Peer Review File

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Reviewer A

Key Comment 1: In line 252, the article's intriguing aspect is the analysis using RCS plots. However, the choice of factors such as age, solid size, and CTR for analysis instead of pathological invasive size or lepidic proportion lacks clarity. The discussion should elucidate the rationale behind this choice. Moreover, considering the widening confidence intervals as MP/S increases, it's crucial to discuss the possibility of the RCS curve rising steeply beyond 20%.

Reply 1: Thank you for your insightful comment. In the RCS model, we selected age as a significant covariate based on its identification in both the univariate and multivariate Cox regression analyses. Additionally, we included solid component size and CTR due to their prognostic value in part-solid lung adenocarcinoma, which constituted the majority of our research samples. However, we have reconsidered this choice in light of your suggestion that pathological invasive size or lepidic proportion should also be considered. After careful consideration, we now believe that adjusting for pathological invasive size is more reasonable than solid size or CTR because solid components identified on CT may not always correspond to pathological invasive patterns. Consequently, we have made changes to Figure 4, selecting age and pathological invasive size for model adjustment. (see Table 2 and Figure 4)

Regarding the widening confidence intervals on the RCS curve (both in the former and revised versions), we acknowledge that the curve may continue to rise (albeit not necessarily steeply) as MP/S increases beyond 20%, especially if more eligible samples are included for analysis. Fortunately, despite having only 12 patients with MP/S >20% tumors, we were able to conduct a sufficiently long follow-up for this group, and no patients were lost to follow-up. It is important to note that making a semi-quantitative assessment of the proportion of a certain pathological subtype is subject to significant subjectivity. In other words, it is challenging to determine which tumors within the 20%-30% MP/S component proportion range precisely have a proportion of 20%, and which ones exceed 20%. Therefore, in the absence of more objective and precise evaluation methods, we believe that the RCS curve is more likely to exhibit a gradual increase after reaching approximately 20% on the x-axis.

Changes in the text:

Page 15, line 276: “These factors included patient age, solid size, CTR, tumor size, invasive tumor size, the presence of lepidic component…”

Page 16, line 297: “The RCS plot, with adjustment for patient age and pathological invasive size…”

Page 16, line 301-306: “As the proportion of MP/S components increased from 0% to 15%, the HR for RFS rapidly grew from 1.0 to 4.3 (95% CI: 2.1 to 8.5). When the MP/S component proportion reached 20%, the HR for RFS increased to 5.1 (95% CI: 2.6 to

9.8). Once the MP/S component exceeded 20%, the HR growth for RFS significantly slowed down, eventually reaching 5.6 (95% CI 2.0 to 15.4) at 30%."

Key Comment 2: The latest WHO classification identifies not only the solid or micropapillary components but also the complex glandular pattern as a high-grade pattern, impacting the tumor's overall grade. Investigating the correlation between the high-grade pattern (including the complex glandular pattern) and survival is essential to understand potential changes in results. If data is unavailable, limitations regarding the exclusion of this pattern from the study should be acknowledged.

Reply 2: Thank you for bringing attention to this limitation. We also considered the complex glandular pattern, which is a rare but significantly prognostic high-grade pattern, for evaluation in our research. To ensure that no tumors with these patterns were overlooked, we performed a thorough examination of our entire dataset consisting of 1499 patients who underwent surgical resection for GGNs. We found only one adenocarcinoma with the cribriform pattern. However, this particular patient was not included in our study cohort because the tumor appeared as pure-solid on CT imaging. We have provided an explanation of this issue in the Results section of our revised manuscript. (see Page 12, line 215, 216)

Changes in the text:

Page 12, line 215,216: "Neither in the MP/S+ group nor in the MP/S- group, were there any patients with adenocarcinoma exhibiting a complex glandular pattern."

