

URINARY “CLUE CELLS” – FINGER-PRINTS AT THE SCENE OF THE CRIME OF URINARY INFECTION

Hypothesis / aims of study

A process in the pathology of urinary tract infection is the adherence of the invading bacteria to the surface of the urothelial cells. In the particular case of *E.coli*, Type 1 Fimbriae have evolved receptors that bind to specific ligands on the cell surface. It is now known that a number of other uropathogens exhibit similar binding properties which are utilised prior to invasion of the cells. An innate immune response of the urothelium, when confronted with invading pathogens, is to increase surface cell shedding and to promote replacement through hyperplastic change. This means that a mixture of normal and infected cells should accumulate in the urine. A number of these cells should manifest bacterial adhesion and various stages of the invasion process. In genitourinary medicine vaginal “Clue Cells”, exhibiting bacterial adhesion are interpreted as markers of infection. A chance finding during a study of urinary spun sediments suggested that a Clue Cell phenomenon might be active in patients with OAB (1) .

The examination of urinary urothelial cell morphology is not a difficult process.

The Shandon Cytospin© is used to concentrate the cells from small volumes of urine. Cells are focalised into a small area on a glass slide with the excess fluid absorbed onto a cardboard filter. Thus a reproducible technique places cells from 0.5 ml of urine onto glass slides, forming 5 mm diameter circles of single cell depth. This permits closer scrutiny and some quantification.

Two stains would seem suitable for this analysis:

Gram staining is important in microbiology for the identification of bacteria. The Gram reaction is based on the structure of the bacterial cell wall and the differences between Gram positive and Gram negative bacteria

The Giemsa stain improves the visualisation of epithelial cells by providing high quality nuclear and cytoplasmic differentiation. Usually the nuclei stain purple and the cytoplasm stains grey to blue. It is useful in the examination of bacterial adherence to cells, in this case, uroepithelial cells. The bacteria generally stain purple whilst the cells stain grey/blue/pink/purple.

Study design, materials and methods

Initially a series of pilot experiments were conducted in order to ascertain the best method of preparation and the preferred stain. It was found the best results were obtained by washing the spun sediment in phosphate buffered saline. The Gram stain provided the best definition. A Clue cell was best realised if it showed bacteria adherence with bacterial division at the site of adherence. (Figures 1 and 2)

A blinded observational method was used. CSU samples from OAB patients, MSU from controls, were examined for pyuria and routine culture was arranged. The samples underwent two centrifuge cycles (694 rcf) to achieve washing with PBS. 1ml of the resulting sample was mixed with 1ml methanol, and 6 drops of this mixture was transferred to cuvettes which were placed in a Shandon Cytospin™ (run for 5 minutes at 1 rcf). The preparations were Gram stained and examined to count the number normal uroepithelial and clue cells.

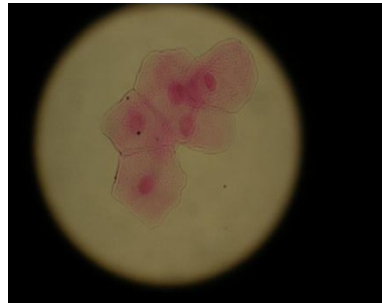
Figure 1

“Clue cell” - Gram stained uroepithelial cell with bacteria adherent and dividing at adherence site.



Figure 2

Some non-clue cells and one cell with bacterial adhesion but no division at adherence site



Results

41 patients (F=32, M=9 mean age=61, sd=17) and 23 normal controls (F=10, M=13, mean age=30, sd=13) were recruited for this study. The patients were symptomatic with an average 24-hour frequency of 9 (sd=4.0), average daily incontinence of 0.74 (sd= 0.93) and average urgency score of 3.62 (sd=3.2) with a normal urgency score being zero. The controls had average daily frequencies of 5.5, nocturia of zero and urgency score of zero.

The data were non-normal and did not respond to transformation. Non-parametric methods of analysis had to be adopted. The Kruskal-Wallis test for unrelated samples was selected to test for significant differences at the 95% level of confidence

Figure 3 compares the proportion of clue cells in relation the urine culture results. It can be seen that there was a significant difference between patients with OAB symptoms and the normal controls. With respect to those with OAB symptoms there was an inverse relationship between the proportion of clue cells and a positive urine culture. (Chi-square = 23, df=2, p<.001). Figure 4 illustrates the same variable in relation to pyuria (Chi-square = 21, df=2, p<.001)

Figure 3 The proportion of clue cells according to the results of urine culture

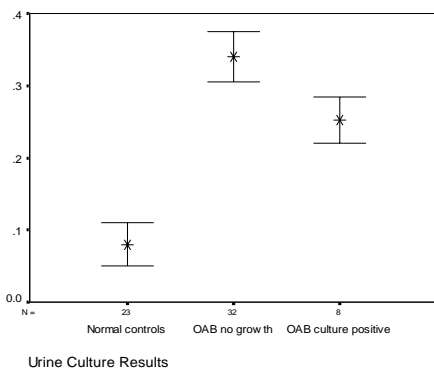
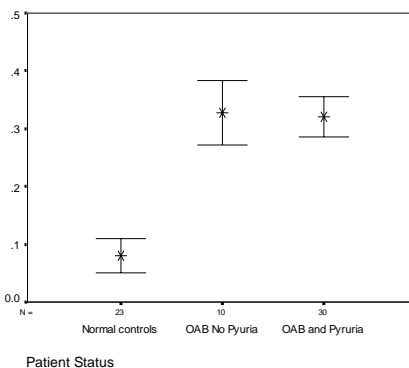


Figure 4 The proportion of clue cells according to the pyuria state



Interpretation of results

The proportion of clue cells clearly differentiates between normal controls and patients with OAB symptoms. There is also a significant difference between OAB patients and those with the same symptoms but positive routine urine culture. A inflammatory infiltrate of the urothelium has been described in OAB patients with or without pyuria and in the presence of negative culture. These data show evidence of increased colonisation of cells associated with OAB with or without pyuria. Perhaps infection does lie at the heart of OAB but that it goes undetected. The inverse relationship with urine culture suggests that bacterial adhesions militate against culture detection for some reason.

Concluding message

The measure of Clue cell expression in the urine of patients with OAB presents a novel, readily applied, method for exploration of this condition. Further evidence of infection aetiology for OAB symptoms is presented here.

References

1. Lunawat R, Khasriya RK, Falzon M, Balachandran L, Malone-Lee J. Spun urinary sediments and Giemsa stain reveal a chronic inflammatory infiltrate in patients with symptoms of OAB. *Neurourol Urodyn* 2009;28(7):776-7.

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| Specify source of funding or grant | Research into Ageing |
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| What were the subjects in the study? | HUMAN |
| Was this study approved by an ethics committee? | Yes |
| Specify Name of Ethics Committee | Morrefields and Whittington Research Ethical Committee |
| Was the Declaration of Helsinki followed? | Yes |
| Was informed consent obtained from the patients? | Yes |