Peer Review File

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Reviewer A

Initial Outcomes

The authors are applauded for writing an interesting paper regarding the feasibility of intraoperative intravesical gemcitabine instillation after bladder cuff closure during RNU. I applaud the authors on their efforts and offer a few points worth discussing further:

1. The comparison of gemcitabine following bladder cuff closure compared to preoperative instillation of doxorubicine and MMC- it is mentioned briefly in the discussion, but it might be helpful to add an extra comment in regards to this .

Reply 1: This is important to discuss and additional comment has been added to the discussion section.

Changes in text: page 9, lines 183 – 194.

2. Patients were included between 2016-2020 (55 patients) it would be interesting to see data regarding the patients that did not receive intravesical chemotherapy during RNU, as one might suspect that more patients underwent RNU during that period.

Reply 2: We were unfortunately unable to directly compare this cohort to patients who underwent RNU without any intravesical chemotherapy during the study timeframe, as the use of intravesical chemotherapy is our standard practice. However, we have included information about published recurrence rates for RNU without intravesical chemotherapy based on the ODMIT-C and THP trials.

Changes in text: page 10, lines 205-209

3. 233 EAU guidelines - spelling error.

Reply 3: This has been fixed

Changes in text: page 13, line 271

Reviewer B

Initial Outcomes

The authors conducted a retrospective study comparing intraoperative gemcitabine vs. MMC/doxorubicin intravesical instillations during robot-assisted nephroureterectomy. Primary endpoints were recurrence-free survival and complications. The topic is of interest, as the exact timing and cytotoxic agent are still open for debate. The manuscript is well written and the figures/tables are of high quality.

However, there are several major limitations:

- Two independent variables were changed in the two arms: Time point (beginning of the procedure vs. directly after bladder-cuff closure) and agent (MMc/doxorubicin vs. gemcitabine). Therefore, it is nearly impossible to attribute differences or no differences

to either the agent or the time point of administration. It might be well possible that MMC is more potent than gemcitabine, but early administration is less effective than post bladder-cuff instillation. Effects might level out each other. Therefore, the study design is not suited to address these questions.

Reply 1: We appreciate the reviewers insightful comments, and agree that if the primary purpose of the study was to specifically compare the efficacy of intravesical gemcitabine to MMC, then it would be important to administer the two agents at the same time point. However, the primary purpose of this paper was to evaluate the safety and efficacy of a single intraoperative gemcitabine instillation immediately following bladder cuff closure so that urologists can be encouraged to employ this data to increase compliance in administering perioperative chemo. We feel that this data supports that intraoperative gemcitabine is similar to postoperative chemotherapy (MMC or doxorubicin) but with significant added benefit of improved workflow with immediate intraoperative instillation. This can leads to higher compliance and improved outcomes for patients. To your insightful point, we have stated in the limitations section of the discussion on page 13, lines 277-279 that the two different time points of administration could confound results.

Changes to text: None

- The groups are small (24 vs. 31 patients), the number of recurrences (n=19) and the number of complications are also low. Therefore, the study seems to be underpowered to address the endpoints. Authors should present a solid statistical explanation, if the study has enough power. If not, authors should present a descriptive study. Of note, groups are quite heterogeneus, with/without neoadjuvant and adjuvant therapies, increasing the uncertainty of the results.

Reply 2: We appreciate the comment. As this was a retrospective study of cases over a pre-specified timeframe (2016 – 2020) and not a prospective randomized controlled trial, we were unable to perform a power calculation. We have added this to the limitations section of the discussion. While we did assess the efficacy and safety of intraoperative gemcitabine compared to postoperative chemotherapy, there is a large descriptive component of this study to encourage urologists to adopt a more affordable chemotherapy option, with a more streamlined workflow, in order to increase the use of perioperative intravesical chemotherapy during RNU.

Changes to text: Page 13, lines 268-270.

Reviewer C

Initial Outcomes

General comments: This retrospective multi-institutional review shows that gemcitabine instilled immediately following bladder cuff during RNU closure is safe and has comparable bRFS compared to established chemotherapy agents

Specific comments:

1. Query to the authors: the manuscript would carry more weight if there is an added cohort of those who did not receive any intravesical therapy after RNU and further evaluation of risk for recurrence (ie., those who had only low-grade disease, pTa vs CIS only vs pT1 vs pT2 and above or node positive disease?).