Key Comment 3: In Table 2, multivariate analyses indicate that solid size was not a statistically significant prognostic factor. The potential correlation between solid size and MP/S components could have influenced these results. It appears that the MP/S factor was treated as a categorical variable (+/- or 5% cutoff), while solid size was considered a continuous variable. Clarification on the rationale behind these variable treatments and the threshold for MP/S proportion is needed, or additional multivariate analyses treating MP/S proportion as a continuous variable should be conducted.

Reply 3: Thank you for your insightful comment. We have indeed conducted multivariate analyses, considering the MP/S proportion as a continuous variable, and we are more than willing to present those results. Please refer to supplementary Table S1 for the relevant findings.

Changes in the text:

Page 15, line 291, 292: "The proportion of MP/S, when included as a continuous variable in the multivariate analysis, was also shown to be a significant prognostic factor (Table S1)."

Minor Comment 1: In line 135, please clarify the methodology used for EGFR mutation analyses.

Reply 1: Thank you for your suggestion. We have incorporated the additional details regarding the methodology used for the detection of EGFR mutations. (see Page 9, line 152-160)

Changes in the text:

Page 9, line 152-160: "With respect to gene testing, DNA extraction from paraffin-embedded tissue was performed using the Autostation FFPE DNA one-step Kit (ACCB Biotech), while qualitative assay of human epidermal growth factor receptors (EGFR) mutations was detected using Human EGFR Gene 18-21 Exon Mutation Assay Kit (ACCB Biotech). Stratagene Mx3000P and ABI7500 (Applied Biosystems) were used to perform amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). No EGFR mutations and EGFR nonsense mutations (T790M) were defined as wild-type, while effective EGFR mutations were defined as mutant type."

Minor Comment 2: In Table 1, there are two numbers in one cell: row of lepidic predominant, and column of MP/S+. Which is correct?
Reply 2: The first number, "3 (3.5)," is the correct value. Additionally, we have noticed several formatting issues in Table 1 that were not consistent with our submitted file. We will make every effort to address these issues and ensure that such mistakes are avoided in our resubmission. (see Table 2.)
Changes in the text: Please refer to the revised Table 1.

Minor Comment 3: In Table 2, results of two multivariate analyses are presented. One appears to include MP/S presence, while the other adopts the proportion of MP/S instead of presence. Please clarify this difference in the first row.
Reply 3: Thank you, we have revised it as your suggestion. (see Table 2.)
Changes in the text: Please refer to the revised Table 2.

Reviewer B
Comment: The authors did not demonstrate invasive tumor size. In GGO pattern, T stage is depending on the invasive tumor size
However, invasive tumor size was not included in this study.
I thought that size is a more prognostic impact than proportion
For example, in my opinion, MP /S size have a more prognostic impact than MP/S+ (>5%)
As authors demonstrated in Figure 1D, there was a significant difference between two groups.
Reply: Thank you for your insightful comment. In this study, we initially evaluated the solid size and CTR of tumors appearing as GGNs on CT rather than the pathological invasive tumor size. Our rationale was that since all tumors included in this study presented as GGNs on CT, there would likely be a strong correlation between these radiological characteristics and patient prognosis. However, upon reevaluation, we have come to the conclusion that it is more reasonable to incorporate the pathological invasive size into our analysis. This decision was prompted by the recognition that the solid component identified on CT may not always correspond to the pathological invasive patterns observed. As a result, we have revised our Cox regression model and RCS model to include the pathological invasive size. (see Page 15, line 276, Page 16, line 297 and Page 16, line 301-306)
Another crucial issue pertains to whether the size of high-grade patterns carries greater

prognostic significance than their proportion. We fully agree with your argument that MP/S size might exert a more substantial impact than MP/S+ (>5%), as tumors of different T stages could exhibit the same proportion of high-grade components but possess distinct prognoses. Thus, there is a pressing need to develop objective and accurate methods for measuring the size of high-risk components, which can replace the current subjective, semi-quantitative measurement approaches. We have duly acknowledged this limitation in the revised manuscript. (see Page 21, line 407-412)

Changes in the text:

