value of >200 bacteria/μl can be used for women (NPV 94.9%), but the cut-off value for men could be raised to >400 bacteria/μl without diminishing the NPV (NPV 95.0%).

1. Introduction

The diagnosis of urinary tract infection (UTI) by urine culture is a time-consuming and costly procedure. For this reason, automated screening methods for urine analysis have been developed. Urine flow cytometers identify particles in urine by scattering and fluorescence. With the use of a fixed cut-off value, urine flow cytometry can be used to reduce the number of cultures [1–6]. Indeed, the introduction of a cut-off value, which determines if urine is subsequently cultured or not, can reduce the number of cultures, while maintaining a low level of false negatives and a high negative predictive value [1–6].

The use of urine flow cytometers as a screening test to reduce unnecessary cultures is well established [1–6]. However, a wide range of cut-off values has been reported (14 [6]–230 [5] bacteria/μl). The large differences among selected cut-off values can (partially) be explained by pre analytical, analytical and post analytical conditions: study population, collection methods, sample handling, criteria that define positive cultures, taking leucocytes into consideration as well, and whether a sensitive or specific cut-off is selected.

Due to anatomic and physiologic differences, UTIs are more common among women than men [7]. Since the length of the human urethra is sex dependent, it is not unreasonable to speculate that cut-off values for bacteria in urine are sex dependent as well. Surprisingly, all studies, but one [2], selected a unisex cut-off value. This may be due to the large number of samples needed, to obtain reliable gender-specific cut-off values.

Most of the above mentioned studies included a limited number of samples. We assessed the ability of urine flow cytometry to identify negative cultures by retrospective analysis of over 7000 urine samples. Due to the size of the data set, the selected NPVs have smaller confidence intervals. Besides determining a reliable unisex cut-off value, it was also assessed if gender-specific cut-off values improve screening results.

2. Materials and methods

2.1. Implementing a cut-off value for UTI screening

To reassess the usability of the Sysmex analyzer as a screening method to identify those urines that do not have to be cultured, 7322 urines, gathered over a period of three years (no sample exclusion), were retrospectively analyzed. Most patients originated from the urology department (76%), followed by Internal Medicine (12%). The remaining samples (11%) originated from all other departments combined. The 7322 urines originated from 4369 outpatients (60%) and 2953 hospitalized patients (40%). The average age was 60 years (Q1 45–Q3 78 years); 3222 patients were male (44%; average age 66 (57–80)), 4100 female (56%; average age 55 (36–75)). All 7322 urine samples were cultured and had their number of bacteria/μl determined on the urine flow cytometer UF5000i (Sysmex Benelux, Etten-Leur, The Netherlands). Automatic data processing, via a SQL-code, was implemented to extract relevant culture results from the electronic hospital information system.

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During the three years of data collecting, a cut-off value of 60 bacteria/μl was in use to reduce the number of cultures. Meaning urines with <60 bacteria/μl were not always cultured. Whether urine samples were cultured at lower bacterial count depended on the physicians request: "Exclusion UTI" requests led to culturing if the bacterial count exceeded the cut-off value of 60 bacteria/μl. "Bacterial Count" requests, were always sent to the microbiological laboratory for culturing. Of the 7322 samples cultured 2671 had a bacterial count <60/μl. As 63% of UTI analyses were "bacterial count" requests, we estimate we did not culture around 1500 urine samples that had a bacterial count <60/μl.

2.2. Sample collection

Midstream urine samples were collected in a sterile container with an integrated transfer straw (BD Vacutainer Collection container, BD Biosciences). For the bacterial culture an UriSwab (Copan Diagnostics, Brescia, Italy) containing preservatives (boric acid) was dipped in the sterile container (storage time under boric acid: during the day on average 2 h (47% of samples), after working hours (53% of samples) on average 11 h, with a maximum of 16 h). For urine flow cytometry, a sample was taken from the vacuum container in a sterile, preservative-free tube (BD Biosciences). Flow cytometry was performed as quickly as possible (usually within 2 h).

2.3. Analysis by urine flow cytometry with a UF500i

A Sysmex UF500i analyzer identified particles in urine by flow cytometry. This flow cytometer has two chambers, where diluted urine can be incubated with a specific dye and lysis reagents. One chamber is used solely for bacteria, while the other counts all other particles (erythrocytes, leukocytes, casts, etc.). After staining, the samples are transported to a flow cell where they are analyzed with the use of a semiconductor laser, and characterized by forward scatter, side scatter, and fluorescence. In this study, only the bacteria count is used.

