

INVESTIGATING THE ORIGIN OF EPITHELIAL CELLS FOUND IN THE URINE OF LUTS PATIENTS USING IMMUNOFLUORESCENCE: CONTAMINATION OR INFLAMMATION?

Hypothesis / aims of study

Epithelial cells are commonly seen in the urine when microscopy is used to examine fresh specimens for pyuria. Without evidence, they have been dismissed as contaminants, exfoliated from the genitalia. However, models of uropathogenic *E. coli* (UPEC) infection have demonstrated increased urothelial cell shedding to be the first-line response to bacterial insult (1). We reported a direct relationship between the number of epithelial cells and the level of pyuria observed in the urine of our chronic LUTS patients (2). Although this evidence supports the murine model of bacteria-induced urothelial inflammation and shedding in LUTS patients, we recognise the requirement for proof of cellular origin. Uroplakin-III (UP3) is expressed solely on the asymmetric unit membrane (AUM) of fully differentiated urothelial cells (3). Therefore, we targeted this glycoprotein to test the hypothesis that the majority of urinary epithelial cells originate in the urinary tract.

Study design, materials and methods

Meticulous MSU samples were cytocentrifuged, fixed in 4% formaldehyde and labelled with anti-UP3 antibody. Post incubation with TRITC- conjugated secondary antibody, the cells were counterstained with DAPI. Vaginal swabs (re-suspended in phosphate buffered saline) were processed identically to act as negative controls. The percentage of UP3-positive cells was calculated by a blinded researcher using epi-fluorescent microscopy. Counts were carried out in triplicate before averaging. Images were also taken using scanning confocal microscopy to demonstrate the staining patterns of UP3 in high resolution.

Results

UP3-positive cells were distinguished easily owing to a distinct staining pattern (**FIG 1**). MSU samples from 22 patients with chronic LUTS (F=22; mean age=51; sd=19) were compared with vaginal swabs from 22 further chronic LUTS patients (F=22; mean age=50; sd=20). The mean percentage of UP3-positive cells in the MSU samples was 72% (95% CI 67 to 77) but only 25% (95% CI 21 to 30) in the vaginal swabs. The proportion of UP3-positive cells found in the urine of chronic LUTS patients was significantly higher than that in the vagina (F (1, 42)=206.7, P<.001) (**FIG 2**).

Interpretation of results

These data show that UP3 immunofluorescence can be used to distinguish cellular origin in MSU samples. The study demonstrates that the vast majority of epithelial cells found in the urine originate in the urinary tract and a properly collected MSU is not unduly contaminated with vaginal contents.

Concluding message

These results show a properly collected MSU sample to be representative of underlying pathology of the urinary tract in women. Increased shedding of urothelial cells is associated with LUTS in women. As with studies of acute UTI in humans and mice, this innate immune response suggests inflammation initiated by low-grade infection in LUTS.

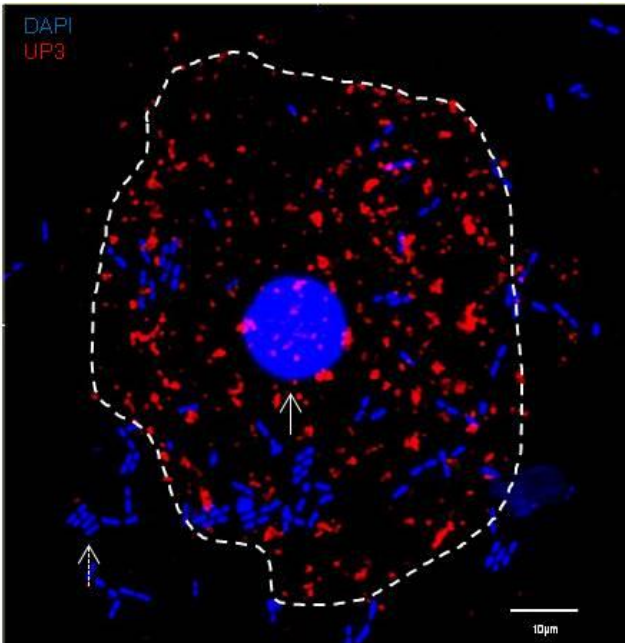


Figure 1. Laser scanning confocal image of a UP3 positive (red) / DAPI (DNA, blue) labelled shed urothelial cell. Cell boundary (broken white line). Nucleus (solid white arrow), bacteria (broken white arrow).

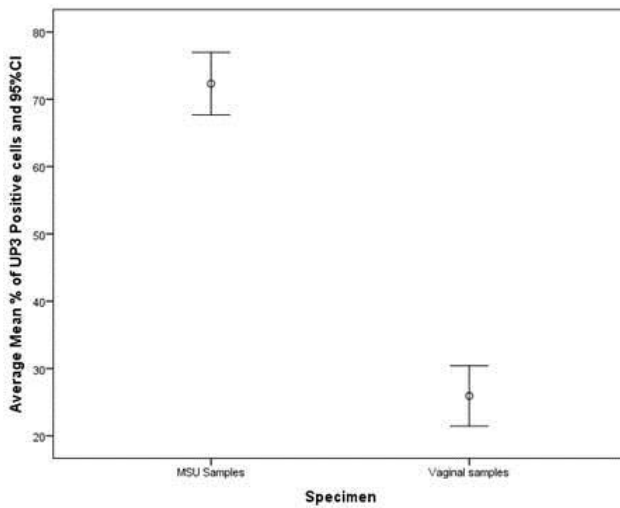


Figure 2. Graph showing the mean proportion (%) and 95% CI of UP3 positive cells found in MSU sample (N=22) when compared with vaginal swabs (N=22) ($F(1, 42)=206.7, P<.001$).

References

1. Infection and Immunity 2008;76(7):2978-90.
2. Neurourology and Urodynamics 2011; 30(6): 1076-77.
3. J Biol Chem 1990 Nov 5;265(31):19170-9.

Disclosures

Funding: No Disclosures **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Moorefields and Whittington Research Ethical Committee **Helsinki:** Yes **Informed Consent:** Yes