Page 15, line 276: "These factors included patient age, solid size, CTR, tumor size, invasive tumor size, the presence of lepidic component…"

Page 16, line 297: "The RCS plot, with adjustment for patient age and pathological invasive size…"

Page 16, line 301-306: "As the proportion of MP/S components increased from 0% to 15%, the HR for RFS rapidly grew from 1.0 to 4.3 (95% CI: 2.1 to 8.5). When the MP/S component proportion reached 20%, the HR for RFS increased to 5.1 (95% CI: 2.6 to 9.8). Once the MP/S component exceeded 20%, the HR growth for RFS significantly slowed down, eventually reaching 5.6 (95% CI 2.0 to 15.4) at 30%."

Page 21, line 407-412: "Objective and precise assessment of the proportion of MP/S component under microscopic examination is challenging, which may to some extent affected the credibility of the RCS model. Particularly, evaluating minor proportions involved a high degree of subjectivity, as the personal inclination of pathologists could greatly influence the determination of whether the MP/S proportion was 1% or 5%."

Reviewer C

Comment: …Based on these reasons, the reviewer suggests the authors to start their manuscript from Figure 4, defining tumors with micropapillary/solid (5% or higher) would have poor prognosis. And then, the authors should compare patients' background and RFS/OS between patients with tumors with micropapillary/solid component (5% or higher) and the rest of the patients.

Reply: Thank you for your insightful advice. After incorporating the inclusion of pathological invasive tumor size, we have reevaluated our analysis strategy (including pathological invasive tumor size in the Cox regression model & adjusting it in the RCS model) and made major revisions to our manuscript. However, we have refrained from altering the fundamental structure and logical progression of the article. We would like to elaborate on why we chose to divide tumors into MP/S+ and MP/S- cohorts instead of MP/S <5% (negative or 1%) and MP/S ≥5%.

The main reason for this arrangement is that our database primarily consists of early-stage lung adenocarcinoma with ground-glass opacity (GGO), where the proportion of MP/S does not exceed 30%, rather than solid lung cancer. As surgeons, we believe that the optimal treatment approach for this patient group is radical resection, and postoperative adjuvant therapy may be unnecessary. However, we also believe that a small subset of patients who experience postoperative recurrence could benefit from more thorough lymphadenectomy and/or adjuvant treatment. This belief is supported

by the significant differences in clinical baseline and pathological characteristics between patients with MP/S+ (≥1%) tumors and those without, as shown in Table 1.
Including tumors with an MP/S proportion of 1% in the control group (MP/S- group) would complicate our analysis. The confidence intervals for the hazard ratios corresponding to the restricted cubic spline (RCS) model curve at MP/S proportions of 1% and 5% both encompass 1. It would be unreasonable to include tumors with an MP/S proportion of 5% in the MP/S+ group while excluding tumors with an MP/S proportion of 1%. Additionally, in the subset of acinar- or papillary-predominant adenocarcinoma, MP/S+(1% or higher) was shown to be a significant factor in the multivariate analysis. This result more accurately reflects the prognostic value of the presence of MP/S subtypes, considering the limited number of patients with lepidic-/solid-/micropapillary-predominant tumors in the MP/S+ group.
It is important to acknowledge that the threshold for "minor" or "minimal" MP/S patterns varies in the literature. In this study, we defined an MP/S proportion of less than 5% as 1% based on the clinical practice of our institution's pathologists. We believe this definition is closer to the actual situation, although it may still be inaccurate. However, we recognize that both 1% and 5% indicate that the MP/S components occupy only a small portion of the entire tumor. The randomness of tissue sampling for pathological slides and the subjectivity of diagnosis can influence whether it is determined as 1% or 5%.
In summary, we have maintained the definitions of MP/S+ and MP/S- tumors in this revised manuscript and conducted subsequent analyses. We look forward to utilizing more accurate methods to assess not only the proportion of MP/S components but also their actual sizes.
Changes in the text: Please refer to the revised Table 2. And Figure 4.

