

## A NOVEL FLUORESCENCE TECHNIQUE TO ILLUMINATE BACTERIAL ADHESION TO UROTHELIAL CELLS IN OAB: A RADIANT DEMONSTRATION OF PATHOLOGY IN ACTION

### Hypothesis / aims of study

The adhesion of invading bacteria to the surface of the urothelial cells is a pivotal step in the pathogenesis of urinary tract infection (UTI). When confronted by pathogenic bacteria, an innate immune response of the urothelium is to increase surface cell shedding. This mechanism ejects infected host cells, and promotes replacement by hyperplasia. This process results in a plethora of urothelial cells in the urine of the patient. These make an attractive target in the search for novel markers of the disease processes underlying the symptoms.

The term 'Clue Cell', borrowed from genitourinary medicine, describes urothelial cells manifesting bacterial adhesion. The enumeration of Clue Cells using Giemsa staining and light microscopy has been reported previously in patients with Overactive Bladder (OAB) (1,2). The abundance of cells demonstrating bacterial adhesion, relative to the total number of urothelial cells, appeared to be a useful marker of UTI in this condition when other tests were negative. The measure discriminated clearly between controls, OAB patients without microscopic pyuria, and OAB patients with pyuria. These three groups represent a spectrum of disease activity in this condition, a progression well depicted in this test's performance.

Whilst not technically difficult, the preparative process has proven to be time consuming. The existing Giemsa staining technique includes incubation for 12 minutes, five wash cycles, and a 10 minute drying period. The discrimination of cells exhibiting bacterial adhesion from the total cell population may also be difficult and would certainly not be up to automation. Additionally, the original method employed catheter specimens of urine, whereas meticulous MSU samples were used in this study.

This work describes the validation of a novel fluorescent staining technique that is rapid, and confers striking clarity to the analysis. The original clinical study was repeated in samples of patients experiencing different disease severities.

### Study design, materials and methods

A meticulous midstream urine specimen (MSU) was obtained and pyuria quantified by light microscopy of the fresh sample. The fluorescent staining process was developed following a series of pilot studies. This preliminary work employed a variety of fixatives, washing methods, and two candidate stains: (a) Baclight LIVE/DEAD and (b) VectaShield/DAPI. This method development phase produced the following staining protocol: Immediate sample fixation, one centrifugation and wash cycle, Cytospin© preparation, VectaShield/DAPI stain.

*Sample fixation:* Immediate fixation with 4% Formaldehyde. *Centrifugation and washing:* Samples were spun at 800rpm for five minutes, the supernatant removed, and the sediment resuspended in the same volume of sterile phosphate buffered saline (PBS). *Shandon Cytospin©:* 50 ul of the washed sample was spun at 800 rpm for five minutes at high acceleration. This produced a single cell layer preparation of urothelial cells. *DAPI staining:* VectaShield/DAPI was dropped directly on to the Cytospin© urothelial cell preparation and a cover slip applied.

The Clue Cell count was enumerated using epifluorescent microscopy and expressed as a proportion of the total epithelial cell count.

### Results

62 patients with OAB (M=13; F=49; mean age=58; sd=18) and 32 controls (M=14; F=18; mean age=37; sd=13) were included in the analysis. The data were non-parametric and the Kruskal-Wallis test was used to assess between group differences in the proportion of Clue Cells. The proportion of Clue Cells discriminated clearly between OAB patients and asymptomatic controls ( $H=17.51$ ;  $df=1$ ;  $p<0.001$ ). Significant differences were also demonstrated between patients without pyuria, pyuria  $1-9 \text{ wbc ul}^{-1}$ , and pyuria  $\geq 10 \text{ wbc ul}^{-1}$  ( $H=17.70$ ;  $df=2$ ;  $p<0.001$ ). This study had a greater than 80% power to detect a significant between group difference ( $\alpha=0.05$ ).

