

## RELATIONSHIP BETWEEN BACTERIURIA AND ATP CONCENTRATIONS IN VOIDED URODYNAMIC FLUID: A 2-YEAR PROSPECTIVE STUDY.

### Hypothesis / aims of study

The normal sensation of fullness in healthy bladders is thought to be related to release of ATP is released from bladder urothelium. Recent studies suggest ATP also contributes to the increased sensation of urgency found in disease states such as detrusor overactivity (1). To date, the effect of bacterial infection on endogenous ATP release in the bladder has not been well studied. Our aim was to determine whether ATP release was alternated in women with bacteriuria compared to those with sterile urine in women with detrusor overactivity (DO) compared to stress-incontinence. We hypothesized that ATP release would be greater in women with bacteriuria compared to those with sterile urine and further, that a different ATP response would be seen in women with detrusor overactivity (DO) compared to stress-incontinent controls.

### Study design, materials and methods

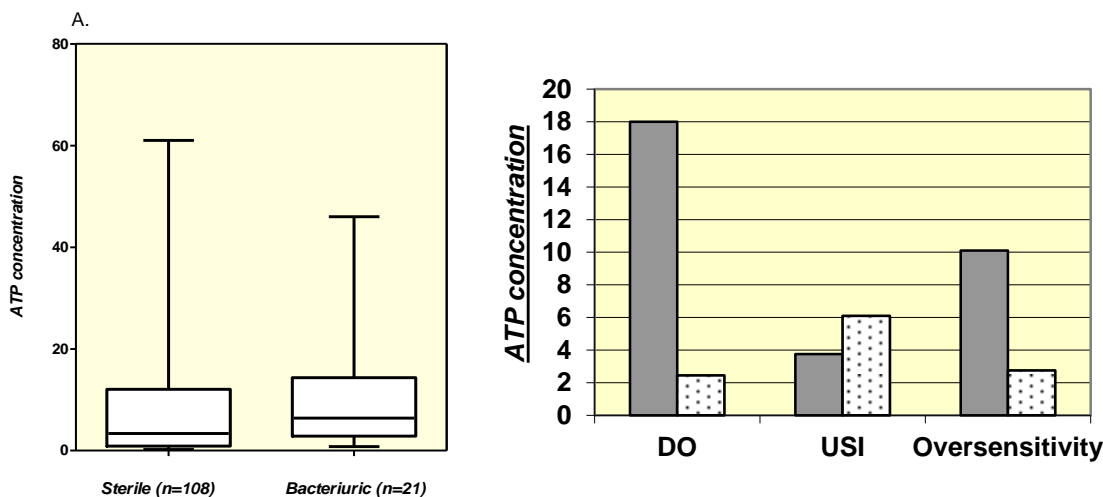
Twin-channel cystometry was performed in a consecutive series of 232 women. A catheter specimen of urine (CSU) was obtained at the start of urodynamics and cultured (threshold  $>10^3$  CFU/ml, Horse Blood Agar/ 35°C in 7% CO<sub>2</sub> and McConkey's agar/ 35°C in air). Bladder volume at first desire to void (FDV) and maximum cystometric capacity (MCC) were recorded during filling cystometry. After uroflowmetry, a sample of the voided urodynamic fluid was snap frozen at -30°C for subsequent ATP analysis.

Frozen voided urodynamic fluid was thawed to room temperature, ATP was measured on a using a standard luciferase bioluminescence assay (Sigma, luminometer). ATP concentration (in nM) for each test specimen was calculated against the ATP standard curve. To check for the effect of freezing, ATP was also measured in 55 freshly collected samples from DO patients, which were then frozen and re-measured. The number of urothelial cells was also determined by haemocytometer. Median ATP concentrations were compared across groups (Mann-Whitney). P value significance was 5% level; Statsdirect statistical package 2.7.2.

