Peer Review File

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Reviewer A

Novel and very welcome for caregivers and their CUP patients.
Throughout the manuscript:
Abstract is lacking a good final conclusion as well as details on the sensitivity and specificity of the developed nomogram.
Results section includes parts that belong on the methods section.
Discussion section is to long 4 pages should be reduced tot 2 pages maximum. Some parts in the discussion section belong in the results section.

We have modified these three sections in the revised article according to the reviewer's suggestions. A final conclusion was added to the Abstract section, and both the Results and Discussion sections were reorganized.

Line 79: the patient population period of incidence is 2010-2016. Are the nomogram results also work for current CUP patients 2018-2020 and/or CUP patients from other registries in the world?

Due to the limitation of the SEER database, data for patients diagnosed between 2018 and 2020 were currently unavailable. In addition, patients diagnosed between 2018-2020 warranted further follow-up before being included in the development of nomograms. The proportion of patients with censored survival data would be high otherwise, and thus led to less robust models. We also did our utmost to obtain data from other clinical databases, such as the National Cancer Database (NCDB). However, we were unable to access other databases apart from the SEER. We admit that the lack of validation in external cohort is one of limitations in this study, which was pointed out in the discussion part of our revised manuscript.

Line 82: Why do the authors exclude patients with only CUP since most patients have had a malignancy in the past before developing CUP. Data/results with regard tot patients with malignancies in the past should be included, preferably by tumor type/category.

We agree with the reviewer that the exclusion of patients with malignancies in the past may introduce selection bias in this study. In the revised manuscript, we included all patients histologically diagnosed with cancer of unknown primary, regardless of the recorded sequence number of CUP in the SEER. According to the SEER manual, the sequence number indicates the sequence of all **reportable** neoplasms over the lifetime of the patient. In fact, most CUP were recorded as "one primary only" or "first of

multiple primaries" in the SEER database according to the above definition. However, it is also noteworthy that the records in this field **may not represent the actual sequence number of malignancies, especially for patients with CUP** since the origin of primaries were unclear. Considering the records of "first primary indicator" may be inappropriate for patients with CUP, we preferred to treat included patients as an entity for further analysis rather than perform subgroup analysis according to the "first primary indicator".

Line 84: Why do the authors exclude patients with less than 1 month survival? Caregivers do not know on forehand a patient' survival time, therefore these patients should be included for nomogram development.

We exclude patients with less than 1-month survival in view of the following reasons. Patients surviving less than 1 month upon diagnosis were coded as having zero time of survival in the SEER, rather than the actual days they having lived. Additionally, many patients with CUP who have zero months of survival time in the SEER were diagnosed at autopsy. Therefore, the recoded survival time of these patients was largely unreliable. According to the NCCN guidelines, CUP entails a median OS of around 8-12 months. However, when we included patients surviving less than 1 month in the survival analysis, the median OS of all included patients was only 3 months (95% CI: 2.893-3.107), which did not match reality (Figure 1R). This result showed that including these patients may introduce bias in the survival analysis.

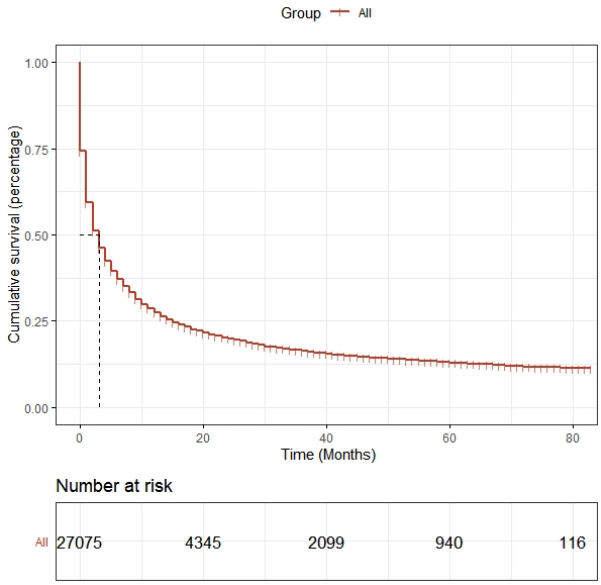

Figure 1R. The survival curve of all patients histologically confirmed with cancer of unknown primary (≥18 years and with active follow-up), including those who having survival time less than 1 month.