Reply 1: We were unfortunately unable to directly compare this cohort to patients who underwent RNU without any intravesical chemotherapy during the study timeframe, as the use of intravesical chemotherapy is our standard practice. This also was not the purpose of the study. However, we have included information about published recurrence rates for RNU without intravesical chemotherapy based on the ODMIT-C and THP trials. In addition, the reported cohort was too small to perform a subgroup analysis for grade and stage of disease.

Changes in text: page 10, lines 205-209

2. It appears as though a significant number received neoadjuvant chemotherapy, what are the final pathologic response after those who received NAC and lesser number who received adjuvant chemotherapy. Any confounding effects especially of NAC on rates of bladder recurrence independent of intravesical instillation? Presumably no patients got immunotherapy during the study period? OS could be affected by systemic therapy but bRFS may not necessarily be

Reply 2: Thank you for the insightful comment. There was no significant difference in the rate of ≥pT2 disease on final pathology between patients who did and did not receive NAC. This has been added to the results section. The reported cohort was too small to perform a subgroup analysis based for NAC and the follow-up period was too short to adequately assess for differences in OS. No patients received neoadjuvant immunotherapy during the study period.

Changes to text: Page 7, lines 126-129.

3. Clarification for the authors: is the pathologic T stage referring to the final pathologic T stage? For those after NAC, a significant number 42% in the non-gem group compared to 29% in the gem group had pT2 and more and while it was not statistically significant (p=0.42), can authors comment on how this could impact bladder RFS?

Reply 3: Pathologic T stage was defined as the final pathologic T stage at the time of nephroureterectomy. The reviewer is correct to note that ≥ pT2 is a positive predictor of bladder recurrence after nephroureterectomy. While there was no significant difference in pathologic stage between the gem and non-gem groups, more patients were pT2 in the non-gem group, and this could possibly impact results in a larger cohort. This had been added to the text.

Changes to text: Page 10, lines 203-205.

4. Perhaps a discussion by authors on practical reasons and solutions on why despite guidelines suggesting Perioperative intravesical chemotherapy with mitomycin or gemcitabine should be considered following nephroureterectomy with cuff of bladder

resection, there is very little uptake. Is it secondary to perceived inefficacy? Irritative effects of MMC? etc.

Reply 4: Most urologist admit that the lack of use of perioperative MMC largely stems from concern about the irritative/toxic effects of MMC, particularly if given postoperatively after a recent cystorrhaphy. This has been added to the text in the introduction section. Additionally, in the discussion section other limitations of postoperative MMC, including cost, lack of availability due to production hurdles, logistical hurdles of needing an additional postoperative visit are discussed.

Changes to text: Page 4, lines 67-69 and Page 12, lines 258-266.

5. Any distinction with sites of the upper tract disease? Ie., renal pelvis, upper, mid or distal ureter? Relationship with recurrence

Reply 5: While tumor location is outlined in table 1, unfortunately the cohort was too small to perform an adequate subgroup analysis on recurrence rates based on tumor location. We have added text discussing previous data that patients with ureteral tumors have a higher likelihood of bladder recurrence, and this will be an interesting point to address in future studies.

Changes to text: Page 10, lines 202-204.

6. Query to the authors as to the choice of gemcitabine, was it because of availability or comfort level or institutional preference?

Reply 6: The use of gemcitabine at our institution is due to several reasons: extrapolating the evidence of adjuvant gemcitabine after TURBT, significantly decreased cost and moderately increased availability compared to MMC, and fewer irritative/toxic effects compared to MMC.

Changes to text: None

7. How and what do the authors propose regarding the best means of prophylaxis since this dataset suggests no difference in gem vs non-gem treatments? It would be ideal to go back in the retrospective dataset to determine how the patients who received no intravesical instillations fared?

Reply 7: We were unfortunately unable to directly compare this cohort to patients who underwent RNU without any intravesical chemotherapy during the study timeframe, as the use of intravesical chemotherapy is our standard practice. This excellent question was unfortunately out of the scope of this study. However, we have included information about published recurrence rates for RNU without intravesical chemotherapy based on the ODMIT-C and THP trials with this insightful comment. We thank you for helpful comments.

Changes to text: page 10, lines 205-209