Estimation of the Causative Bacterial Group from Bacterial Scattergrams of the Fully Automated Urine Particle Analyzer UF-1000i

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Symptoms of Urinary tract infection

- Frequent urination, small amounts of urine
- Unable to control bladder and leaks urine
- Burning sensation when urinating
- Urinating blood
- Cloudy or foul smelling urine
- Malaise or the general feel of unwell
- Severe pain the lower abdomen region
- Mild fever
Pathogenesis of urinary tract infection

- If the inflammatory cascade continues, tubular obstruction and damage occur, leading to interstitial edema.
- This may lead to interstitial nephritis, causing acute kidney injury (AKI).

- Infection of the renal parenchyma causes an inflammatory response called pyelonephritis.
- While infection of the renal parenchyma is usually the result of bacterial ascension, it can also occur from hematogenous spread.

- Once sufficient bacterial colonization occurs, bacteria may ascend on the ureter towards the kidney.
- Fimbria may aid in the ascension process.
- Bacterial toxins may also play a role by inhibiting peristalsis (reducing the flow of urine).

- Fimbria allow bladder epithelial cell attachment and penetration.
- Following penetration, bacteria continue to replicate and may form biofilms.

- Pathogen colonizes the periurethral area and ascends through the urethra upwards towards the bladder.
Classification of Urinary tract infection

Uncomplicated UTI

No underlying disease

Complicated UTI

Systemic or localized underlying disease

Uncomplicated and complicated UTI are handled differently, as they differ in the disease type, causative organisms, symptoms, treatment policy, and the patients' response to treatment.
Urine culture for UTI Diagnosis

1. Midstream urine
2. 0.001mL定量接種環
3. MacConkey flat plate and Blood plate
4. 37°C for 24 hours
5. Colony counting
6. Gram staining
7. UTI
   - G (+) >10^4CFU/mL
   - G (-) >10^5CFU/mL
Diagnose of Urinary tract infection

- As it takes a few days to get the results of urine bacterial culture, resorting to empiric treatment with antibiotic agents right from the patient's first visit is unavoidable.

- The patient has complicated UTI, such initial empiric antibiotic therapy is often ineffective, and currently, physicians can identify the primary disease only when the results of urine culture become available, a few days after the initial consultation.
Fully automated urine particle analyzer

- Measure RBC, WBC, epithelial cells, casts, and bacteria
- Flow cytometry
- Non-centrifuged urine
- Quantitative
Fully automated urine particle analyzer - UF 1000i

Semiconductor laser
(wavelength $\lambda = 635$ nm)

Detection unit

Incubation

SEARCH - BAC

PACK-BAC

SEARCH - SED

PACK-SED

SEDIMENT Channel

BACT Channel
UF II PACK-BAC contains a cationic surfactant that lyses blood cell components.

**Fig. 3** An example of scattergram displayed (sediment channel)

- Sediment channel
- RBC are shown as red dots
- WBC are blue dots
- Epithelial cells are orange dots
- In the BACT channel, the area close to the intersection of the two axes is magnified, and the **RBC components** are **hemolyzed** with a surfactant.
The staining fluid UF II SEARCH-BAC used in the BACT channel contains a polymethine dye that specifically stains the nucleic acid of bacteria, and it does not stain microfragments of non-bacterial cells in the urine, which are of about the same size as bacteria.
**Fig. 4** UF-1000i analysis screen *(acute uncomplicated cystitis)*

This method uses the BACT channel scattergram, shown at bottom right. The dots representing bacteria are distributed in the zone of less than 30° angle from the x-axis (Low Angle Pattern).

1. Table at the third row from bottom, on the left side of the screen: The WBC count in the urine was elevated at 387.1/HPF.
2. Enhanced image of WBC distribution in the sediment channel (graph at second column from the right, second row from the bottom) shows a large number of blue dots.
3. Table at the third row from bottom, on the left side of the screen: The bacterial count in the urine was elevated at BACT = 1.5 × 10⁸/mL.
4. BACT channel
How to approximately identify the bacterial species from the BACT scattergrams

UF-1000i uses flow cytometry for bacteriological analysis. The resulting pattern is displayed graphically in the form of the scattergram shown below. The identity of gram negative bacilli can be assumed from such scattergrams.

**Gram negative bacteria** were isolated from 81/86 (94.2%) of the specimens that had angle of the scattergram pattern < 30°.
**Gram positive bacteria** were isolated from 33/44 (75.0%) of the specimens that had angle of the scattergram pattern ≥ 30°.

**Patient who is under treatment with penicillin or cephem antibiotic often shows gram negative bacilli with antibiotic-induced filaments in the sample.** In that case, the scattergram of the data points has a steeper angle. There have been cases where bacteria of the genus Enterococcus gave scattergram with an angle of less than 30° or gram negative bacilli gave scattergram of about 40° angle, although the reasons for this are not well understood.

**The number of data points on the scattergram generally depends on the concentration of bacterial cells.**

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**Examples of gram positive cocci**

- A coagulase negative Staphylococcus
- Gram positive bacilli such as corynebacteria, and gram negative bacilli that developed filaments also produce similar scattergrams.