We also referred to the selection criteria of other studies that based on the SEER database. Many investigators also exclude patients with less than 1-month survival to eliminate patients who diagnosed at autopsy alone and died in the immediate

postoperative period. The representative references were listed as follows:

1. Karanth S, Rajan SS, Sharma G, Yamal JM, Morgan RO. Racial-Ethnic Disparities in End-of-Life Care Quality among Lung Cancer Patients: A SEER-Medicare-Based Study. J Thorac Oncol. 2018;13(8):1083-1093. doi:10.1016/j.jtho.2018.04.014
2. Shah CP, Mramba LK, Bishnoi R, Unnikrishnan A, Duff JM, Chandana SR. Survival trends of metastatic small intestinal neuroendocrine tumor: a population-based analysis of SEER database. J Gastrointest Oncol. 2019;10(5):869-877. doi:10.21037/jgo.2019.05.02
3. Feng SS, Li HB, Fan F, et al. Clinical characteristics and disease-specific prognostic nomogram for primary gliosarcoma: a SEER population-based analysis. Sci Rep. 2019;9(1):10744. Published 2019 Jul 24. doi:10.1038/s41598-019-47211-7
4. Bishop AJ, McDonald MW, Chang AL, Esiashvili N. Infant brain tumors: incidence, survival, and the role of radiation based on Surveillance, Epidemiology, and End Results (SEER) Data. Int J Radiat Oncol Biol Phys. 2012;82(1):341-347. doi:10.1016/j.ijrobp.2010.08.020

Line 98: Can the authors explain why the variable Marital status and insurance status is related tot CUP survival, and is therefore an important factor in the development of the nomogram?

Marital status was found to be associated with the survival of patients with CUP in the previous literature. According to Urban et al, patients with CUP who were married had a better overall survival than those who were divorced, widowed, or separated (HR=1.20, 95%CI: 1.17-1.23, p-value<0.001) and those who were single (HR=1.17, 95%CI: 1.14-1.21, p-value<0.001) in the multivariate analysis (1). Insurance status was also found to be associated with cancer prognosis according to several previous studies (2-6), although no evidence has showed its association with CUP so far. Based on the above evidence from past studies, we included these two factors as potential variables for statistical selection. These two variables were eliminated based on the result of statistical selection with the criteria of likelihood ratio, and were not included in the final nomogram in the revised manuscript.

Line 105: The histological subtype "adenocarcinoma" should be subdivided according to differentiation status.

We understand that it would be better to subdivide the adenocarcinoma group according to differentiation status. According to the NCCN guideline, poorly differentiated or undifferentiated CUP seem to be highly responsive to cisplatin-based combination chemotherapy, which are different from the well- to moderately differentiated. However, since around 89% records of tumor grade were missing in the database, the differentiation status of most cases was unclear. Therefore, we had to treat adenocarcinomas as one group. We also pointed out this limitation in the discussion part in the revised manuscript.

Line 110-113: Why only these metastatic (single) sites? Combinations of metastatic sites as well as metastatic patterns are also informative for caregivers and patients?

In the SEER database, the data for metastatic sites were only available at bone, brain, lung, and liver. We set the variable "Number of metastatic organs" to represent the combinations of metastatic sites, and the variable "visceral metastases to represent the metastatic patterns of patients with CUP.

Line 120: Why did the authors set the endpoint on 6-9 months OS? Why not 3, 5 or 12 months?

CUP confers a poor prognosis with median OS of 6-12 months according to previous literature. Our study cohort demonstrated a median OS of around 7 months. We therefore chose the endpoint on 6 and 9-month rather than 1-year or 3-year mainly due to the worse prognosis of CUP compared with other malignancies. In addition, since the database was submitted in 2018, the follow-up time for patients in the 2016 would be otherwise too short to validate the survival probability.

Line 323: In the conclusions section the authors do not describe overall predictors for CUP patient survival according to the nomogram, as well as, details on the sensitivity and specificity of the developed nomogram.

We have revised the Conclusion section according to the reviewer's advice. However, since the Harrell's C-index was preferred to ROC curve in the evaluation of nomogram performance according to TRIPOD statement, we presented the Harrell's C-index instead in the conclusion to show the discriminate ability of the nomogram.

Reviewer B

The authors have prepared a manuscript proposing the potential clinically prognostic factors and prognostic nomogram of CUP. This study results are based on large number of CUP cases, and this is a merit of this study. However, there are serious methodological concerns for this manuscript to be considered for potential publication. In detail, the most serious technical and clinical problem is that the cases in this study were not divided into favorable subset and unfavorable subset. This stratification is the most important in clinical setting, and not considering this grouping should confound the appropriate interpretation of the overall data derived from this study. In addition, very important clinical factors for CUP prognosis such as performance status, blood markers including LDH or lymphocyte counts, or metastatic patterns limited to multiple lymph nodes are not considered in this study (as shown Haratani et al. JITC 2020). The reviewer understand that the authors could not obtain these clinical data from SEER database, but science should not allow the inappropriate data to be accepted.
The following are additional comments to improve the manuscript.

We understand that the data unavailability of favorable/unfavorable subset, performance status, blood markers, and metastatic patterns limited to multiple lymph nodes led to limitation of this study. However, we still insist that this study had its merits and value, since this is the first nomogram for patients with CUP who lacks standard staging system, and we construct this nomogram based on a large population. We performed a risk stratification based on this nomogram, which we hope can be helpful for clinicians to identify favorable and unfavorable patients, especially when considering questions related to treatment. We also emphasized these limitations in both the abstract and main text in the revised manuscript. We hope the gap can be filled by further investigations.

