PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects on Renal Outcome of Concomitant Acute Pyelonephritis, Acute Kidney Injury, and Obstruction Duration in Obstructive Uropathy by Urolithiasis: Retrospective Cohort Study
AUTHORS	Lee, Eung Hyun; Kim, Su-Hyun; Shin, Jung-ho; Park, Sung Bin; Chi, Byung Hoon; Hwang, Jin Ho

VERSION 1 – REVIEW

REVIEWER	Daniel Fuster Bern University Hospital, Inselspital, University of Bern Department of Nephrology and Hypertension Freiburgstrasse 15 CH-3010 Bern Switzerland
REVIEW RETURNED	26-Apr-2019

GENERAL COMMENTS	This is an interesting, timely and well conducted study. The manuscript is clearly written, methods are sufficiently described, analyses and data acquisition appear carefully conducted and conclusions drawn are supported by the data. One minor comment: I was not able to access the original dataset in "Mendeley" by the link given in the manuscript nor was I able to find it manually. Please clarify.

REVIEWER	Dr Nissar Shaikh Hamad Medical Corporation/Doha-Qatar
REVIEW RETURNED	24-May-2019

GENERAL COMMENTS	Add a few lines from the publication Hamadi et al .severe postrenal acute kidney injury, post-obstructive diuresis, and renal recovery. BJU int 2012;110:1027-34.

REVIEWER	Tron Anders Moger University of Oslo Norway
REVIEW RETURNED	14-Jun-2019

GENERAL COMMENTS	Thank you for an interesting paper. I am not a specialist in uropathy or chronic kidney disease, but was asked to focus on the statistical methods, which I have done. **Major comments:** Statistical analysis p.7: Use of log-rank tests and hazard ratios should be mentioned in this section, you only mention Kaplan-

	Meier. And how are the HRs estimated? As you mention a multivariate analysis in the abstract (should also be included in Statistcal analysis), I assume you are doing Cox regression? A more detailed description of the decision tree model would also be beneficial. What was the purpose? What was the structure of the tree and how did you use the data and survival analysis to estimate parameters in the tree? Still, my main concern is the survival analysis. In all figures with more than two survival curves, what do the p-values and HRs in the text refer to? A log-rank test is typically for comparing two groups, are you referring to an overall p-value, a score-type test perhaps? And similarly for the hazard ratio (which definitely compare only two groups), what two curves do these refer to? And here is the main problem: in several of the figures the survival curves cross, and this makes it hard to interpret the hazard ratios as well – clearly the empirical data do not give constant effects over time (I think 2a, 3c and 3d are the best examples where it could be a real problem, for the others you quickly get quite small sample sizes, so it is more a visual problem than a real problem). Or perhaps you only estimate the hazard ratios for group comparisons where the hazards are approx. proportional (or survival curves do cross), but then you should clearly state so in the paper. Still, the same variables/categories are used for the multivariate model in Table 5, e.g. AKI stage, so the fact that you have shown the crossing survival curves for AKI stage in 3c also undermines the analysis in Table 5. If this is a difficult problem to solve, I was wondering if the paper really lose much information by skipping the hazard ratios and multivariate analyses altogether, and only present Kaplan-Meier and non-parametric p-values (log-rank etc). But you probably need the multivariate model for parameters in the decision tree analysis? (which is difficult to say at present, as the description of how the tree is estimated is missing). You clearly have some challenges here that needs to be adressed before accepting the paper for publication. **Minor comments:** Abstract: «1607 patients from a urolithiasis-related obstructive uropathy cohort of 2314» reads somewhat strange. I first thought it was a typo, and that 2314 should be a year, not the gross number of participants. Strengths/limitations: “Our study firstly investigated” -> This is the first study to investigate Methods: I think I would move the description of exclusion criteria here, instead of including them in Results. I think the reader would like to know right away what they are, and all subsequent analyses are done on the 1607, so the exclusions are not really relevant for the Results section.

