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Reviewer A

Comment 1: The authors try to use serum protein/albumin/alb to glob ratios to predict response to afatinib and the likelihood of side effect development. From the data presented, the side effect aspect holds up to the authors' assumption.

- Reply 1: Thank you for the valuebale comments.
- Changes in the text: N/A

Comment 2: The authors did not state when the "baseline" labs were drawn. Was it prior to each line of therapy or prior to 1st line treatment? If it was prior to 1st line treatment, then the conclusions with regards to treatment response for 2nd line treatment with afatinib are probably not valid. Response to TKI differs greatly depending on line of treatment, as is well-known. There are far more 2nd line patients in this study than 1st line. Hence, the ROC in supplementary figures 1 and 2 are not quite convincing. This is a major issue the authors need to address.

- Reply 2: For patients on first-line afatinib, the baseline laboratory results were those taken before the initiation of afatinib. For patients on seond-line afatinib, the baseline laboratory results were those taken before the initiation of afatinib. So the laboratory results represented the status right before afatinib treatment and reflected the association of serum protein/albumin/albumin to globulin ratios and the responses and adverse effects of afatinib instead of any prior TKI/treatment.
- Changes in the text: This sentence was added in the manuscript: “For baseline laboratory results, the laboratory tests right before the initiation of afatinib were taken as the baseline for both patients on first and second line afatinib.” (Line 121-123)

Comment 3. References 5 and 12 are incomplete.

- Reply 3: The two references are updated.
- For reference 5, the latest position is changed to reference 8 – “Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29(Suppl 4):iv192-iv237.”
- For reference 12: “International BI. Giotrif® Full Prescribing Information2013.

Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/201292s000lbl.pdf".

- Changes in the text: LINE 411 – 413 for reference 5 and LINE 425 – 426 for reference 12.

Reviewer B

This study aims to investigate the role of serum protein level in the treatment efficacy and adverse event profile in patients with advanced stage EGFR-mutant NSCLC. However, there are still some questions needed to answer.

Comment 1. For logistic regression, the author must describe the cut point of each biomarker and also the rationale of setting such value.

- Reply 1: Thanks to the reviewer's comment. The statistical methods for univariate and multivariate logistic regression were updated as per suggestions.
- Changes in the text:
  - Line 145 – 148 – "To identify whether serum protein and albumin levels and their ratios were associated with clinical response to Afatinib and specific adverse effects from Afatinib, univariate logistic regression analyses were performed with the protein, albumin and globulin level and their ratios being continuous variable in the univariate logistic regression analysis. Multivariate analysis was conducted with adjustment for potential confounding factors including age, gender, smoking status, *EGFR* mutation and performance status."

Comment 2. Previous cohort studies had demonstrated that baseline characteristics, including tumor size, nodal status, presence of distant metastasis (brain, liver), will affect the treatment efficacy of EGFR-TKI. In the multivariate analysis of responsiveness, the author should put all these factors into regression analysis and define whether the protein level or protein ratio could be the independent prognostic factor.

- Reply 2: Multivariate logistics regression analysis with different variables, including tumor size, nodal status and presence of distant metastasis, was performed.
- Changes in the text:
  - Line 195-197: With multivariate analysis adjusted for age, gender, smoking status, *EGFR* mutation, tumor size, lymph node metastasis and presence of distant metastasis, the association was significant with an OR of 1.045 (95% CI = 1.002 – 1.090, p = 0.038).

- Line 202 - 204: With multivariate analysis adjusted for age, gender, smoking status, *EGFR* mutation, performance status, tumor size, lymph node metastasis and presence of distant metastasis, the association was significant with an OR of 1.101 (95% CI = 1.016 – 1.160, p = 0.015).

- Line 209 - 212: With multivariate analysis adjusted for age, gender, smoking status, *EGFR* mutation, performance status, tumor size, lymph node metastasis and presence of distant metastasis, the association was not significant with an OR of 3.933 (95% CI = 0.913 – 16.950, p = 0.066).