### Results

#### A. The relationship between bacteriuria and ATP release in frozen voided urodynamic fluid

From Jan. 2008 – Dec. 2009 232 consecutive cystometry tests were performed. The overall incidence of bacteriuria on the day of urodynamic testing was 9.5% (22/232). Of the 232 women, 99 with sterile urine were previous reported (3) and are excluded here in. Remaining was 21 patients with bacteriuria and 108 with sterile urine (*Figure 1*). Overall, we found no significant difference in ATP concentrations in washings from the bacteriuric bladders compared to the sterile bladders (6.4 nM versus 3.4 nM respectively;  $p=0.09$ ; median difference 1.8 nM; 95% CI -0.5 to 4.7, *Figure 1*). Women with pure DO had significantly higher ATP levels in the presence of bacteriuria compared to those with sterile urine (18.0 nM versus 2.45 nM respectively;  $p=0.048$ , *Figure 2*). Women with bladder oversensitivity, also showed higher ATP levels in the presence of bacteriuria compared to sterile cases, (10.1 nM versus 2.7 nM respectively;  $p=0.36$ , *Figure 2*). In women with pure urodynamic stress incontinence, USI), the presence of bacteriuria did not influence ATP concentration ( $p=0.52$ , *Figure 2*). The presence of bacteriuria did not influence FDV or MCC in the total group or in any of the diagnostic sub-groups.

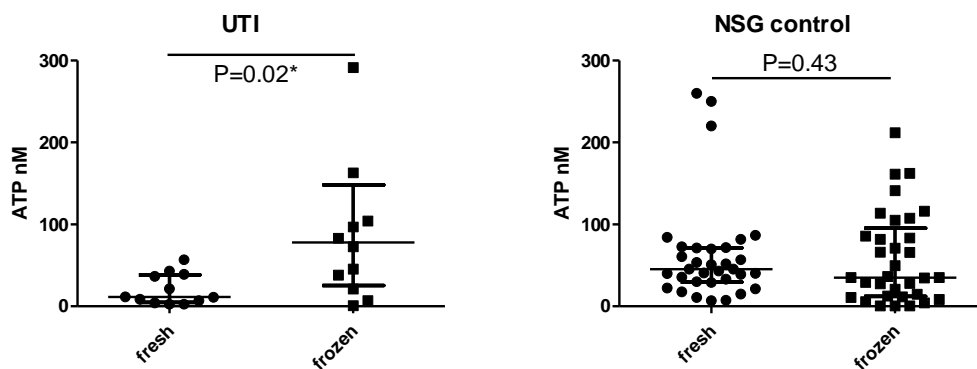


**Figure 1**

**Figure 2** (Dark bars = bacteriuric, Light bars = sterile)

#### B. Comparison of ATP concentration in fresh and frozen voided urodynamic fluid

In DO patients with bacteriuria the concentration of ATP was significant higher in frozen samples ( $p=0.02$ , Wilcoxon, *Figure 3*) compared to fresh samples, but remained unchanged in DO patients without bacteriuria.



**Figure 3.** Comparison of ATP concentration measured between fresh and frozen in UTI patients (A) and no significant growth controls (B)

As we expected, there were more exfoliated live cells counted in fresh bacteriuric sample (25,465 cells/ml) than non-infected samples (107 cells/ ml). Only a small number of these live cells remained after thawing frozen samples (638 cells/mL in bacteriuric, 11 cells/mL in non infected).

Interpretation of results

Our results show that in women with severe urgency (DO/ bladder oversensitivity) the presence of bacteriuria significantly increased ATP concentrations in frozen intravesical fluid (after thawing). This was not seen in women with pure USI, in whom bacteriuria did not affect ATP concentrations. ATP was increased after freezing in DO patients with bacteriuria, but not in those with sterile urine. In addition a higher urothelial cell count was found bacteriuric patients. Our results comparing the cell count before and after freezing suggest that cells burst during freezing and thawing.

Concluding message

To our knowledge, this is the first report of the effect of bacteriuria on ATP concentrations *in vivo* in women with urinary incontinence. Those with severe urgency show an increased ATP concentration with bacteriuria. This is not seen in women with stable bladders.

References

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3. Cheng, Y., Mansfield, K. J., Allen, W., Walsh, C. A., Burcher, E. & Moore, KH. 2010. 'Does adenosine triphosphate released into voided urodynamic fluid contribute to urgency signaling in women with bladder dysfunction?' *J Urol*, 183:3, 1082-6.

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| <b>Specify source of funding or grant</b>               | NHMRC   |
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| <b>What were the subjects in the study?</b>             | HUMAN   |
| <b>Was this study approved by an ethics committee?</b>  | Yes   |
| <b>Specify Name of Ethics Committee</b>                 | South East Area and Illawarra Health Service Human Ethics Committee |
| <b>Was the Declaration of Helsinki followed?</b>        | Yes   |
| <b>Was informed consent obtained from the patients?</b> | Yes   |