2.4. Microbiological analysis

The UriSwab was sent to the laboratory for medical microbiology. The UriSwab was centrifuged, and after Gram staining, 10 μl was plated on a Brilliance UTI Clarity Agar (Oxoid, Basingstoke, UK) as well as a blood agar plate containing 5 μg/ml colistin and 2 mg/ml aztreonam (CAP agar). Both plates were examined for growth after 18–24 h (incubation at 35 °C; blood agar plates are used to better identify group B β-hemolytic *Streptococcus*). Grown colonies were identified by color or VITEK2® (bioMerieux, France) if necessary. Positive cultures are defined by thresholds ranging from 10^3–10^5 cfu/ml urine, depending on the species found [8]. In this study a positive culture is first defined by a fixed rule (>10^5 cfu/ml). Next, a different (not fixed) gold standard to define a positive culture was used: the addition of an antibiogram by the microbiologist to the culture result. This expert opinion considers both the cfu/ml and the type of pathogen found.

3. Results

3.1. Implementing a cut-off value for UTI screening

At the introduction of the Sysmex urine flow cytometer our laboratory originally introduced a conservative cut-off value of 60 bacteria/μl [1]. This cut-off was selected after analyzing about 300 urines (40 positive) from outpatients of the Urology department. Due to the small number of samples included in this initial evaluation, this cut-off was selected to not miss any positive urine (negative predictive value (NPV) 100%). Three years later, the cut-off value was reassessed with 7322 urines (44% male, 56% female). Retrospectively, the bacteria count of each sample is compared to its culture result (all 7322 samples were cultured: N = 189 no growth (3%), 4021 < 10^3 cfu/ml (55%), 707 10^3–10^4 cfu/ml (10%), 543 10^4–10^5 cfu/ml (7%), and 1862 > 10^5 cfu/ml (25%)). One thousand eight hundred and sixty-two (1862; 25%) of these cultures were positive (>10^5 cfu/ml). The organisms identified were mostly *Escherichia coli* (59%), followed by *Klebsiella pneumonia* (8%), *Enterococcus faecalis* (7%), and *Proteus mirabilis* (4%). The resulting receiver operator characteristic (ROC) curve is plotted in Fig. 1A (black circles). This reevaluation led to a raise of the cut-off value to 200 bacteria/μl. With this cut-off value a unisex sensitivity of 96.9% and specificity of 64.1% were obtained, avoiding the culture of 49% of all samples. This cut-off value together with a gold standard of >10^5 cfu/ml, results in a NPV of 98.4%. By implementing a higher cut-off value of 200 bacteria/μl some patients will be falsely classified as "negative" (N = 58, 2% of negative cultures; data not shown).

Defining a culture positive by a fixed threshold of >10^5 cfu/ml may not be most optimal. Depending on the species found a culture could be considered positive at lower cfu/ml [8]. To include all clinical relevant cultures, the addition of an antibiogram by the microbiologist was also considered as gold standard for a positive culture. Besides the cfu/ml this expert opinion also considers the pathogen found. In this scenario, 1933 (26%) of the 7322 cultures were positive. The additional positive cultures comprise a large group of group B β-hemolytic *Streptococcus* (5% of all positive cultures; 31% of all cultures with an antibiogram and <10^5 cfu/ml). The remaining additional cultures contain the same four pathogens previously mentioned, but at >10^4 cfu/ml. All other urine samples that resulted into growth of >10^3 cfu/ml are considered negative (these contain nonpathogenic micro-organism or mixed flora).

In our hospital the addition of an antibiogram rather than a cfu/ml threshold is used as the gold standard for a positive culture. The resulting receiver operator characteristic (ROC) curve for this scenario is plotted in Fig. 1B (black circles). Differences with the threshold of >10^5 cfu/ml are small, as the same cut-off is selected: 200 bacteria/μl (sensitivity 93.0%, specificity 63.5%, NPV 96.2% avoiding the culture of 49% of all samples; Table 1). By selecting a cut-off value of 200 bacteria/μl some patients will be falsely classified as "negative" (N = 136, 4% of negative cultures; data not shown). To get insight into sensitivity, specificity, NPV, PPV and culture reduction obtained with our data set, a range of cut-off values, that takes into consideration some of the previously reported cut-off values, is listed Table 1.

3.2. Cut-off values to rule out UTI should be gender-specific

Due to anatomic and physiologic differences, UTIs are more common among women than men [7]. Therefore it was assed if gender-specific cut-off values could improve screening results (addition of an antibiogram is considered positive). ROC curves were constructed separately for men and women (Fig. 1A and B; dark gray squares (men) and light gray triangles (women)). The area under the curve (AUC) was 0.948 for men and 0.880 for women (Fig. 1B). The 95% confidence boundaries are 0.939–0.957 and 0.867–0.893, respectively. The curves are significantly different (P < 0.05). The unisex cut-off value of 200 bacteria/μl resulted in a sensitivity of 92.1% with a specificity of 88.5% for men (NPV 97.0%) and a sensitivity of 93.5% with a specificity of 43.7% for women (NPV 94.9%; Table 1). Especially the difference in specificity, i.e. lower number of false positives in men, is striking between genders (Table 1).

