Peer Review File

Article information: https://dx.doi.org/10.21037/tau-23-561

Reviewer A

I have thoroughly reviewed your manuscript titled "Effect of a variant histology on the oncological outcomes of Japanese patients with upper tract urothelial carcinomas after radical nephroureterectomy: A multicenter retrospective study." Your study addresses a significant gap in the existing literature on upper tract urothelial carcinoma (UTUC), particularly focusing on the Japanese population. The multicenter approach and the large sample size enhance the validity of your findings. Below are my comments and suggestions for improvement:

Literature Review: I appreciate your effort in briefly discussing existing literature on similar studies in other populations. This provides a good context for understanding the uniqueness of your findings. You might consider expanding slightly on how your findings align or differ from these studies, to further emphasize the significance of your work in the global context.

Methodology and Analysis: Your methodological approach is sound and well-executed.

Statistical Analysis: The statistical methods are appropriate and well-applied.

Results and Comparative Analysis: The results are clearly presented and the analysis is thorough.

Limitations: The limitations section is adequately addressed. Further elaboration on how these limitations might impact the interpretation and applicability of the findings would be beneficial.

Your manuscript is well-written and provides important insights into the prognostic significance of variant histology in UTUC, particularly in the Japanese population. The suggestions above are aimed at further refining and strengthening your paper. I recommend acceptance of the manuscript after considering these minor revisions.

Reply 1: Thank you for your heartful comments. As the reviewer pointed, to compare the results of our study with the previous studies is important. However, we already described the differences in the manuscript (Page 13, Line 203- Page 15, Line 239).

Changes in the text: None.

Reviewer B

The incidence of upper urothelial carcinoma varies greatly from one race to another, so it is very useful to examine Japanese data with such a large number of cases; the examination of variant histology is also highly significant, as the genetic background may differ greatly from one race to another.

Let me make a few comments.

1. The data are very convincing that pathological Ta/is/1 is less common in Variant

UC than that in Pure UC. On the other hand, although the clinical diagnosis of upper urinary tract cancer on imaging is difficult, it is often possible to differentiate between pathological Ta/is and muscle-invasive cancer. Despite this, the number of patients in the variant UC group who underwent lymph node dissection is so small that it does not differ from pure UC. How might this have affected the prognosis?

Reply1: As noted in the text, the decision to undertake lymph node dissection is left to each medical facility's discretion. Generally, it is performed in cases where lymph node enlargement is observed in imaging studies, but there is no strict protocol in place. This background may have influenced the results of this study.

2. Similarly, as a factor influencing lymph node dissection, do you have any data on the results of preoperative urine cytology, which you believe has a significant impact?

Reply2: Urinary cytology does not necessarily influence the decision to perform lymph node dissection.

3. The presence of tissue diagnosis by ureteroscopy is considered a strong factor in intravesical recurrence; if the number of high grade tumors in the Variant UC group is high and ureteroscopy is often omitted due to a confirmed diagnosis by urine cytology, it may have a significant impact on intravesical recurrence. The number of ureteroscopic biopsies undergone should also be considered.

Reply3: This aspect is being examined in our recent study (PMID: 38151321).

4. In a previous report on your institution, "Prognostic significance of subclassifying pathological T3 upper tract urothelial carcinoma: Results from a multicentre cohort study", UC variant was the predominant factor in the multivariate analysis of overall survival. Can you explain why it did not have an impact this time?

Reply4: First, we would like to express our gratitude for reviewing our previous reports. In the earlier study, we conducted an analysis exclusively on pT3 cases, leading to a different cohort, and as a result, the variant may have remained a significant factor in overall survival (OS). However, in terms of Intravesical Recurrence-Free Survival (IVRFS), Recurrence-Free Survival (RFS), and Cancer-Specific Survival (CSS), the variant was not a significant factor in either study. Therefore, we believe it is reasonable to interpret that in the context of oncological outcomes, the variant does not become a significant factor in multivariate analysis.

5. It is well understood from the results of the multivariate analysis that variant histology does not have a significant impact on Recurrence free survival, Cancer specific survival and Overall survival. However, it would be easier for readers to understand visually if there was a Kaplan-Meier curve combined with a propensity score. Please consider this if possible.

Reply5: Indeed, as the reviewer points out, creating visually clear survival curves is possible through propensity score matching. However, given the small number of variant pathology in our study, we would prefer to address this using only multivariate

analysis.

Reviewer C

I read with great interest the paper on Effect of a variant histology on the oncological outcomes of Japanese patients with upper tract urothelial carcinomas after radical nephroureterectomy.

Below my suggestions to improve the manuscript:

Abstract: Structure : The abstract i could benefit from a review to ensure greater consistency and fluidity.

Reply: Thank you for your comment.

Results:

-specify median follow-up

Reply: We have already mentioned the median follow-up period, and it is also indicated in Table 1. (Page 5, Line 139-141).

- specify sites of metastasis, any difference in utuc vs. variant?

Reply: We apologize for the inconvenience, but our database does not contain detailed information on the sites of metastasis. This limitation in data granularity restricts our ability to provide specific insights into the metastatic patterns. We acknowledge this as a limitation of our study and suggest it as an area for future research with more comprehensive datasets. Thank you for highlighting this important aspect.

- why didn't authors explore the percentage of varaint hystology and its impact on rfs css irfs and os?

Reply: As mentioned in the Limitations section, we did not conduct a central pathology review in this study, which prevented us from conducting a detailed examination of the extent to which variants are included.

Discussion:

- a paragraph on the differences between your results and BCa variants should be added. authors may rely on the following paper doi: 10.3390/jcm12051776.