Reviewer D

This paper investigated the influence of the presence of micropapillary and solid components as poorly differentiated components on the prognosis in early stage lung adenocarcinoma with GGO. What was previously reported in solid lung cancer without GGO was similarly confirmed in early lung adenocarcinoma with GGO. Additionally, this paper reports the extent to which the relationship between the presence of lepidic components, which have been established as factors for good prognosis, and the presence of micropapillary/solid components, which are factors for poor prognosis, influences prognosis. This is a very interesting result that has been carefully considered, and I believe that it is worthy of acceptance.

Reply: We would like to thank you for carefully reading our manuscript. We have made major revision to our manuscript according to editor’s and reviewers’ suggestion. We remain committed to addressing any further concerns you may have and ensuring the quality of our research.

Reviewer E

Comment 1: First, the title needs to indicate “underwent curative resection” and the prognosis outcome, as well as the clinical research design of this study, i.e., a

retrospective cohort study.

Reply 1: Thank you for your suggestion. We agree with your suggestion to indicate in our study cohorts that the patients underwent curative resection. To make the research design and outcomes more explicit, we believe it would be informative to include them in the title. However, in order to maintain a balance between professionalism and readability, we have decided to address the research design and outcomes in the abstract instead. As a result, we have revised the title to "The presence of micropapillary/solid subtypes is an independent prognostic factor for patients undergoing curative resection for stage I lung adenocarcinoma with ground-glass opacity".

Changes in the text:

Page 1, line 2: "**The Presence of Micropapillary/Solid Subtypes is an Independent Prognostic Factor for Patients Undergoing Curative Resection for Stage I Lung Adenocarcinoma with Ground-Glass Opacity"**

Comment 2: Second, the abstract needs some revisions. The background did not describe the knowledge gap on the prognostic role of MP/S. The methods need to describe the inclusion of subjects, the assessment of baseline clinical factors, follow up procedures, and measurement of prognosis outcome. The results need to first briefly summarize the clinical characteristics of the study sample and the HR and accurate P values for the prognostic role of MP/S+. The conclusion needs comments for the clinical implications of the findings.

Reply 2: Thank you for your insightful comment. We have revised the abstract meticulously, addressing each point individually.

Changes in the text: Please refer to the revised Abstract section (Page 3, 4)

Comment 3: Third, in the introduction of the main text, the authors did not review why MP/S+ as a potential prognostic factor is important and why it is ignored in prior studies. The authors need to analyze why it is more clinically relevant than other known prognostic factors. Analysis on the limitations of prior studies is also necessary.

Reply 3: Thank you for your advice. We have made significant modifications to the introduction section, which reviewed the prognostic value of MP/S components in lung adenocarcinoma and their association with other morphological features that also hold prognostic value. Furthermore, we highlighted that those previous studies predominantly focused on solid tumors, which created knowledge gaps regarding the prognostic impact of MP/S on subsolid tumors.

Changes in the text: Please refer to the revise Introduction section (Page 6, 7)

Comment 4: Fourth, in the methodology of the main text, please clearly indicate the clinical research design, sample size estimation, assessment of baseline clinical and pathological factors, and follow up details of this study. Please indicate the primary prognosis outcomes. In statistics, please describe the details of ascertaining the independent prognostic role of MP/S+ in particular the covariates adjusted.

Reply 4: Thank you for your insightful suggestions. We have revised the Materials and Methods section as per your suggestion, providing additional explanations about the

research design, assessment of baseline clinical and pathological factors, and follow-up strategy. We acknowledge the importance of sample size estimation in retrospective studies. However, in this study, we did not estimate the sample size, and therefore, relevant information regarding sample size estimation was not available in the methodology. We did not perform sample size estimation due to two main reasons:

First and foremost, there is a scarcity of existing literature on the prognostic value of high-grade patterns in tumors appearing as GGO. As a result, we lacked previous studies or pilot data to guide us in determining an appropriate sample size.

