Peer Review File

Article information: http://dx.doi.org/10.21037/tau-20-1259

Reviewer: 1

#

Authors could mention and refer to these 2 papers:

Response

Thank you for your insightful comment. We have added two reference (reference 26 and 63) in the text.

#

In expert comments, authors state even normal appearing non-malignant urothelium may shed cfDNA with alterations- this may reduce PPV?

Response

We appreciate the reviewer's insightful comment, which we have addressed in the Challenges and future directions for urinary cfDNA analysis section (page 23, line 7).

In Challenges and future directions for urinary cfDNA analysis:

Though this might reduce positive predictive value, patients with mutated cfDNA in urine without visible tumor have a high likelihood of developing tumors and should be followed carefully.

#

Future directions could touch on if this technology may assist in bladder preserving approaches for muscle invasive bladder cancer?

Response

We appreciate the reviewer's meaningful comment, which we have addressed in the Challenges and future directions for urinary cfDNA analysis section (page 23, line 10).

In Challenges and future directions for urinary cfDNA analysis:

Furthermore, it might be useful for patients with MIBC after bladder preservation therapy by urinary cfDNA analysis.

Reviewer: 2

#

The authors fail to convey some important nuances with regard to the detection of low grade and high grade tumors. Low grade tumors are usually papillary and detectable on cystoscopy/ureteroscopy and/or other studies. They may be difficult to biopsy. High grade tumors may be papillary or flat CIS lesions, the latter of which is difficult to detect and for which cytology/ancillary testing are generally more important. Current thinking on urinary cytology, as established by The Paris System, indicates that urinary cytology is best at detecting high grade lesions and not really meant anymore to detect low grade lesions. It is true, as the authors state, that sensitivity and specificity (actually specificity and NPV) of urinary cytology for high grade lesions are very high. There isn't really a "low sensitivity" of urinary cytology for low grade lesions among those that practice cytology, since it is not a test used to detect low grade lesions. This may not be so important given that the problem is the detection of high grade lesions (especially CIS). The authors should include these concepts when they discuss urinary cytology and also briefly mention The Paris System. If they do not wish to read TPS book, a very simple review can be found here https://doi.org/10.1111/cyt.12345. For another review discussing urinary ancillary tests in the context of TPS, the following may be useful: doi: 10.1159/000499027

Response

Thank you for your meaningful advice. We have incorporated your advice in the Introduction and references (page 5, line 13).

In Introduction:

For the detection of UC, urine cytology **established by The Paris System** has high sensitivity for high-grade tumors (84%), but low sensitivity for low-grade tumors (16%) (21, **22).** Since current procedures for diagnosis or surveillance, such as cystoscopy or ureteroscopy (for patients after KSS) are invasive and costly, less invasive and reliable follow-up methods are necessary for patients with **(especially for low-grade)** UC**.**

#

The authors do not really discuss problems in differentiating low grade lesions from high grade lesions using ancillary tests, including molecular tests discussed in this review. While this allows for detection of both low and high grade lesions, I have not encountered a test (other than urinary cytology) that confidently and specifically identify high grade lesions when present.

Response

We appreciate the reviewer's meaningful comment, which we have addressed in the Genetic analysis section (page 10, line 5).

In Genetic analysis:

Given these facts, urinary cfDNA would help to differentiate low-grade tumors from high-grade tumors by analyzing *TP53* and *FGFR3* mutations.

#

The authors fail to mention that cfDNA analysis in urine is likely to be oversensitive and detect abnormalities that are not clinically relevant. For instance, see reference 101 as an example of false positive results that likely correspond to dysplasia or other pre-clinical alterations, which are not actionable. This is an important limitation of this sort of testing.

Response

We appreciate the reviewer's meaningful comment, which we have addressed in the Challenges and future directions for urinary cfDNA analysis section (page 23, line 7).

In Challenges and future directions for urinary cfDNA analysis:

Though this might reduce positive predictive value, patients with mutated cfDNA in urine without visible tumor have a high likelihood of developing tumors and should be followed carefully.

#

The manuscript uses proper English grammar but the abstract is not worded as well.

Response

Thank you for your constructive comment. We have proofread by native speaker.

#

"Urine can be collected in sterile collection containers without any specialized skills or equipment." - this is true, but the collection of sterile urine is not that easy. As the authors state in the next paragraph, voided urine can contain contaminants. If a urine specimen is being sent for expensive molecular testing, it might be best collected in a sterile manner (cystoscopy, catheterized, etc. sample).

Response

Thank you for your insightful comment. As you pointed out, sterile urine collection is the ideal method for molecular analysis, but it takes cost and invasiveness for patients by catheter induction. Furthermore, because even the urine collected by catheter is also susceptible to crystal precipitation or bacteria while storage at 2 to 8 °C, we think that voided urine collection is one of the feasible manners in real world. We have incorporated your advice in the Overview of the cell-free DNA section (page 14, line 4).

In Overview of the cell-free DNA:

Urine can be collected in ~~sterile collection container~~cups without any specialized skills or equipment

#

The authors mention at the beginning that urine cytology has high sensitivity (for high grade lesions) but several times later in the manuscript state that urine cytology has low

sensitivity, without qualifying that they are including low grade lesions. I would rather this be stated to instead say that urinary cytology cannot reliably detect low grade lesions.

Response

We appreciate the reviewer's meaningful comment. We have clearly stated the problems of urinary cytology for low-grade tumor. We have incorporated your advice in the Challenges and future directions for urinary cfDNA analysis section (page 22, line 9).

In Challenges and future directions for urinary cfDNA analysis:

First, urine cytology has low sensitivity **for low-grade tumor**;

Urine cytology has a ~~low sensitivity but~~ high specificity and a long history of clinical use.

#

"We investigated the diagnostic and prognostic 9 potential of urinary cfDNA in patients with localized UTUC (110). In their report, 10 the sensitivity of 3 hotspot gene mutations in urinary cfDNA was only 55.4%, but 11 the sensitivity increased..." Who is "we"? If "we" is the authors, shouldn't the next sentence read "In our report" instead of "In their report"?

Response

Thank you for your meaningful advice. We have corrected it.

#

"BCG-induced inflammatory changes in the urothelium are often mistaken for CIS or other malignant manifestations" - this sentence should end "manifestations on cystoscopy" to clarify that it does not mean cytology. Cytology does not confuse BCG changes with carcinoma, generally speaking.

Response

We appreciate the reviewer's meaningful advice. We have corrected it.