	Statistical analysis, use of Mann-Whitney tests and medians: Out of curiosity, are all continuous variables skewed, or is it standard in this field to present medians instead of means? It is somewhat uncommon to only see medians. Results, table 2 and 4: I hope there is a clinical reviewer on this paper who can assess the relevance of all the variables, as you have a lot of descriptive information. Is really all of it important? And so many subgroups, some with limited sample size? Tables, general: Include Group 1 and Group 2 in headings for better readability.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

This is an interesting, timely and well conducted study. The manuscript is clearly written, methods are sufficiently described, analyses and data acquisition appear carefully conducted and conclusions drawn are supported by the data.

One minor comment: I was not able to access the original dataset in "Mendeley" by the link given in the manuscript nor was I able to find it manually. Please clarify.

➔ I uploaded the dataset in Mendeley four months ago, but I found that the current status was moored in a draft in my mistake. I activated the publish process, and it has been published recently. Reviewers and readers can access our dataset through the following address;
http://dx.doi.org/10.17632/5phfg9dd48.1

Reviewer: 2

Add a few lines from the publication Hamadi et al .severe postrenal acute kidney injury, post-obstructive diuresis, and renal recovery. BJU int 2012;110:1027-34.

➔ Thank you for your comment. As you recommended, we further described in the introduction section that postobstructive diuresis may occur with complications associated with urolithiasis (page 5).

Reviewer: 3

Thank you for an interesting paper. I am not a specialist in uropathy or chronic kidney disease, but was asked to focus on the statistical methods, which I have done.

Major comments:

Statistical analysis p.7: Use of log-rank tests and hazard ratios should be mentioned in this section, you only mention Kaplan-Meier. And how are the HRs estimated? As you mention a multivariate analysis in the abstract (should also be included in Statistcal analysis), I assume you are doing Cox regression? A more detailed description of the decision tree model would also be beneficial. What was the purpose? What was the structure of the tree and how did you use the data and survival analysis to estimate parameters in the tree?

⇨ Thank you for your important comment. We fully agree with the lack of sufficient description in the statistical analysis section. So, based on the things you pointed out, we supplemented the content as follows:

"The analyses and calculations in this study were performed using SPSS Statistics V20.0 (IBM Corporation, Armonk, NY, USA), and R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables did not satisfy normality tests, so nonparametric tests (Mann-Whitney U) were performed and median (min-max) was provided. For categorical variables, data were expressed as number (percentage) and compared using the Chi-squared test. Renal outcome-free survival rates were also performed, using the Kaplan-Meier method, and comparison between groups was performed using the log-rank test. Building tree-based regression and classification models (decision and survival tree analysis) were performed by recursive partitioning using party package. Input variables were age, sex, APN, AKI stages, and obstruction duration-based groups."
. The Cox proportional hazard model was used to identify independent risk factors for the renal outcome, and to calculate the HR and 95% CI. Statistical significance was set at the level of $p < 0.05$."

Still, my main concern is the survival analysis. In all figures with more than two survival curves, what do the p-values and HRs in the text refer to? A log-rank test is typically for

comparing two groups, are you referring to an overall p-value, a score-type test perhaps? And similarly for the hazard ratio (which definitely compare only two groups), what two curves do these refer to? And here is the main problem: in several of the figures the survival curves cross, and this makes it hard to interpret the hazard ratios as well – clearly the empirical data do not give constant effects over time (I think 2a, 3cand 3d are the best examples where it could be a real problem, for the others you quickly get quite small sample sizes, so it is more a visual problem than a real problem). Or perhaps you only estimate the hazard ratios for group comparisons where the hazards are approx. proportional (or survival curves do cross), but then you should clearly state so in the paper.

⇨ We performed log-rank test for all the Kaplan-Meier survival analysis. In the previous version of our manuscript, we presented the log-rank test results only calculated by setting pooled ov er strata even in more than two groups. As you pointed out, we cannot confirm the inter-group significance, so we added significant results of the p-values obtained by pairwise over strata t o each figure:

"When the prognosis was evaluated by the quartile of obstruction duration of all patients, the longer the obstruction duration, the greater the likelihood of a decrease in GFR more than 30% (log-rank p for pooled analysis=0.052, pairwise analysis; p=0.009 for 1Q vs. 3Q, p=0.037 for 2Q vs. 3Q, figure 2A), and a decrease in GFR of more than 50% (log-rank p for pooled analysis=0.016, pairwise analysis; p=0.002 for 2Q vs. 3Q, p=0.022 for 2Q vs. 4Q, figure 2B) respectively."

