Peer Review File

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Reviewer A

This is an interesting study with a thorough review of their cohort of patients. The authors have sought to identify factors that may predict for prolonged hematospermia. They have re-identified that abnormal imaging is associa0ted with prolonged hematospermia but also identified the urinary pH as a potential factor.

I have some concerns regarding the use of urine pH in this manner. The values reported are based on urine dipstick assessment, as opposed to laboratory testing, and reported in whole numbers. The overall median pH was reported at 6.0 with a wide standard deviation, that included a significant portions of both cohorts.

Reply: We appreciate these important comments from the reviewer. As noted, urinary dipstick pH assessments have been reported to differ by as much as ±0.9 pH from those measured with a benchtop laboratory pH device in some cases (Ref. 27). Therefore, it cannot be denied that even among patients classified into the same UpH group in the present study, some may have values that were greatly different. We understand that this is a limitation, so have added the following sentence to the revised version for better understanding by the readers.

Changes in text: A litmus strip method was used to provide integer values for UpH levels to be used in analyses, though those may differ greatly from the actual value in some cases (27). (Lines 277-278)

The difference in duration of hematospermia between the two groups was statistically significance but translates to a time period of only 2 weeks which is unlikely to be a significant clinically difference. This is in contrast to the time difference between the normal and abnormal imaging groups which was almost 4 months in duration.

Reply: As the reviewer pointed out, even when statistical significance is observed, it is necessary to thoroughly determine whether it has significant effects on clinical findings. At this time, it is not possible to conclude how a median difference of two weeks is clinically informative for individual patients. Therefore, we would like to emphasize the significance of UpH level by showing that it is possible to make better predictions when using that in combination with imaging findings, rather than using it alone. The following has been added to the Conclusions section in the revised manuscript.

Changes in text: By combining these two factors in analyses of affected patients, more accurate prediction of hematospermia duration was found to be possible. (Lines 293-295)

it similarly is not clear if there is a range of durations of hematospermia for the different pH levels, i.e. is a pH of 5 much shorter than a pH of 7, which might be shorter than pH of 9.

Reply: Based on the reviewer's helpful suggestion, Kaplan-Meier curves for the five UpH level groups (UpH 5-9) based on the PSM cohorts were created. Those findings

confirmed that hematospermia persistence tended to be prolonged as UpH level increased. These results are now shown in Figure 3A and have been incorporated into the text of the revised manuscript. While we do not think this represents a complete solution, it is considered to be helpful for the readers to understand the results more clearly.
Changes in text: In comparisons of the five groups divided by UpH level (UpH 5-9), it was also noted that the persistence of hematospermia tended to be prolonged as the level increased (Fig. 3A). (Lines 215-217)

While I appreciate the authors have acknowledged the limitations of pH and the lack of information regarding dynamic pH levels, I think to come to their conclusion of it being a predictor, we also need the information to identify if correcting it improves things.
Reply: We appreciate these important comments. As noted in the Discussion section, we consider that an underlying urinary tract infection is associated with elevated urinary pH and delayed healing of hematospermia. Therefore, investigating changes in pH over the course of treatment may support this hypothesis. In the Limitations section of the revised version, please note the following modifications.
Changes in text: In addition, UpH level was measured in spot urine rather than 24-hour urine samples, as it has been reported that the pH value of a spot urine sample may correlate well with that of a 24-hour sample (14, 28), though the latter is considered to possibly provide more accurate values. Additionally, the effects of dynamic UpH value on clinical outcome were not evaluated. Although the effects of UpH level on recurrent and persistent urinary tract infections have been investigated, how pH level changes over time during a urinary tract infection remains unclear (29, 30). We consider that UpH level may change over the course of treatment when infection or inflammation in the prostate and surrounding area are factors related to elevated UpH level and delayed healing of hematospermia. (Lines 278-286)

When the authors use propensity match scoring to compare groups they excluded quite a number of patients. The average duration of disease within this PSM cohort was only 1.8 months, very similar to that reported in general literature. It fails to address the more clinically concerning cohort of those who have a much more prolonged hematospermia (>2 months).
Reply: We agree that this is a very important point. Therefore, patients were categorized according to the duration of hematospermia (<2, 2-6, >6 months) and the proportion of patients in each group with both factors was calculated. Those results showed a significantly higher proportion of patients with the two factors in the 2-6 months group and especially the ≥6 months group, as compared to the <2 months group. We consider that this also indicates the possibility of applying the combination of these two factors for prediction of patients who will show prolonged hematospermia.
Changes in text:
In addition, the proportion of patients with these two factors present simultaneously was significantly higher in the group with hematospermia for two months or more, and especially with a duration of greater than six months, than in the group with a duration

of less than two months. (Lines 45-48)
In addition, the proportion of patients with these two factors was found to be significantly greater in the group with hematospermia for two months or more, and especially with a duration greater than six months, as compared to the group with a duration of less than two months (Fig. 4). (Lines 224-227)