The fluorescent staining technique was associated with a shorter preparation time, and the identification of Clue Cells was augmented through improved visualisation of adherent bacteria. Figure 1 demonstrates a monochrome image of the results of a standard staining technique using Giemsa, examined by light microscopy (x630); adherent bacteria can be seen in small numbers (arrowed). Figure 2 is a colour image of four Clue Cells, prepared using a VectaShield/DAPI fluorescent stain and examined by epifluorescent microscopy (x630). Figure 3 demonstrates a similar preparation in monochrome which produced remarkable discrimination; the contrast between cells exhibiting bacterial adhesion and those that do not (arrowed) is striking.

Fig. 1

Fig. 2

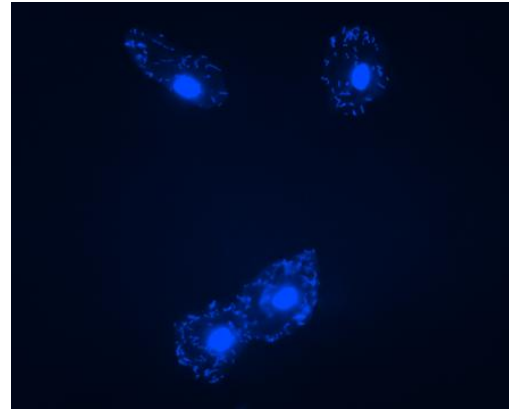
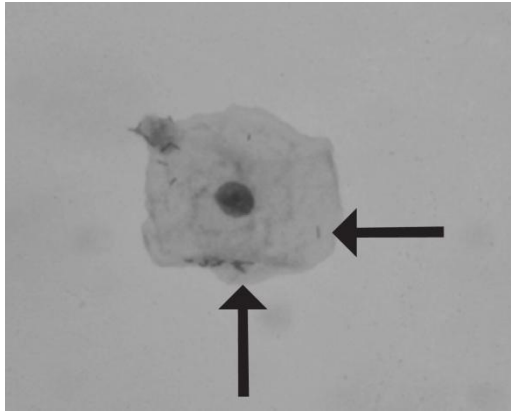
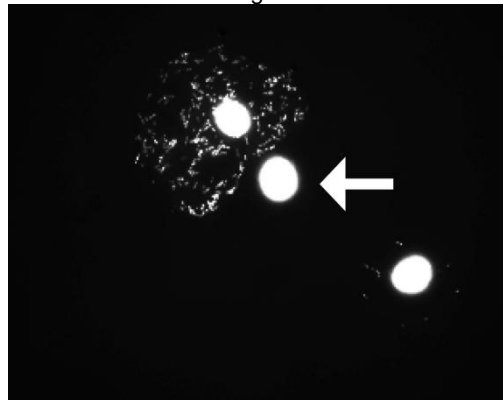


Fig. 3



Interpretation of results

Fluorescent staining represents a significant refinement of the existing staining method. The technique confers clear identification of urothelial cells exhibiting bacterial adhesion, allowing a more rapid assessment of disease activity. The results of this analysis mirror earlier studies which demonstrated the potential of this measure; it appears to be capable of unearthing evidence of disease in a group of patients who do not demonstrate a contemporary pathological signal.

Concluding message

The quantification of urothelial Clue Cells offers a novel method of disease exploration in OAB. It is able to quantify disease activity in a group of patients who may not manifest orthodox evidence of infection, such as bacteriuria, or significant microscopic pyuria. This new method of cell enumeration is another step forward on the road to more sensitive measures of urinary infection and inflammation.

References

1. Neurourol Urodyn 2009;28(7):776-7.
2. Proceedings of the Joint Annual Meeting of the International Continence Society and International Urogynaecological Association 2010: 333.

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<b><i>Is this a clinical trial?</i></b>	<b>No</b>
<b><i>What were the subjects in the study?</i></b>	<b>HUMAN</b>
<b><i>Was this study approved by an ethics committee?</i></b>	<b>Yes</b>
<b><i>Specify Name of Ethics Committee</i></b>	<b>Whittington and Moorfields Research Ethics Committee</b>
<b><i>Was the Declaration of Helsinki followed?</i></b>	<b>Yes</b>
<b><i>Was informed consent obtained from the patients?</i></b>	<b>Yes</b>