"However, patients with severe AKI of grade II or III, the probability of GFR reduction >30% (log-rank p for pooled analysis <0.001, HR 1.58, 95% CI 1.37-1.82, pairwise analysis; p<0.001 for No AKI vs. AKI stage II or III, and AKI stage I vs. stage II or III, figure 3C) and >50% (log-rank p for pooled analysis <0.001, HR 2.62, 95% CI 2.05-3.34, pairwise analysis; p<0.001 for No AKI vs. AKI stage II or III, p=0.035 for AKI stage I vs. II, p<0.001 for AKI stage I vs. III, p=0.001 for AKI stage II vs. III, figure 3D) was significantly higher than the others.

The prognosis was best when neither AKI nor APN was present, and the prognosis was progressively worse with AKI alone, APN alone and both AKI and APN,

consecutively (log-rank p for pooled analysis <0.001, HR 1.50, 95% CI 1.33-1.71, pairwise analysis: p=0.029 for AKI(-)APN(-) vs. AKI(+), p=0.027 for AKI(-)APN(-) vs. APN(+), p<0.001 for AKI(-)APN(-) vs. AKI(+)APN(+), and p<0.001 for AKI(+) vs. AKI(+)APN(+), figure 3E; log-rank p<0.001 for pooled analysis, HR 2.18, 95% CI 1.75-2.71, pairwise analysis: p=0.024 for AKI(-)APN(-) vs. AKI(+), p<0.001 for AKI(-)APN(-) vs. AKI(+)APN(+), and p<0.001 AKI(+) vs. AKI(+)APN(+), figure 3F)."

*Additional log-rank test results of some figures.

Figure	P for Pooled Analysis	P for Pairwise Analysis
Fig 2A	P=0.05	1Q vs 3Q P=0.009, 2Q vs 3Q P=0.037
Fig 2B	P=0.02	2Q vs 3Q P=0.002, 2Q vs 4Q P=0.022
Fig 3C	P<0.001	No AKI vs AKI stage 2 P<0.001, No AKI vs AKI stage 3 P<0.001, AKI stage 1 vs. 2 P<0.001, AKI stage 1 vs. 3 P<0.001
Fig 3D	P<0.001	No AKI vs AKI stage 2 P<0.001, No AKI vs AKI stage 3 P<0.001, AKI stage 1 vs. 2 P=0.035, AKI stage 1 vs. 3 P<0.001,

		AKI stage 2 vs. 3 P=0.001
Fig 3E	P<0.001	AKI(-)APN(-) vs. AKI(+) P=0.029 AKI(-)APN(-) vs. APN(+) P=0.027 AKI(-)APN(-) vs. AKI(+)APN(+) P<0.001 AKI(+) vs. AKI(+)APN(+) P<0.001
Fig 3F	P<0.001	AKI(-)APN(-) vs. AKI(+) P=0.024 AKI(-)APN(-) vs. AKI(+)APN(+) P<0.001 AKI(+) vs. AKI(+)APN(+) P<0.001

Still, the same variables/categories are used for the multivariate model in Table 5, e.g. AKI stage, so the fact that you have shown the crossing survival curves for AKI stage in 3c also undermines the analysis in Table 5. If this is a difficult problem to solve, I was wondering if the paper really lose much information by skipping the hazard ratios and multivariate analyses altogether, and only present Kaplan-Meier and non-parametric p-values (log-rank etc). But you probably need the multivariate model for parameters in the decision tree analysis? (which is difficult to say at present, as the description of how the tree is estimated is missing). You clearly have some challenges here that needs to be adressed before accepting the paper for publication.

- ⇨ Thank you for your valuable comments. Table 5 is a multivariate analysis for the occurrence o f eGFR decrease of > 50%. The multivariate analysis in Table 5 was calculated using the Cox proportional hazard model. The risk for each AKI stage was calculated using the No AKI grou p as a reference. The AKI groups for table 5 correlates with Figure 3D. In figure 3D, the cross ing survival curves were seen between No AKI and AKI stage I, and p for pairwise analysis re sult also showed insignificant between those groups. Through this revision, we hope that the pairwise analysis information that we newly provided would broaden you and other readers' u nderstanding. We are grateful that many of the scarcities in the statistical analysis seem to ha

ve been complemented by your valuable advice.