There are two potential errors within the manuscript. The phrasing on line 165+168 "and 76 cases had any of these findings" seems unnecessary as already documented. in line 198 the manuscript states that spontaneous improvement of haematospermia in the PSM cohorts were seen in 115 cases, however in the accompanying table 3 only 105 cases are noted to have had improvement.
Reply: Thank you for pointing out these mistakes. We have deleted or corrected parts of the text.
Changes in text: Other abnormal imaging findings, such as urethral cysts or intraprostatic hematoma, were noted in seven (3.5%). (Lines 163-164)
Spontaneous improvement of hematospermia was seen in 105 (87.5%) of the 120 patients, with a median disease duration of 1.8 months. (Lines 208-209)

Reviewer B
The authors performed a retrospective study to evaluate predictor factors of persistent hematospermia. This is an interesting study; however, some aspects deserve attention to further improve the quality of the manuscript.
Comment 1: How hematospermia was defined? Macroscopically, microscopically or both? If microscopically, which RBC cutoff was used?
Reply 1: Thank you for the important comments and questions. The diagnosis of hematospermia was based on the gross color of the semen sample. We have added the following to the ”Evaluation and definition of clinical characteristics” subsection in the revised version of the manuscript.
Changes in text: Hematospermia was defined as reddish or dark brown semen found to be contaminated with blood by macroscopic observation. (Lines 113-114)

Comment 2: Was there any protocol for hematospermia treatment in the institutions involved in the study?
Reply 2: At our institution, there is no strict treatment protocol and temporary empirical treatment is determined for individual patients by the attending physician. Nevertheless, in principle, an examination to determine whether symptoms have improved will be performed from two to four weeks after the start of treatment. The following text has been added to the “Treatment and follow-up” sub-section.
Changes in text: Generally, the presence or absence of symptom improvement was determined from two to four weeks after the start of treatment. The need for medication during that period was decided by the attending physician. (Lines 136-138)

Comment 3: Did any participants received 5areductase inhibitors as treatment?
Reply 3: None of the patients received temporary empiric therapy with a 5α-reductase

inhibitor.
Changes in text: For temporary empirical treatment, some patients received antibiotics and/or hemostats for approximately two weeks, though none received a 5α-reductase inhibitor. (Lines 138-139)

Comment 4: How many participants that received empiric treatment had improvement of hematospermia? How do they compare to the participants that were not treated?
Reply 4: Thank you for these questions. Persistent hematospermia was identified in 10.5% (12/114 cases) of empirically treated patients, which was not significantly different from the untreated patients (11.9%, 10/84 cases) (p=0.760). A similar trend was noted in comparisons of the hemostatic treatment alone and antibiotic treatment alone patient populations. These results are comparable to those reported in previous studies and indicate that empirical treatment may not lead to improvement of hematospermia. The following has been added to the revised Results section.
Changes in text: It has been reported that empirical treatment may not be necessary for improvement of hematospermia (7). In the present study, hematospermia improvement was confirmed in 89.5% (102/114 cases) of the empirically treated patients, which was not significantly different from the untreated patients (88.1%, 74/84 cases) (p=0.760). (Lines 171-174)

Comment 5: The participants should be classified into groups based on the treatment offered (no treatment x antibiotics x hemostats x both modalities) and the ROC analyses should be redone, because these are different cohorts of participants.
Reply 5: As the reviewer pointed out, it is important to consider whether the patient received empirical treatment and what type. We also consider that it would be best to perform separate ROC analyses for groups based on treatment given (no treatment, antibiotics, hemostats, both modalities). On the other hand, there is concern that such type of group subdivision often leads to reduced analysis accuracy due to an insufficient number of samples. Also, when different cutoff values are calculated from different ROCs, choosing an appropriate cutoff value can be a problem. Fortunately, in the present study, as noted in the reply to Comment 4, it was found that the presence or absence of empirical treatment was not related to improvement of hematospermia. Furthermore, multivariate analysis results showed no relationship with presence or absence of treatment or its content, or symptom persistence. Thus, it was confirmed that there was no relationship between the two, thus we consider it may be possible to analyze them as a single cohort.
Nevertheless, this is an important point, so some text has been added to the Results section, as shown following.
Changes in text: Subsequently, the optimal threshold for UpH value associated with improvement of hematospermia was examined. The entire cohort was subjected to ROC analysis without dividing by treatment, as it was considered that the effects of treatment on improvement or persistence of hematospermia could be excluded. (Lines 189-192)
In addition, the following has been added as an important limitation.
Even when using an optimal cut-off value for UpH value, it may be better to compare

various patient groups, such as those with and without treatment. (Lines 286-287)
We trust that the reviewer will understand our explanation.

Reviewer C

The manuscript TAU-23-108-CL (Urine pH and imaging findingstimetate useful predictors of prolonged duration of hematospermia) aims to identify clinical factors associated with the duration of hematospermia. The major strengths of this article are that it is an interesting original article, based on a suited methodology, with a discussion that outlines the clinical factors linked to hematospermia. Nonetheless, this paper needs minor modifications before publishing, basically the English grammatical errors. I recommend to an English native speaker editor to improve it.

Reply: Thank you for reviewing our manuscript. As the reviewer suggested, a native speaker of English has checked the paper, including the revised portions.