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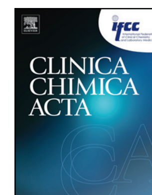

Does flow cytometry have a role in preliminary differentiation between urinary tract infections sustained by gram positive and gram negative bacteria? An Italian polycentric study

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ABSTRACT

Background: Urine culture is the most frequently requested test for a Microbiology Lab. A reliable screening tool would be of paramount importance both to clinicians and laboratorians, provided that it could get fast and accurate negative results in order to rule-out urinary tract infection (UTI).
Materials and methods: We evaluated 1907 consecutive urine samples from outpatients. Culture was performed on chromogenic agar with 1 μL loop, using 10^5 CFU/mL as a limit of positive growth. Using Sysmex Uf-1000i analyzer we evaluated bacteria forward scatter (B_FSC) and fluorescent light scatter (B_FLH) in a preliminary discrimination step for UTI caused by Gram+ or Gram− bacteria.
Results: We got 512 positive samples. A mono-microbial infection was observed in 490 samples; two bacterial strains were isolated in 22 samples, so 534 bacterial strains were found: 392 Gram−, 133 Gram+ and 9 yeasts. Comparing Gram+ and Gram− bacteria we observed a statistically significant difference for B_FSC but not for B_FLH. In this application experimental cut-off value for B_FSC was 25ch. Using this cut-off to perform a presumptive identification of UTI sustained by Gram-+ bacteria, we observed a SE 0.68, SP 0.84.
Conclusion: Our data although preliminary suggest that B_FSC could be useful in presumptive exclusion of UTI caused by Gram-positive bacteria.

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1. Introduction

Among transmissible diseases, urinary tract infections (UTI) are only second in frequency to upper respiratory tract infections, and urine culture is the most common bacteriological test in a clinical laboratory [1].

However, a number of unresolved problems still exist. The overall yield of positive culture results, even from patients with typical symptoms of UTI, is low despite the heavily labor- and time-consuming procedures [2]. The generally accepted definition of significant bacteriuria in voided urine specimens is $\geq 10^5$ CFU/mL of a single microorganism [3], but lower limits were suggested for children, men, patients with underlying diseases, or when "fastidious" microorganisms are involved [4].

In the vast majority of patients, UTI are caused by Gram negative bacteria, (*Enterobacteriaceae* first, e.g. *Escherichia coli*, then non-fermenting Gram negative rods, such as *Pseudomonas* spp.); Gram positive bacteria (*Enterococcus* spp., *Streptococcus* spp. and *Staphylococcus* spp.) are involved in about 25% UTI [5,6].

In patients with UTI, the Turnaround Time (TAT) for a test result is no less than 48 h: you simply cannot get a 2-day time-span before starting antibacterial treatment in a symptomatic patient. So physicians usually start a blind, empiric therapy based on the sensitivities to chemotherapeutic agents known to be active against the bacteria most commonly involved in UTI. Unfortunately, the most active agents against Gram-negative bacteria are not very effective against Gram-positive bacteria: an idea of the Gram characteristic of the germ involved in suspected UTI would certainly enhance the efficacy of empirical therapy [7–11]. Some evidence exists that the evaluation of "dimensional parameters" derived from the distribution histograms in the bacterial channel (bacteria forward scatter: B_FSC) of the modern cytometers can be useful in a rough, but extremely rapid etiological

Abbreviations: AUC, Areas Under Curve; BACT, Bacteria; B_FSC, Bacteria Forward Scatter; B_FLH, Bacteria Fluorescent Light Scatter; CFU, Colony Forming Units; CI, 95% Confidence Interval; CPS ID3 agar, Chromogenic Agar Plates; DA, Diagnostic Accuracy; FN, False Positive; FP, False Negative; IQR, Inter Quartile Range; LEU, Leukocytes; ME, Median; NPV, Negative Predictive Value; PPV, Positive Predictive Value; ROC, Receiver Operating Characteristic; SE, Sensitivity; SP, Specificity; TN, True Negative; TP, True Positive; UTI, Urinary Tract Infections

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differentiation [12]. The aim of this retrospective study was to evaluate the feasibility of a rapid presumptive identification of UTI caused by Gram negative bacteria using bacteria forward scatter (B_FSC) and bacteria fluorescent light scatter (B_FLH).

2. Materials and methods

Three hospital-based clinical laboratories were involved in this study, according to the prerequisites of using dip-stick automated analyzers for routine chemical urinalysis and a flow cytometer Sysmex Uf-1000i for formed particle examination.

2.1. Sample collection

We considered 1907 adult outpatients (775 males and 1132 females, age 18–70 years) submitted to our institutions for suspected UTI. Urines were collected in a sterile container (100 mL), fully equipped for sampling by vacuum tubes (Vacutest Kima, Arzergrande PD, Italy). Two separate tubes without preservative solution were immediately sampled, one for microbiological examination and one for Sysmex UF-1000i examination and kept refrigerated until analysis. Plate inoculation and Sysmex UF-1000i analysis were performed within 4 h from sample collection [13,14].