Major points

1. The authors should not exclude cases who lived less than one month. This lead to serious selection bias and did not reflect real world data.

We exclude patients with less than 1-month survival in view of the following reasons. Patients surviving less than 1 month upon diagnosis were coded as having zero time of survival in the SEER, rather than the actual days they having lived. Additionally, many patients with CUP who have zero months of survival time in the SEER were diagnosed at autopsy. Therefore, the recoded survival time of these patients was largely unreliable. According to the NCCN guidelines, CUP entails a median OS of around 8-12 months. However, when we included patients surviving less than 1 month in the survival analysis, the median OS of all included patients was only 3 months (95% CI: 2.893-3.107), which did not match reality (Figure 1R). This result showed that including these patients may introduce bias in the survival analysis.

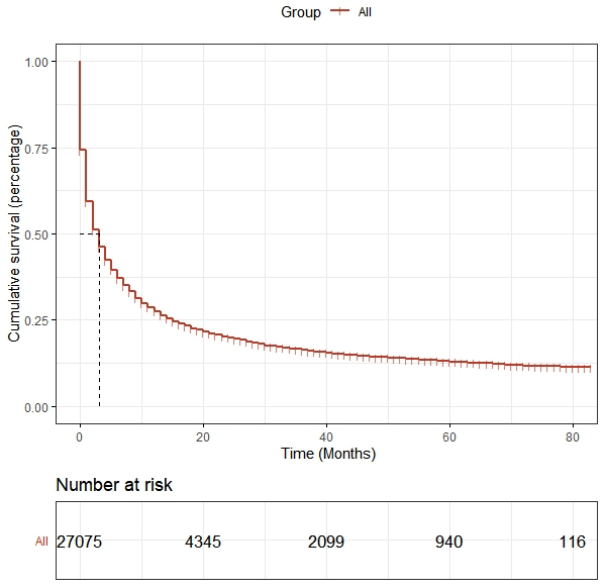

Figure 1R. The survival curve of all patients histologically confirmed with cancer of unknown primary (≥18 years and with active follow-up), including those who having survival time less than 1 month.

We also referred to the selection criteria of other studies that based on the SEER database. Many investigators also exclude patients with less than 1-month survival to eliminate patients who diagnosed at autopsy alone and died in the immediate postoperative period. The representative references were listed as follows:

1. Karanth S, Rajan SS, Sharma G, Yamal JM, Morgan RO. Racial-Ethnic Disparities in End-of-Life Care Quality among Lung Cancer Patients: A SEER-Medicare-Based Study. J Thorac Oncol. 2018;13(8):1083-1093. doi:10.1016/j.jtho.2018.04.014
2. Shah CP, Mramba LK, Bishnoi R, Unnikrishnan A, Duff JM, Chandana SR. Survival trends of metastatic small intestinal neuroendocrine tumor: a population-based analysis of SEER database. J Gastrointest Oncol. 2019;10(5):869-877. doi:10.21037/jgo.2019.05.02
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2. Stepwise method should not be used in this type of clinical study. The authors should choose covariates clinically.

Covariates were chosen based on both clinical and statistical considerations in this study. We have to point out that although clinical relevance was of vital importance in the selection of covariates in this type of study, statistical consideration should not be omitted in the development of a nomogram. Experience from clinical practice was not reliable enough to generate this prognosis model. In addition, the fitness and collinearity have to be considered in the development of a prognosis model, great bias would be otherwise introduced to the results in its further application. We first selected potential variables according to clinical relevance, and then used backward elimination method for variable selection based on the recommendations from statisticians (7, 8).

3. The authors must add sentences in the Abstract as follows. “This nomogram or prediction model cannot be used in a clinical practice, because very important clinical factors such as favorable/unfavorable subset, performance status, LDH, blood cell counts, or metastatic patterns limited to multiple lymph nodes could not be considered due to lack of availability of these data”.

We do agree with the reviewer that this study had some limitations due to the lack of availability of above data, which were critical factors to be considered in the further development of nomogram for patients with CUP. We also made amendments to the Abstract part in the revised article according to the reviewer’s suggestions.

Minor points

1. The reviewer does not understand what the descriptions or sentences in the Introduction section (lines 61-67) is meaning. The reviewer does not agree with these descriptions, and the current manuscript does not seem to be more accurate or integrative than the previous studies. These descriptions should be removed or improved.

   We have revised these descriptions in the manuscript.

2. The authors should explain why 3408 cases were chosen, in the Results section.

   All eligible patients with available data for metastatic status at bone, brain, liver, and/or lung (the information of metastases was only available at the above four sites in the database) were included in the further nomogram study to optimize the generalizability of the nomogram. According to the advice from reviewer A, we explained this in the Methods section in the revised manuscript.

3. The reviewer cannot understand what the lines 163-165 are meaning.

   We only included patients histologically confirmed with CUP in the revised manuscript. These sentences were therefore removed.