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UNACCEPTABLE CONTAMINATION, WITH WHITE CELLS, OF CLEAN-CATCH MIDSTREAM URINE SAMPLES FROM WOMEN WITH OAB SYMPTOMS

Hypothesis / aims of study

The diagnosis of overactive bladder (OAB) presumes the absence of proven urinary infection. Recent studies have demonstrated that standard laboratory midstream urine specimen analysis, relying on a diagnostic threshold of 10^5 colony forming units (cfu) ml^{-1}, has been shown to miss over 50% of infections [1] in patients with lower urinary tract symptoms. An alternative threshold of 10^2 cfu ml-1 has been advocated but most laboratories are not equipped to apply this. Another biomarker of significant urinary infection has been sought and contemporary studies confirm the pre-eminence of pyuria ≥10 white blood cells (wbc) μl^{-1} as the best surrogate indicator of urinary infection.[2]. In applying this to patients with OAB symptoms, culture negative pyuria ≥10 wbc μl^{-1} has been found in over 50% of patients presenting with OAB symptoms.

Whilst the significance of this finding has yet to be elucidated, doubts should be raised by the fact that the samples in that study were collected by the clean catch midstream sample (MSU) method. It is possible that white cells from other sites, particularly the vagina, may have contaminated the MSU samples. There are no published data that address this concern, whilst bacterial contamination of MSU samples is well recognised.

This study tested the hypothesis that clean-catch MSU samples are unacceptably contaminated by extrinsic white cells when compared to samples collected by the catheter specimen of urine (CSU) method.

Study design, materials and methods

The null hypothesis was that the proportion of pyuria positive samples was identical whether collection was by MSU or CSU. A clinically significant difference in proportions was estimated as 0.33 (specifically, 0.66 versus 0.33). A sample size of 40 pairs of data was calculated to have power of 85.3% to yield a statistically significant result.

40 newly presenting, female patients with symptoms of OAB were recruited and gave consent. They provided an MSU sample and up to three days later, during which time they went untreated, they provided a CSU. The sequence was used to avoid CSU introduction of new infection. The sample was analysed immediately on collection. A pipette was used to introduce 0.9µl of urine into the counting chamber of a haemocytometer (Improved Neubauer, Hawksley) and the specimen was examined using a x25 objective with x10 eyepiece with a light microscope (Leitz Dialux 20EB). The leucocyte count (wbc μL^{-1}) was enumerated by counting cells in five large squares and multiplying by 2.2. If a cell overlapped a dividing line, it was included, provided the line ran along the top or right side but ignored if the line ran along the bottom or left side. The investigators were blinded to the previous results.

The data were collated and analysed. The between method differences in proportions of positive (pyuria) and negative (no pyuria) samples were assessed by the Chi squared test. The between method differences in white cell counts were compared by the paired t-test.

Results

40 women were recruited. Their mean age was 60 (sd=18). They all had symptoms of overactive bladder. The results from the different sampling methods are illustrated in table 1.

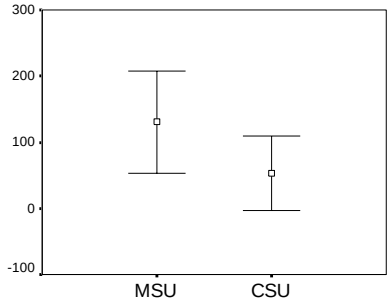
Table 1 – The comparison of pyuria detection between the sampling methods

Method of sampling	No pyuria detected	Pyuria detected
MSU	3	37
CSU	24	16

37 (93%) of the MSU samples showed pyuria but only 16 (40%) of CSU samples (X^2 =25, df=1, p<0.001). The differences in absolute white blood cell count between sampling methods is illustrated in Figure 1.

Figure 1

Comparison of absolute pyuria counts obtained from MSU and CSU samples obtained from the same patients.



There was a significant difference in mean pyuria count between the two groups with higher counts evident from MSU samples (95% CI of difference = 18 to 144, p=0.013, t=2.6).

Interpretation of results

These data support the hypothesis that MSU samples may be contaminated by extraneous white blood cells, possibly from the vagina. This could lead to over-diagnosis of urinary infection and studies that involve the analysis of pyuria in association with symptoms must take this into account. This was a blinded study but the method did not randomise the sampling because of concerns that a catheterisation of the bladder might introduce infection or perturb the bladder in some way. It may be that the difference in counts could be explained by spontaneous resolution during the time between sampling. Given the numbers it is not probable but nevertheless, despite the risk of catheter contamination, these data will require verification in a trial using a randomised order for sampling.

Concluding message

With the increased interest in the role of urothelial inflammation in the exacerbation of lower urinary tract symptoms the detection of pyuria attracts greater significance. However, before accurate conclusions can be drawn, the sampling methods must be thoroughly validated. Many methods that are taken for granted have not been subject to the appropriate ratification.
These data suggest that in the case of women, a CSU is a much more reliable sampling method for enumerating pyuria.

References

1. N.Engl.J.Med. 329(18), 1328-1334. 28-10-1993
2. Urol.Clin.North Am. 11(1), 95-101. 1984

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**