Author's response to reviews

Title: Significant Correlation between Urinary N1, N12-diacetylspermine and Tumor Invasiveness in Patients with Clinical Stage IA Non-small Cell Lung Cancer

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Version: 4 Date: 8 December 2014

Author's response to reviews: see over
Response to the Editor and the Referees

Dear Ryan M. Relox,
Academic Editor, BMC CANCER

I enclosed the revised manuscript entitled “Significant Correlation between Urinary N$^1$, N$^{12}$-diacetylspermine and Tumor Invasiveness in Patients with Clinical Stage IA Non-small Cell Lung Cancer” (MS: 7929952551406451). Please note that the title of the manuscript has been altered to avoid the use of the term “predict” in accordance with your and the #2 Referee’s comments. We are grateful to you and the Referees for the comments and valuable suggestions that have helped us to improve the quality of our paper considerably. As indicated in our responses as follows, we have taken all of your comments and suggestions into account in the revised version of our paper. We would like you to see the point-by-point response to the comments of Referees as below.

Yours sincerely,
Yusuke Takahashi, M.D., Ph.D.
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Response to Referee #1

Comment 1: In the abstract, TDR should be spelled out.
Response 1: In accordance with this comment, we have spelled out TDR in the abstract.

Comment 2: In a paragraph titled "Measurement of tumor disappearance rate on chest computed tomography (p12, line 1-5)," please clarify TDR or urinary DiAcSpm to be predictive for what?
Response 2: We would like to test the correlation of pathological invasiveness with TDR or urinary DiAcSpm. Therefore, we have revised the following sentences: “When we applied 0.75 as the cut-off value of TDR to test correlation with pathological invasiveness...with the best predictive accuracy of 70.2%.” (page 13, lines 12-13) and “The urinary DiAcSpm had an
accuracy of 60.8%, sensitivity of 69.4%, and specificity of 57.4% for prediction of pathological invasiveness...161.8 for females.” (page 13, lines 14-17).

**Comment 3:** The sentence (p17, line 2-4: When the two factors were.) in the discussion should go to the result section.

**Response 3:** We have moved the sentence, “Moreover, the predictive value of non-invasive tumor was 94.9% (37of 39) when the TDR and urinary DiAcSpm were inversely combined.” into page 13, lines 17-18 in the Results section.

**Comment 4:** The authors consistently analyzed the levels of urinary DiAcSpm by dichotomisation (i.e., high vs. low in Table 2) rather than using absolute values. They may want to analyze the urinary DiAcSpm values and other parameters by applying the Pearson's correlation test (CEA, tumor size as continuous variables), or by t-test (N0 vs 1-2, vasc invasion pos vs. neg, ly invasion pos vs. neg, as categorical variables) if the urinary values shows a statistical normal distribution.

**Response 4:** We are grateful for the suggestive comment of Referee #1. We have analyzed the correlation between absolute value of urinary DiAcSpm and CEA, tumor size as a continuous variable using Pearson’s test. Both CEA and tumor size were not significantly correlated with urinary DiAcSpm (p=0.785 and p=0.404, respectively). This may seem contradictory to table 2, which shows that high urinary DiAcSpm significantly correlates with elevated serum CEA level (p=0.023). On closer examination of the scattergram showing the correlation between urinary DiAcSpm and CEA, however, we confirmed the following relationship in serum CEA value: high DiAcSpm case < low DiAcSpm case among the normal CEA subgroup, while high DiAcSpm case > low DiAcSpm case among the high CEA subgroup (figure A).
Then, we analyzed the association between the absolute value of urinary DiAcSpm and serum CEA level (≤5.0 mg/dL vs. >5.0 mg/dL), lymph node metastasis (N0 vs. N1-2), vascular invasion (positive vs. negative), lymphatic permeation (positive vs. negative), histological type (adenocarcinoma vs. others), tumor size (≤2.0 cm vs. >2.0 cm), and TDR (≥0.75 vs. <0.75), using Mann-Whitney U test because the urinary DiAcSpm value did not show a statistic normal distribution. Urinary DiAcSpm of the N0 group was significantly lower than that of N1-2 group (p=0.014). Urinary DiAcSpm of the normal serum CEA group was significantly lower than that of elevated serum CEA group (p=0.044). Urinary DiAcSpm of the negative lymphatic permeation group was significantly lower than that of positive lymphatic permeation group (p=0.038). Similarly, urinary DiAcSpm of the negative vascular invasion group was significantly lower than that of positive vascular invasion group (p=0.002). In contrast, DiAcSpm was not significantly different between adenocarcinoma and others, tumor size ≤2.0 cm and >2.0 cm, and TDR ≥0.75 and <0.75, respectively.
These data are consistent with the original data presented in our manuscript that CEA level (≤5.0 mg/dL vs. >5.0 mg/dL), lymph node metastasis (N0 vs N1-2), vascular invasion (positive vs negative), and lymphatic invasion (positive vs negative) were correlated with high urinary DiAcSpm group.

Therefore, we have added the sentences as follows:

“Mann-Whitney U test to compare between … and TDR (≥0.75 and <0.75 (p=0.489).” (page 14, lines 8-page 15, line 2)

Comment 5: The authors may want to add figures for showing distributions of the absolute values of the urinary DiAcSpm by tumor size, histology, etc. if they are informative (e.g., box-plot figures).