Secondly, the MP/S patterns we focused on in this study are relatively rare occurrences within this special lung adenocarcinoma population. Although our sample size for the entire dataset was considerably large, the MP/S+ group only consisted of 86 samples. Consequently, obtaining a larger sample size would have been time-consuming. The limited sample size of MP/S+ tumors was one of the major limitations discussed in our manuscript.

While we acknowledge the potential limitations of our study's sample size, we believe that our findings provide valuable insights into the prognostic value of MP/S patterns in GGO and may have implications in clinical decision-making.

Thank you once again for your valuable feedback, which has undoubtedly enhanced the clarity and quality of our work. We remain committed to addressing any further concerns you may have and ensuring the integrity of our research.

Changes in the text:

Page 7, line 111-115: "In this retrospective cohort study, we retrospectively screened 1,499 patients who underwent surgical resection for pathological stage I GGOs in our institution between January 2014 and December 2016."

Page 10, line 163-167: "All eligible patients were followed up starting from the day of pulmonary resection. Physical examination, lung cancer biomarker testing, and chest CT scans were performed every 6 months during the initial 2 years, and annually thereafter. Doctors had the option to perform other imaging examinations or blood testing for circulating tumor DNA (ctDNA) on people with suspicious clinical symptoms."

Page 10, line 177: "Otherwise, patients were censored at the time of the last follow-up (if available)."

Page 10-11, line 181-185: "Patient demographic and clinicopathological characteristics were reported as medians with interquartile ranges (IQRs) or frequencies with percentages. To compare these characteristics between the MP/S+ group and the MP/S- group, we utilized the Mann-Whitney U test for continuous variables and either the Chi-squared test or Fisher's exact test for categorical variables."

Page 11, line 191-193 and line 195-199: "The primary endpoint was recurrence-free survival (RFS, duration from surgery to any tumor relapse or death). The second endpoint was overall survival (OS, duration from surgery to death). Survival statistics, including OS and RFS, were estimated using the Kaplan-Meier method and evaluated using the log-rank test. The Cox regression hazard model was employed to determine the independent prognostic value. The significant clinicopathological factors identified in the univariable model would be included in the multivariable model. Factors

demonstrating a significant hazard ratio (P< 0.05) with a 95% confidence interval (CI) entirely below or above 1.00 would be considered to have an independent prognostic impact."

Comment 5: Finally, please cite several related papers:
1. He B, Song Y, Wang L, Wang T, She Y, Hou L, Zhang L, Wu C, Babu BA, Bagci U, Waseem T, Yang M, Xie D, Chen C. A machine learning-based prediction of the micropapillary/solid growth pattern in invasive lung adenocarcinoma with radiomics. Transl Lung Cancer Res 2021;10(2):955-964. doi: 10.21037/tlcr-21-44.
2. Hou Y, Song W, Chen M, Zhang J, Luo Q, Um SW, Facchinetti F, Bongiolatti S, Zhou Q. The presence of lepidic and micropapillary/solid pathological patterns as minor components has prognostic value in patients with intermediate-grade invasive lung adenocarcinoma. Transl Lung Cancer Res 2022;11(1):64-74. doi: 10.21037/tlcr-21-934.
Reply 5: We carefully considered your suggestion to include reference to [He B, Song Y, Wang L, Wang T, She Y, Hou L, Zhang L, Wu C, Babu BA, Bagci U, Waseem T, Yang M, Xie D, Chen C. A machine learning-based prediction of the micropapillary/solid growth pattern in invasive lung adenocarcinoma with radiomics. Transl Lung Cancer Res 2021;10(2):955-964. doi: 10.21037/tlcr-21-44.]. After a comprehensive review of this paper, we have determined that it presents a valuable tool for preoperative prediction of the presence of MP/S patterns in lung adenocarcinoma. However, it is important to note that these findings are not directly relevant to our own research. We are more than willing to cite the second paper that you have recommended, which has been referenced as number 35 in our manuscript.
Thank you once again for your constructive feedback.
Changes in the text: There is not any change to be noted. \