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Campbell N L[1], Cheng Y[2], Lochhead A[1], Mansfield K J[1], Moore K H[2]
1. *University of Wollongong,* ***2.*** *St George Hospital, UNSW*

CAN WE DISTINGUISH BETWEEN CELL TYPES PRESENT IN MIDSTREAM AND CATHETER URINE SAMPLES FROM WOMEN WITH INCONTINENCE?

Hypothesis / aims of study

Urine samples may be obtained from patients with Lower Urinary Tract Symptoms (LUTS) by either midstream urine (MSU) or catheter specimens of urine (CSU). Most commonly urine microbiology and microscopy is performed on MSU specimens, so-called 'clean catch' urine specimens. These samples potentially contain both urothelial cells and squamous epithelial cells of vaginal origin. In contrast, CSU are more difficult to obtain but should only contain cells from a single source, that is urothelial cells from the bladder. The aim of this study was to identify variations in cell types present in MSU and CSU specimens. We have employed 3 staining methods in order to compare the differences in morphology and staining characteristics between MSU and CSU. We aim to establish a protocol to identify the origin of cells in MSU (vaginal and bladder) and CSU (bladder only) specimens. These findings would be important in future efforts to examine the prevalence of occult bacterial cystitis in women with refractory detrusor overactivity, which needs to be distinguished from the report of "mixed growth due to vaginal contamination".

Study design, materials and methods

Paired MSU and CSU specimens were obtained from 10 women (age range 32 to 79 years) who presented to our centre for investigation of incontinence. Patients were asked to arrive with a comfortably full bladder bladder and then to provide a MSU specimen on arrival. All patients were then catheterised by the continence nurse 15-20 minutes later and a CSU specimen was collected just prior to urodynamics testing.

Aliquots of paired MSU and CSU specimens were preserved for urine cytology. Samples were centrifuged to concentrate the urothelial or vaginal epithelial cells; the cells were fixed with formalin. Fixed cells were then centrifuged onto microscope slides by cytospin.

Cells preparations were subjected to 3 different staining techniques:

A) Wright's Giemsa stain (usually used for identification of white blood cells),

B) Papanicolaou stain (usually used for cervical cytology, differentiates keratinised and non-keratinised epithelial cells [1]) and

C) Cytokeratin 20 (CK20) immunocytochemistry (used as a marker for superficial umbrella urothelial cells [2]).

Wright's staining and Papanicolaou staining was performed on all specimens and CK20 immunocytochemistry on paired specimens from five patients. The proportion of keratinised and non-keratinised cells in Papanicolaou stained paired MSU and CSU specimens was estimated in four low-power fields by two investigators independently.

Results

In MSU specimens a number of individual cell types were identified using Papanicolaou stain. The first was a large spreading cell with pale, blue-staining cytoplasm (non-keratinised) (Figure 1, arrows) containing a large round to oval blue-staining nucleus (Figure 1A) or a dense orange-staining nucleus (Figure 1B and C). In addition, polygonal shaped cells, with orange-stained cytoplasm (keratinised) and a small, dense nucleus were also identified (Fig 1B and C, arrowhead). The third distinct cell type, a smaller cell with densely blue or orange-staining cytoplasm and a blue/ purple stained nucleus (Figure 1A and C, *) were also visible in some MSU specimens. In MSU specimens, there was great variability in the proportion of the cell population that were orange-staining, keratinised cells (10 to 80%).

On Wright's stain, cells of similar shape and size could be seen, although these were unable to be distinguished by their staining. In general, much fewer epithelial cells were isolated from CSU specimens. In CSU specimens the most prominent cell type was the non-keratinised cells with a blue-stained cytoplasm (similar to those shown by the arrows in Figure 1) although in approximately 50% of samples, cells with orange-stained cytoplasm (keratinised cells, similar to those shown by the arrowhead in Figure 1B and C) were still present. These cells represented approximately 0 to 30% of the cells stained in CSU specimens.

CK20 immunocytochemistry staining patterns in both MSU and CSU samples were indistinguishable, indicating the presence of superficial urothelial cells in both urine sources.

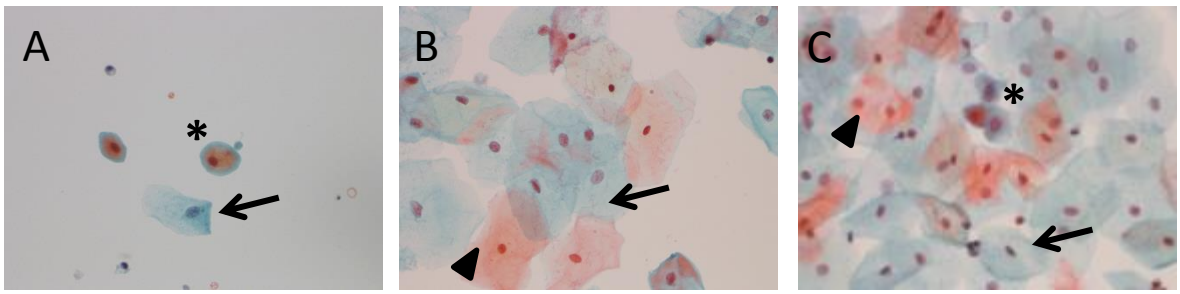

Figure 1. Papanicolaou staining of MSU specimens (40 x magnification) identified large spreading blue-staining (non-keratinised) epithelial cells (arrow in A and B) as well as large orange-staining (keratinised) epithelial cells (arrowhead in B and C). In addition smaller, round blue or orange-staining cells were also identified (* in A and C).

Interpretation of results

Based on the results of this preliminary study, MSU specimens contain both orange-stained, keratinised, and blue-stained, non-keratinised cells, that is cells presumed to be of both bladder and vaginal origins. In CSU specimens there was a significant

reduction in the presence of keratinised cells in support of the hypothesis that these were of vaginal rather than urothelial origin. However, it was interesting that these keratinised epithelial cells were still present in approximately 30% of CSU specimens (where we would assume the cells were of bladder origin alone). The presence of these orange-stained, keratinised epithelial cells in CSU specimens could be explained by the presence of trigonal urothelial cells which are known to be squamous keratinised epithelium in up to 50% of women [3].
Wright's staining was unable to distinguish between keratinised and non-keratinised cells although cellular morphology was similar in both Papanicolaou and Wright's staining. Based on this result Papanicolaou staining appears to be superior in distinguishing between urothelial and vaginal epithelial cells when comparing cell preparations of MSU and CSU urines. CK20 immunocytochemistry demonstrated the presence of urothelial umbrella cells in specimens from both MSU and CSU.

Concluding message

This study indicates that Papanicolaou stain appears to be the best protocol to identify the origins of cells in MSU and CSU specimens, although this is complicated by the presence of keratinised trigonal cells in some patients. These findings could have implications for the manner in which specimens from women with LUTS are analysed in the future. Due to the difficulty of distinguishing cells on a purely morphological basis, establishment of a protocol combining a number of stains and a definitive urothelial cell marker would be valuable in studies where the urothelial origin of the cells being examined is important.

References

1. J Urology 2013; 189 (1), 343-351
2. BMC Urology 2011; 11, 5
3. J Urology 1979; 122, 317-321

Disclosures

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