Response 5: We again thank Referee #1 for his helpful suggestion. In accordance with the referee’s note, we have analyzed the distribution of the absolute values of urinary DiAcSpm and compared the distribution between high and low CEA groups, positive and negative vascular invasion and so on. As described in the Response 4 above, the results were consistent with the results summarized in Table 2, but we think it appropriate to note this just in the text rather than showing the results in numerous box-plot figures (Figure B·E, as below), for the sake of conciseness of the manuscript.
Response to Referee #2

Comment 1: This is a descriptive manuscript detailing the urinary diacetylspermine values in lung cancer patients. Authors took a big leap of faith in claiming that this urine test predicts invasiveness in stage IA lung cancer patients, which is no way supported by their data. Authors should be strongly consider to rewrite the manuscript with no definitive or novel claims except for the descriptive nature.

- Authors claim predictive ability, the data supports prognostic nature, but not predictiveness

Response 1: We are grateful for the insightful comments of Referee #2. First, we agreed that there was certain leap of logic in the discussion section. We provide following point-by-point response.
Comment 2: Does the data include consecutive patients or selected patients?
Response 2: We have added that the current cohort consisted of the consecutive patients. Therefore, we revised the pertinent sentence as follows: “Among them, 171 consecutive patients with clinical stage IA were consistent with our study cohort.” (page 7, line 18).

Comment 3: Authors ignore sentinel publications while discussing limited resection versus lobectomy for stage IA lung cancer patients, for example. Nitadori et al JNCI 2013
Response 3: In accordance with the comment, we have added the following sentence in page 6, line 18 to page 7, line 2: Moreover, a recent report described that the presence of a micropapillary component was independently associated with the risk of recurrence in patients with stage I NSCLC treated with limited resection [9].

Comment 4: Discussion and conclusions should be toned down to reflect the data, claiming a predictive test for stage IA lung cancer by urinary test is beyond the data provide.
Response 4: In accordance with Referee’s comment, we have changed the title into “Significant Correlation between Urinary N¹, N¹¹²-diacetylsperrmine and Tumor Invasiveness in Patients with Clinical Stage IA Non-small Cell Lung Cancer” Then, we have also revised some sentences to tone down our claim as follows: “We have successfully shown the significant correlation between urinary DiAcSpm and pathological tumor invasiveness in patients with clinical stage IA NSCLC.” in Abstract section: “We therefore considered the current result reasonable that urinary DiAcSpm value is significantly associated with pathologically proven tumor invasiveness in stage IA NSCLC.” (page 19, lines 10-11), and “They were proven to be independently correlated with ...and urinary DiAcSpm was strongly correlated with pathological invasiveness.” (page 20, lines 6-9)

Response to Editor’s comment
Comment: I agree with the comments provided above, particularly the criticism of strong language used in the manuscript to describe the results of what is a retrospective and small cohort of patients. The results support a correlation
but support of predictability would require more robust statistics such as a correlation coefficient. I agree with the suggestion to soften the language and conclusions or add further statistical analysis to demonstrate the strength of the correlation.

I suggest the authors limit the evaluation to adenocarcinoma and use the accepted parameters on invasiveness that are described in the IASLC/ATS/ERS Classification Guidelines for Adenocarcinoma. This would maintain clarity and consistency in the interpretation of the results.

Response: We deeply appreciate the Editor for the helpful comment. As explained in the Response to the comment of Referee #2, we have toned down the description in Discussion and Conclusion section as described above. We have also analyzed the correlation between histological invasiveness defined in IASLC/ATS/ERS classification and clinicopathological characteristics in clinical stage IA adenocarcinoma cases. Supplementary table 1 shows that male gender (p=0.010), Smoker (p=0.033), a tumor size >2.0 cm (p<0.001), serum CEA >5.0 mg/dL (p=0.006), high urinary DiAcSpm (p<0.001), and TDR>0.75 (p=0.023) were more frequent in patients with invasive tumors than in those with non-invasive tumors. Supplementary table 2 shows that a tumor size >2.0 cm (Risk ratio (RR) =3.249, 95% confidence interval (CI): 1.380-7.650, p=0.007), high urinary DiAcSpm (RR=8.208, 95%CI: 3.470-19.417, p<0.001), and TDR<0.75 (RR=2.783, 95% CI: 1.090-7.108, p=0.032) were independent predictors for invasive tumors. However, gender, smoking history, and serum CEA did not independently correlated with histological invasiveness defined in IASLC/ATS/ERS classification in clinical stage IA adenocarcinomas.

The outcome of this analysis was essentially the same with the results of analysis in the original patient cohort. This provides for a strong support for the results on the original patient cohort.

Therefore, we have added the sentences, “Of 171 NSCLC lesions, 140 adenocarcinomas were...defined in this classification and clinicopathological factors as described above.” (page 11, lines 1-8), “Of 171 NSCLC lesions, 140 adenocarcinomas were...correlated with histological invasiveness defined in IASLC/ATS/ERS classification in clinical stage IA adenocarcinomas.” (page 16, line 6-18), and “Our further analysis on the correlation between...” (page 16, line 8-10).
clinicopathological characteristics and histological invasiveness defined in IASLC/ATS/ERS classification in adenocarcinomas (Supplementary table 1, 2), strongly supported the results of analysis on the original patient cohort (table 2-4). In fact, the latter analysis revealed a clearer correlation between histological invasiveness and urinary DiAcSpm.” (page 20, lines 9-14),