**Examples of gram negative bacilli**

- E. coli
- E. pneumoniae

Gram negative cocci like gonococcus also show a similar pattern.
In the present study, we examined the possibility of broadly estimating the bacterial group (whether it is a bacillus or a coccus) from the UF-1000i bacterial scattergram pattern of patients' urine samples, to gather data for use in the selection of suitable antibiotics at the first visit to the hospital.
Materials and Methods

- Urine samples: 81 subjects
  - 23 males and 58 females, mean age 59.6, and age range 17 to 89
  - selected from among persons who were examined in the Department of Urology.
  - 78 persons whose urine had ≥10 WBC/μL as analyzed by UF-1000i and the bacterial species present in whose urine were identified by urine bacteria culture
  - 3 persons who were positive for Neisseria gonorrhoeae when tested by transcription-mediated RNA amplification.
Fig. 1 Distribution of cases

Age group-wise

- < 20 years old: 2%
- 20-29 years old: 7%
- 30-39 years old: 5%
- 40-49 years old: 10%
- 50-59 years old: 18%
- 60-69 years old: 33%
- 70-79 years old: 18%
- ≥80 years old: 18%

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Underlying disease-wise

- Acute cystitis: 48%
- Prostatitis: 23%
- Prostate enlargement: 6%
- Intermittent catheterization infection: 12%
- Urinary bladder fistula: 5%
- Urethral catheterization infection: 2%
- Bladder stones: 2%
- Others: 2%
Fig. 2 Bacteria detected in urine culture
Result

89% Gram-negative bacilli

69% Gram-positive cocci

79% Polymicrobial infection

70% E. coli

32% Multidrug resistant bacteria

Fig. 5-A. Low Angle Pattern
Dots representing bacterial clusters are distributed in a zone below the line with 30° angle.

Fig. 5-B. High Angle Pattern
Dots representing bacteria are distributed above the 30° line.

Fig. 5-C. Wide Pattern
The dots are distributed in the areas both above and below the 30° line.
Table 1  Bacterial species detected by urine culture of each case that showed the Wide Pattern, listed in the order of decreasing counts from left to right.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Species</th>
<th>Additional Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>86 F</td>
<td>F</td>
<td>E. faecalis</td>
<td>S. haemolytics MRS*</td>
</tr>
<tr>
<td>84 F</td>
<td>F</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>82 F</td>
<td>F</td>
<td>E. coli ESBL*</td>
<td>S. haemolytics MRS*</td>
</tr>
<tr>
<td>81 M</td>
<td>M</td>
<td>K. pneumniae</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>79 F</td>
<td>F</td>
<td>P. mirabilis</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>79 F</td>
<td>F</td>
<td>MRSA*</td>
<td></td>
</tr>
<tr>
<td>75 F</td>
<td>F</td>
<td>K. pneumniae</td>
<td>E. aerogenes</td>
</tr>
<tr>
<td>74 M</td>
<td>M</td>
<td>K. pneumniae</td>
<td>E. faecalis</td>
</tr>
<tr>
<td>73 F</td>
<td>F</td>
<td>E. coli</td>
<td>S. agalactiae</td>
</tr>
<tr>
<td>72 F</td>
<td>F</td>
<td>E. coli ESBL*</td>
<td>S. salivarius</td>
</tr>
<tr>
<td>72 M</td>
<td>M</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>72 M</td>
<td>M</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>70 F</td>
<td>F</td>
<td>Streptococcus spp.</td>
<td></td>
</tr>
<tr>
<td>66 F</td>
<td>F</td>
<td>E. faecalis</td>
<td>Lactbacillus spp.</td>
</tr>
<tr>
<td>44 F</td>
<td>F</td>
<td>S. epidermidis</td>
<td></td>
</tr>
<tr>
<td>39 M</td>
<td>M</td>
<td>M. morganii</td>
<td>Streptococcus spp.</td>
</tr>
<tr>
<td>36 M</td>
<td>M</td>
<td>E. coli</td>
<td>Alcaligene spp.</td>
</tr>
<tr>
<td>23 F</td>
<td>F</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>22 F</td>
<td>F</td>
<td>E. coli</td>
<td>S. epidermidis MRS*</td>
</tr>
</tbody>
</table>

* Multidrug resistant bacteria
Discussion

• The results of the urine culture test are not available at the first visit, and currently a few days are required to arrive at definite diagnosis.

• The results of microscopy of urine sediments and gram staining, apart from blood cell counts in urine, must be given a predominant role in the diagnosis of UTI.

• The gram-negative/positive differentiation of the bacteria by gram staining is a useful method.

• When only a small number of bacteria are present, their presence cannot at times be confirmed by microscopy.
• **UF-1000i** can display the RBC count, WBC count, and bacterial count in the urine through its basic cell analysis function in a **very short time** and can thus contribute to diagnosis of UTI.
• A typical **Low Angle Pattern** means that the patient can be fully cured by short-term oral administration of ordinary wide spectrum antibiotics.

• The **High Angle Pattern** and the **Wide Pattern** suggest the possibility of complicated UTI.

• The **Wide Pattern group** had significantly higher incidence of involvement of **multidrug resistant bacteria** such as methicillin resistant staphylococci (MRS) and ESBL-producing E. coli.
• Wide Pattern scattergrams could arise from complicated UTI that could not be fully cured by earlier antibiotic treatment.

• With such patients, it is important to avoid empiric antibiotic therapy, except in cases that require urgent intervention because of high fever or septicemia detected at the time of the first visit itself, and give priority to identification of the underlying condition that led to the urinary tract disease.
• Low Angle Pattern had a high incidence of infection by a bacilli and the majority of cases with the High Angle Pattern had infection by cocci, whereas the majority of cases with the Wide Pattern had complicated UTI involving more than one bacterium.

• The method described here enables a broad judgment as to whether the UTI is caused by a bacillus, coccus, or more than one bacterium, within about one minute on the day of the consultation itself without having to wait for the results of urine culture, and therefore can be used as a guide in the selection of antimicrobial agents for treating UTI patients.
Thank you for your attention