Reviewer's report

Title: Urinary Levels of Hepatocarcinoma-intestine-pancreas/Pancreatitis-associated Protein as a Diagnostic Biomarker in Patients with Urothelial Carcinoma

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Reviewer: Virginia Urquidi

Reviewer's report:

The authors investigated the potential utility of HIP/PAP as a biological marker for detecting urothelial carcinoma (UC). HIP/PAP expression was monitored in tissue specimens, cell lines and in urine samples. The urinary levels were monitored by ELISA, and were found to be elevated in cancer patients. Urinary HIP/PAP levels also correlated positively with pathological stages and were predictive of recurrence risk to some extent. The HIP/PAP assay detected UC with a sensitivity of 82.5%, and a specificity of 78.7%, which outperformed the commercial NMP-22 test in the same cohort. The authors conclude that urinary levels of HIP/PAP may be a novel biomarker for the detection of UC.

The study is well designed and had a good sized cohort with a range of stage/grades. There are some gaps in information that need to be strengthened.

Minor essential revisions:

1. Information on the study cohort needs to be mentioned in the abstract.

2. Page 4, Background. Authors state that BTA/NMP22 tests are unable to correctly predict bladder tumors. These biomarker tests can detect UC with performances better than voided urine cytology. They are not perfect, particularly when detecting low stage/grade UC, but the literature shows that NMP22 achieves sensitivity/specificity values close to those achieved by HIP/PAP in this study. The performance of these biomarkers needs to be more fairly described.

3. Page 5, the authors describe the fact that they have reported higher HIP/PAP levels in patients with painful bladder syndrome/interstitial cystitis. The authors state that this was the basis for investigating HIP/PAP in UC, but the utility of HIP/PAP as a UC biomarker may be severely compromised because many patients with urinary tract infections may be falsely positive with this test. With this knowledge, there should have been an effort to include UTI cases in the cohort, but of the 119 control cases, only 6 have cystitis. This limitation needs to be discussed.

4. There is no mention of correlation of HIP/PAP with hematuria. The other common interference with urinary tests is the presence of blood in the urine. Since HIP/PAP is present in serum/plasma (Mol Cell Proteomics 2004, 3(4):311-326) the investigation for such correlation is pertinent. Was there any
correlation of HIP/PAP with blood in cancer and/or controls? If not tested, this limitation needs to be discussed.

5. NMP-22 tests usually state that they should be performed on fresh urine samples. In this retrospective study, samples were processed and frozen for storage. This may explain the weaker performance of NMP22 in this study than described in the literature. This needs to be considered and commented on.

6. The legend of Figure 3A indicates that ‘Bars represent median levels’; however there are no bars in Figure 3A.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'