2.2. UF-1000i analysis

Samples were processed on Sysmex UF-1000i Analyzer (Dasit, Milano, Italy). Briefly, a flow cytometer that counts, separates and analyzes microscopic particles suspended in a fluid stream. It also performs simultaneous, physico-chemical, multi-parametric analyses on single cells flowing through a detection system, in order to obtain adequate classification of urinary particles. The measured parameters are converted into electric signals, and the signal analysis enables classification and quantitation of each particle accordingly. All measurements are shown as a scattergram by means of a software (version 0018). Particle counts include erythrocytes, WBCs, epithelial cells, casts, bacteria, crystals and yeasts. UF-1000i has a separate analytical channel for bacteria, where urine specimen is mixed at 42 °C to a diluent that increases cell wall permeability and allows specific staining of bacterial nucleic acids with a dedicated polyethnic fluorescent dye. Particles are classified and quantified by considering their size- (impedance) and staining-characteristics using the forward scatter and the intensity of fluorescent light. Two additional parameters are available in this channel: the bacteria forward scatter (B_FSC) and the bacteria fluorescent light scatter (B_FLH), reported in arbitrary units (analytical channel — ch) and providing information about size (B_FSC) and nucleic acid contents (B_FLH).

2.3. Microbiological analysis

Quantitative urine culture was performed by using a 1 mL inoculation loop. Urine samples were routinely cultured for pathogens using the commercial chromogenic agar medium CPS ID3, (Biomerieux, Milano, Italy). Culture plates were incubated aerobically at 35 °C for 18–24 h. Quantification, in CFU/mL, was obtained multiplying the colonies numbered on the agar plate by the dilution factor. The culture was labeled as positive if containing $\geq 10^5$ CFU/mL [15,16]. Standard biochemical identification and susceptibility tests to anti-microbic drugs were performed by using Vitek 2 analyzer (Biomerieux, Milano, Italy) [15].

2.4. Statistical analysis

Statistical tests were performed using a dedicated software (Analyse-it© version 2.03). A nonparametric statistical approach was adopted, evaluating median and 90% confidence intervals (CI 90%), Variance analysis was expressed by the interquartile range (IQR). A Kruskal–Wallis test was performed for comparison of data. Receiver operating characteristic (ROC) curves were drawn by plotting sensitivity versus 1-specificity, to define the best cut-off values the Youden index was evaluated, and the areas under curve (AUCs) were measured. Finally, specificity (SP), sensitivity (SE), positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) were calculated.

3. Results

3.1. Microbiological analyses

Bacterial isolates of this study are reported in Table 1. 553 out of 1907 samples (29%) showed a bacterial growth $\geq 10^5$ CFU/mL. A mono-microbial infection was observed in 490 samples: 362 Gram negative, 119 Gram positive and 9 yeasts; two bacterial strains were isolated in 22 samples (8 had two Gram negative bacteria; 14 a Gram positive plus a Gram negative), 41 patients showed a poly-microbial flora and were considered as contaminated.

3.2. Presumptive differentiation between Gram positive and Gram negative bacteria by UF-1000i

For B_FSC and B_FLH median and quartiles have been calculated, as reported in Table 2, by considering Gram positive alone (119), Gram negative alone (370), a Gram positive and a Gram negative strain (mixed) (14), yeast (9), and contaminated samples (41). In Gram negative bacteria B_FSC median value was 20.60ch (1st quartile = 14.50ch, 3rd quartile = 33.03ch, IQR 18.53ch). In Gram positive bacteria B_FSC median value was 40.70ch (1st quartile = 27.80ch, 3rd quartile = 59.93ch, IQR 32.13ch). A high statistically significant difference ($p < 0.001$) in B_FSC between Gram positive and Gram negative was observed. A lower, but still significant difference ($p < 0.01$) in B_FSC remained between Gram negative bacteria and yeast and contaminated samples but not in samples with mixed growth of Gram positive and Gram negative bacteria (data reported in Table 2). Gram negative bacteria had a B_FLH median value = 85.50ch (1st quartile 77.62ch, 3rd quartile 112.35ch, IQR 34.73ch). B_FLH median value for Gram positive bacteria = 98.00ch (1st quartile 71.30ch, 3rd quartile 115.77ch, IQR 28.43ch). Here no statistically significant difference was detected ($p > 0.5$), Scattergram patterns from B_FSC channel, for Gram positive, Gram negative, association of a Gram positive plus a Gram negative and contaminated samples are reported in Fig. 1.

Table 1
Isolated strains from positive ($\geq 10^5$ CFU/mL) urine samples.

	Strains
Candida spp.	9
Citrobacter spp.	11
Enterobacter spp.	11
Enterococcus spp.	63
Escherichia coli	279
Klebsiella spp.	53
Morganella spp.	4
Proteus spp.	11
Pseudomonas spp.	23
Staphylococcus spp.	29
Streptococcus spp.	41

1907 consecutive samples from adult outpatients. 553 samples. In 490 samples was observed a mono-microbic infection, in 22 samples were detected two pathogens and in 41 samples a polymicrobic growth was observed, these samples were considered as contaminated.

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