Reply: This paper focuses on Upper Tract Urothelial Carcinoma (UTUC), and we have chosen not to discuss the differences in variants between UTUC and Bladder Cancer (BCa) in this discussion. The reason for this is the emergence of recent studies suggesting that variant histology may not impact prognosis in BCa. Given that the findings in BCa are not yet conclusive, we believe including them would unnecessarily complicate the discussion.

Reviewer D

the authors report an important addition to the literature. Unlike the bladder cancer experience, they found variant histology for upper tract urothelial carcinoma did not worsen survival outcomes

Reply 1: Thank you for your review comment.
Changes in the text: None

Reviewer E

Overall, the paper is nice and well-written. The aims and methodology are simple and clear, with well-presented results.

Some suggestions:

Why intravesical recurrence (IVR) was defined as urothelial recurrence in the bladder, contralateral ureter, or contralateral renal pelvis? Why intravesical if the recurrence is in the ureter or renal pelvis?

Reply: Thank you for your astute observation regarding the use of the term 'vesical.' You are correct that 'vesical' refers specifically to the bladder, and it would be inappropriate to include the ureter or pelvis in this context. Actually, in this study, we did not observe any cases of recurrence in the contralateral ureter or renal pelvis. Therefore, our discussion of recurrences is confined to the bladder (intravesical recurrence). We appreciate your attention to this detail and will revise our manuscript accordingly to reflect this more accurately as follows.

Changes in the text: Intravesical recurrence (IVR) was defined as urothelial recurrence in the bladder, contralateral ureter, or contralateral pelvis.

→Intravesical recurrence (IVR) was defined as urothelial recurrence in the bladder.

Please discuss more in detail if variant histology could be associated with LVI and T3-4 rates...moreover please discuss if variant histology could be associated with poorer adjuvant treatments

Reply: Thank you for your feedback. We have already addressed the relationship between pathological T stage, Lymphovascular Invasion (LVI), and variant histology in the discussion section of our manuscript (Page 6, Line 184-188).

Line 238: is not EUA but EAU

Some typos in the text, please revise English with a mother tongue.

Reply: Thank you for your comment.

Changes in the text: Page 8 Line 238.

Reviewer F

This study investigates the impact of the variant histology on oncological outcomes in Japanese UTUC patients. They concluded that the variant histology does not add to the prognostic information by multivariate analysis. The manuscript is well-written, however, there are several concerns described below.

Major: None

Minor:

1. The proportion of histological variant is low (3.9 %) compared to the other previous reports. This problem may come from the lack of central pathology. This issue is often seen in many observational multi-center study, but the author should mention that central pathology would find additive value of histological variant in the limitation section.

Reply: Thank you for your comment. We have already acknowledged the limitations of not conducting a central pathology review in the limitations section of our manuscript (Page 8, Line 242-244).

2. Describe the title and units (months) for the X-axis in Figure 1.

Reply: Thank you for updating the X-axis definition. As you've indicated, the title of the graph, which reflects the Y-axis information, should make the overall presentation clear. This approach ensures that the graph is informative and easily understandable.

3. Describe 95 % CI for HR in the main text and Fig. 1.

Reply: Regarding the survival analysis presented in Figure 1, we are examining the differences using the log-rank test. Consequently, it is not necessary to include the Hazard Ratio (HR) in this instance. The log-rank test is adequate for the purpose of comparing survival curves between the groups, as it specifically evaluates the equality of these curves over the entire follow-up period. We appreciate your attention to this detail and have ensured that our analysis aligns with the appropriate statistical methodologies.

Reviewer G

The presented study examines retrospectively >800 UTUC regarding the occurrence of variant histologies and their prognostic significance.

It is a well and clearly written work that is certainly important for the community. However, in my opinion, some points could be improved.

1. Material and methods: Here it would certainly be good to work out the defined forms of the histological variants of urothelial carcinoma according to the WHO classification 2016. In what percentage were these alternative histologies seen alongside the classic morphology and assessed as present?

In the meantime, however, the new WHO classification (5th ed., 2022) has been published, which should actually be used.

Reply1: As mentioned in the Limitations section, we did not conduct a central pathology review in this study, which prevented us from conducting a detailed examination of the extent to which variants are included.

2. Results: There were 32 (3.9%) cases seen with variant histology, including 23 (2.8%), 5 (0.61%), 3 (0.36%), and 1 (0.12%) cases with squamous, glandular, sarcomatoid, and both squamous and glandular differentiation, respectively.

In what percentage (range) was the variant histology present?
Were no cases seen with at least a proportion of other divergent differentiation (such as micropapillary, nested type, lipid-rich, etc.)? What was the proportion of pure squamous cell carcinomas or pure adenocarcinomas?
Reply: Thank you for your comment. There were 32 (3.9%) cases seen with variant histology. No case of the other divergent differentiation was seen.

3. Regarding the conclusions: According the authors variant histology correlated with advanced T-stage and lymphatic invasion. Univariate analysis showed that variant histology was an independent risk factor for suboptimal RFS, CSS and OS. However, significance was lost in multivariate analyses. However, this does not necessarily mean that variant histology in UTUC has no prognostic value. Firstly, the "correlation analyses" already show an association of variant histology with prognostically unfavorable parameters. Secondly, it is possible that no significance is achieved in the multivariate analysis due to the small number of cases with variant histology. In this case, it might be good to carry out a matched-pair analysis (1:1-3).

Reply3: Indeed, as the reviewer points out, creating visually clear survival curves is possible through propensity score matching. However, given the small number of variant pathology in our study, we would prefer to address this using only multivariate analysis.

4. No HR and p-values should be given in the discussion.

Reply4: Thank you for your feedback. Following the reviewer's suggestion, we have removed the Hazard Ratio (HR) and p-values from the discussion section of our manuscript.