Although the American Society of Microbiology recommends a NPV > 98% [9] (results in this study: unisex cut-off 20 bacteria/μl, male 70 bacteria/μl, female 2 bacteria/μl), in our hospital a NPV of 95% was considered acceptable for a rule out strategy. The current unisex cut-off barely qualifies for women, whereas it could be increased considerably for men. At 400 bacteria/μl, a NPV of 95% is still obtained. Due to the lower number of male urines, and the small amount of female urine with <200 bacteria/μl, the reduction in cultures is relatively small (N = 112, 1.5%; Table 1).

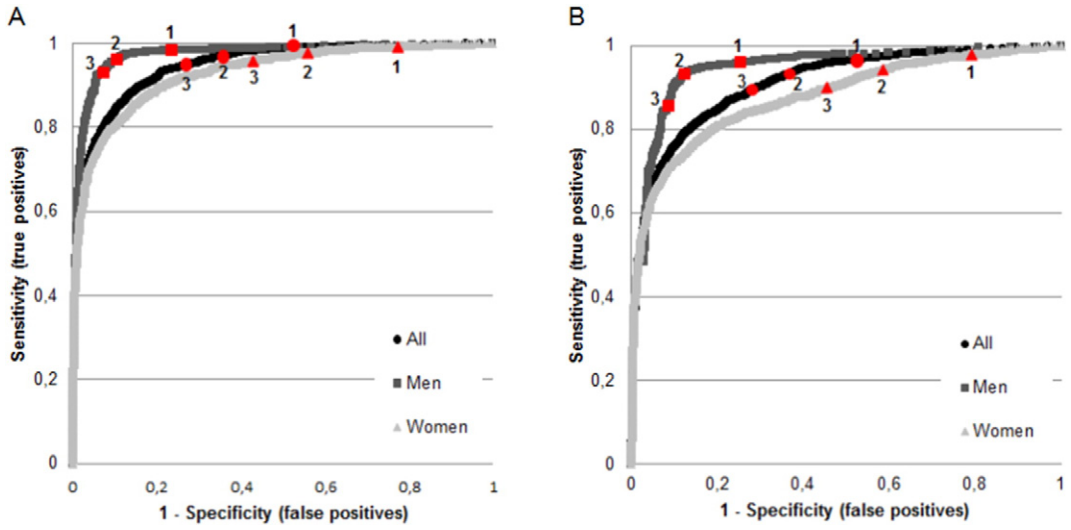

Fig. 1. Culture results A. ROC curve overall (black circles), for men (dark gray squares) and for women (light gray triangles) obtained with urine flow cytometry (positive culture defined by $>10^5$ cfu/ml). B. As A, but the gold standard for a positive culture is the addition of an antibiogram. The cut-off values mentioned in the main document are highlighted and marked by numbers (1. 60, 2. 200, and 3. 400 bacteria/μl).

4. Discussion

4.1. Implementing a cut-off value for UTI screening

In this study, we retrospectively assessed the power of the Sysmex urine flow cytometer to exclude urinary tract infections. The feasibility of using flow cytometry to reduce the number of cultures, by screening for urine samples that will lead to no, or no significant growth has been analyzed by multiple groups [1–6], but to our knowledge never with this amount of samples. Based on over 7000 urines with bacteria count and culture results, the cut-off value was set at 200 bacteria/μl (NPV 96.2%; by decreasing the cut-off value to 100 bacteria/μl the NPV only marginally increases (97.2%)). Due to the size of this analysis, it also became clear that in hindsight the previously selected conservative cut-off of 60 bacteria/μl did not result in a NPV of 100% (63 (2%) of 2671 urines with <60 bacteria/μl did result into a positive urine culture).

The implementation of a cut-off value might not be suitable for all patient groups. For instance children, pregnant women and patients that are immunocompromised or have urological diseases may need to be excluded from the screen-routing. To bypass the routine urine screening, the physician can request "Exclusion UTI pregnant/immunocompromised". With this request a urine is always cultured, regardless of bacteria count. Except for these patient groups our data indicate that with a cut-off value of 200 bacteria/μl few UTIs will be missed. From this small group of false negative samples, no evidence was derived to indicate another patient group to exclude from the screen-routing (always culture urine samples), nor did they entail specific pathogens. It could be that some of these samples originate from patients with a UTI in its early stages or patients pretreated with antibiotics (few bacteria present in both cases). To cater to this specific unknown group of patients, and their physicians, our laboratory introduced a new request: "continuous suspicion of UTI with low bacteriuria". With this request a urine is always cultured, regardless of bacteria count. By introducing this additional option, a NPV below recommendation of the American Society of Microbiology (NPV 95% instead of >98%) was considered acceptable for a rule out strategy.