Line 216 - 218: At multivariate analysis adjusted for age, gender, smoking status, *EGFR* mutation, performance status, tumor size, lymph node metastasis and presence of distant metastasis the association was still significant with 1.077 (95% CI = 1.022 – 1.136, p = 0.006)

- Line 223 - 225: However, the result was statistical insignificant after adjustment for age, gender, smoking status, *EGFR* mutation, tumor size, lymph node metastasis and presence of distant metastasis in multivariate analysis, with an OR of 1.055 (95% CI = 0.986 – 1.128, p = 0.121).

- Line 231 - 233: The result was significant after adjustment for age, gender, smoking status, *EGFR* mutation, tumor size, lymph node metastasis status and presence of distant metastasis with an OR of 1.199 (95% CI = 1.029 – 1.397, p = 0.02).

- Line 239 - 242: The result was still significant after adjustment for age, gender, smoking status, *EGFR* mutation, tumor size, lymph node metastasis, presence of distant metastasis, first line EGFR-TKI used and response to first line EGFR-TKI with an OR of 1.108 (95% CI = 1.016 – 1.209, p value = 0.021).

- Line 247 - 250: The results remained significant after adjustment for age, gender, smoking status, *EGFR* mutation, tumor size, lymph node metastasis, presence of distant metastasis, first line EGFR-TKI used and response to first line EGFR-TKI with an OR of 11.111 (95% CI =1.003 – 1.231, p = 0.043).

Comment 3. The patients enrolled in present study received afatinib therapy after 2014 and more than half of patients received afatinib as second line therapy. What first-line therapy does these patients receive? Does this population receive any EGFR-TKI before? If yes, which drug and how about the efficacy? Does previous TKI exposure affect the treatment efficacy of second line afatinib? The author should clarify all the question above.

- Reply 3: The treatment details for patients on second line afatinib was included in the Results section. While prior EGFR-TKI exposure and corresponding responses were included in multivariate analysis for patients on second line afatinib, which was

stated in last comment reply section.

- Changes in the text: Line 172 - 175: Among patients on second line afatinib, 85 (67.5%) patients received gefitinib and 41 (32.5%) received erlotinib in first line setting. Among patients treated with gefitinib and erlotinib as first line, 1 (0.8%) had primary progressive disease, 58 (46.0%) had stable disease, 66 (52.4%) had partial response and 1 (0.8%) had complete response.

Comment 4. For the analysis of adverse event, the author should state what doses of afatinib does the patients receive. Does the dose deescalation affect the severity of adverse event? Does the protein level had similar role in adverse event among patients received different doses of afatinib? Also, the baseline characteristics, including age, gender, performance status, might also affect the adverse event. The author should added these factors into multivariate analysis.

- Reply 4: The dosage information of afatinib was included as below in the revised manuscript. The protein level, albumin level and the alobumin/globulin ratio were not found to be associated with the incidence of dose reduction. The association of baseline characteristics, including age, gender, performance status and adverse events, were included in the Results section.

- Changes in the text:

- Line 167 – 170. Regarding the starting dose of afatinib, 7 (3.2%), 1 (0.5%), 77 (35.5%), 40 (59.0%) and 4 (1.8%) were started on afatinib as 20 mg daily, 20/30 mg alternate day, 30 mg daily, 40 mg daily and 50 mg daily respectively. 53 (24.4%) of patients required dose reduction because of adverse effects.

- Line 257 – 258: With multivariate analysis adjusted for age, gender and performance status, the result was significant with OR of 1.083 (95% CI = 1.007 – 1.165, p = 0.031)

- Line 267 – 269: With multivariate analysis adjusted for age, gender and performance status, the result was significant with OR of 1.149 (95% CI = 1.016 – 1.298, p = 0.027).

- Line 272 – 274: With multivariate analysis adjusted for age, gender and performance status, the result was significant with OR of 1.077 (95% CI = 1.005 – 1.154, p = 0.035).