Minor comments:

Abstract: «1607 patients from a urolithiasis-related obstructive uropathy cohort of 2314» reads somewhat strange. I first thought it was a typo, and that 2314 should be a year, not the gross number of participants.

⇨ As your valuable comment, we deleted the part, “of 2314” to clarify our intention (page 2).

Strengths/limitations: “Our study firstly investigated” -> This is the first study to investigate

⇨ There were the editor’s comments in writing the “Strength and limitations of this study” section , and the sentence was deleted as the contents were modified. Please refer to it.

Methods: I think I would move the description of exclusion criteria here, instead of including them in.

Results. I think the reader would like to know right away what they are, and all subsequent analyses are done on the 1607, so the exclusions are not really relevant for the Results section.

⇨ Thank you for your insightful comment. We have modified these two parts you pointed out tog ether. Information on 707 exclusion patients has been moved from the results section to the m ethods section. It seems to be structured so that readers can accept it more clearly.

Statistical analysis, use of Mann-Whitney tests and medians: Out of curiosity, are all continuous variables skewed, or is it standard in this field to present medians instead of means? It is somewhat uncommon to only see medians.

⇨ We conducted Shapiro-Wilk test for normality test in all the continuous variables. The results

are as follows:

variables	Mean	Median	Tests of Normality (Shapiro-Wilk)		
			Statistic	df	Sig
Age	52.63	54.0	0.991	1607	P<0.001
Obstruction duration	16.57	6.0	0.281	1607	P<0.001
Peak CRP	54.75	5.9	0.674	528	P<0.001
Baseline SCr	0.8392	0.8	0.796	1607	P<0.001
Baseline eGFR	97.67	93.6	0.704	1607	P<0.001
SCr at admission	1.777	1.0	0.013	1607	P<0.001
eGFR at admission	75.1767	74.5	0.658	1607	P<0.001
Peak SCr	1.853	1.0	0.014	1607	P<0.001
Lowest eGFR	75.6174	72.0	0.08	1607	P<0.001
Final SCr	0.9767	0.9	0.449	1607	P<0.001
Final eGFR	85.8282	84.4	0.916	1607	P<0.001
Stone size	7.652	6.5	0.746	1411	P<0.001

We also added the sentence, "Continuous variables did not satisfy normality tests, so nonparametric tests were performed and median (min-max) was provided." in Statistical Analysis section, because readers might have the same question as you.

Results, table 2 and 4: I hope there is a clinical reviewer on this paper who can assess the relevance of all the variables, as you have a lot of descriptive information. Is really all of it important? And so many subgroups, some with limited sample size?

⇨ We fully understand your concerns. Clinically, inflammation and infection (APN) and AKI are c ommon complication of obstructive uropathy caused by urolithiasis. Because this study was a retrospective study, we considered that the time required for obstruction release was somewh at affected by these complications. So even if some groups had a limited sample size that cou ld be statistically insignificant, we concluded that providing information on the duration of the o bstruction release as well as the presence of APN and AKI would provide clinicians with more useful information.

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Tables, general: Include Group 1 and Group 2 in headings for better readability.

⇨ The text is mainly described as group 1 and group 2, but the table does not, so we added gro

up 1 and group 2 headings.

VERSION 2 – REVIEW

REVIEWER	Tron Anders Moger University of Oslo Norway
REVIEW RETURNED	31-Jul-2019

GENERAL COMMENTS	Thank you for the revision. I think the clarification in Methods and the added analyses in Results have made the paper sufficiently transparent in terms of the analyses you have performed, and sufficiently clear on limitations, to be accepted for publication.

VERSION 2 – AUTHOR RESPONSE

Thank you for the opportunity to revise our manuscript for publication in your journal.

We have revised out title and stated more clearly the objectives in the abstract section as you recommended.

Also, we thoroughly reviewed our manuscript several times.

We hope that this version of the manuscript will be considered more suitable for publication.