Table 1
Sensitivities, specificities, negative predictive values and predicted culture reduction at different cut-off values overall, for men and for women. Suggested cut-offs are indicated in bold. Gold standard for positive culture — addition of an antibiogram[a].

	Cut-off: Bacteria/μl	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Culture reduction (%, N)[b]
Overall	15	98.3	31.2	98.2	34.2	24%, N = 1761
	50	97.0	44.4	97.6	38.5	34%, N = 2454
	100	95.7	54.4	97.2	43.0	41%, N = 3017
	200	93.0	63.5	96.2	47.8	49%, N = 3559
	400	89.3	71.9	94.9	53.2	56%, N = 4076
	600	86.8	76.7	94.2	57.2	60%, N = 4390
Men	15	97.6	58.5	98.6	45.2	43%, N = 1388
	50	95.9	74.3	98.1	56.7	56%, N = 1804
	100	94.9	83.0	97.9	66.1	63%, N = 2024
	200	91.9	88.4	96.9	73.6	68%, N = 2179
	400	**86.4**	**91.2**	**95.0**	**77.4**	**71%, N = 2291**
	600	84.0	92.5	94.3	79.6	73%, N = 2342
Women	15	98.8	12.0	96.5	29.1	9%, N = 374
	50	97.8	20.8	96.3	31.1	16%, N = 648
	100	96.3	31.7	95.9	34.0	24%, N = 993
	200	**93.5**	**43.7**	**94.9**	**37.8**	**34%, N = 1380**
	400	89.2	56.5	93.5	42.8	44%, N = 1810
	600	86.6	64.2	92.9	47.0	51%, N = 2074

[a] Of the urine samples with an antibiogram (1933 positive cultures) 88% were positive at $>10^5$ cfu/ml, 8% at $>10^4$ cfu/ml, and 4% at $>10^3$ cfu/ml.

[b] Expected culture reduction. After introducing the unisex cut-off value of 200 bacteria/μl a reduction of 20% in negative cultures was obtained. By renaming the UTI screening requests unambiguously and introducing gender-specific cut-off values a further reduction in negative cultures is expected.

4.2. Cut-off values to rule out UTI should be gender-specific

With the length of the human urethra being sex dependent, it is not a far stretch to speculate that reference values for bacteria in urine should be sex dependent. Indeed, analysis of this data set resulted in significantly different cut-off values for men and women, where the cut-off value for men was considerably higher (>400 bacteria/μl) than for women (>200 bacteria/μl). Surprisingly, the one gender-specific set of cut-offs reported had a higher value for women than men (630 vs. 40 bacteria/μl; combined sensitivity 97%, NPV 99%) [2]. These values were based on the Youden index, rather than an acceptable NPV. By considering the Youden index as optimal, the data set presented here leads to a cut-off of 270 bacteria/μl for men (NPV 96.3%) and 1440 bacteria/μl for women (NPV 91.9%). It should however be noted that in the aforementioned study a smaller amount of urines was included (184 positives, 1094 total), the selected cut-off value considered leukocyte count as well and a different gold standard was used (positive at different cfu/ml depending on symptoms at presentation and patient group). The Youden index is a statistical analysis to capture the performance

of a diagnostic test. The UF500i is implemented as a screening method. For a rule out strategy a higher sensitivity is preferred (low number of false negatives). The screening route was implemented to exclude the presence of an UTI and reduce the amount of unnecessary cultures. Therefore, together with the Urologists the decision was made to select a cut-off based on a high NPV. However, the fundamental differences between the two studies make a true comparison difficult, and possibly it is not the method of selecting a cut-off (Youden or NPV) that results into the different cut-off values, but rather a combination of study population and pre analytical, analytical and post analytical conditions like, collection methods, sample handling, criteria that define positive cultures, and taking leucocytes into consideration.

In conclusion, urine flow cytometers can reduce the number of cultures by screening urines, while maintaining a high NPV. The resulting ROC curves, and therefore cut-off values, are not the same for men and women. Introducing gender-specific cut-off values improves screening results. Equally high NPVs can be obtained for men and women at different cut-off values, thereby reducing the number of cultures. After the introduction of a male cut-off value, 71% of men suspected of an UTI could be safely (NPV 95%) ruled out by screening alone.

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