Author's response to reviews

Title: Annexin A1 in plasma from patients with bronchial asthma: its association with lung function

Authors:

Sun-Hye Lee (s2sund@gmail.com)
Pureun-Haneul Lee (puruenhanul@hanmail.net)
Byeong-Gon Kim (byeonggone@naver.com)
Hyun-Jeong Seo (svamp@naver.com)
Ae-Rin Baek (arbaek@schmc.ac.kr)
Jong-Sook Park (newstart1221@naver.com)
June-Hyuk Lee (junehyuk@schmc.ac.kr)
Sung-Woo Park (mdswpark@gmail.com)
Do-Jin Kim (kdj@schmc.ac.kr)
Choon-Sik Park (mdcspark@hanmail.net)
An Soo Jang (jas877@schmc.ac.kr)

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Author's response to reviews:

Dear Editor:

Manuscript ID PULM-D-17-00094 entitled "Annexin A1 as a potential biologic marker for bronchial asthma".

We wish to express our appreciation the opportunity to revise and re-submit our manuscript. We enclose a point by point response to the reviewers’ comments. We believe the revised manuscript now fulfills the high standards of BMC Pulmonary Medicine.

With my best regards,

Correspondence
Reviewer reports:

Reviewer 1

C1- Caption of figure 1 should be changed to "plasma Annexin-A1 (ANXA1) level in healthy control, stable state and exacerbated state of asthmatic patients.

R1. The authors have changed as you suggest.

Fig. 1 Plasma Annexin-A1 (ANXA1) level in healthy control, stable state, and exacerbated state of asthmatic patients.

C4- In caption of figure 3, H & E staining was not mentioned.

R4. The authors have changed as you suggest.

Fig. 4 (Figure number changed.) Hematoxylin and eosin (H&E) staining and immunohistochemical staining of mouse lung paraffin sections.

C5- Table 1 was not cited in text.

R5. The authors have changed as you suggest. Also we added followings in the method session. Fifty asthmatic patients were recruited and followed for 6.6 ± 3.6 years, and plasma ANXA1 levels were determined during the stable and exacerbated states (Table 1). The population characteristics are shown in Table 1.

C6- Reference 34 was cited in text while it was not exist in the references list.

R6. The authors have changed as you suggest.

GC induce expression of FPR2 in human myeloid cells [34], → GC induces the expression of FPR2 in human myeloid cells [33].

Thank you
Reviewer 2

C1. mouse model: AHR results and BALF/histology quantification should be shown. Also did the model display Th2 activation? Finally Annexin A1 should be measured in mouse plasma.

R1. The authors have changed as you suggest.

Methods - Airway hyperresponsiveness and differential cell counts in mouse asthma model

Airway hyperresponsiveness (AHR) was increased in the OVA sensitized and OVA challenged mice (OVA-OVA mice group) compared with saline sensitized and air challenged mice (Control mice group) (Fig. 3A). Inflammatory cells, including eosinophils, macrophages, lymphocytes and neutrophils were increased in the BAL fluid in OVA-OVA mice compared with control mice group (Fig. 3B). The results demonstrated that induced the infiltration of inflammatory cells into the BAL fluid of the OVA-OVA challenged mice.

This model well is known to asthma model, TH2 type. And the authors have checked the mouse lung Annexin A1 plasma comparable to mouse plasma.

C2. subjects numbers, why =50 and n=25, this needs to be justified statistically, or the numbers be changed to concur with a power calculation.

R2. Thank you for your suggestion. Statisticians have reviewed our study subjects and have no concern about statistic power.

C3. the abstract is not written well, the results contain a statements that are duplicated, no subject numbers are shown, the moths are not well described (eg plasma? definition of asthma and exacterbation, mouse model details, etc)

R3. The authors have changed as you suggest.

The authors have rewritten abstracts including subject numbers. Also we added followings in the method session.

Asthma exacerbation was analyzed in subjects who had completed regular follow-up for at least 2 years. Asthma exacerbation was defined by the GINA guidelines as episodes of progressively increasing shortness of breath, cough, wheezing, chest tightness, or some combination of these symptoms, accompanied by decreased expiratory airflow and use of systemic corticosteroids (tablets, suspensions, or injections) or an increase in dose from the stable maintenance dose for at least 3 days and a hospitalization or emergency department visit due to asthma, requiring systemic corticosteroids.

Female 6-week-old BALB/c mice (6 weeks of age, weighing 20-24 g) were purchased from Charles River Korea (Orient Bio Inc., Seongnam, Korea).
C4. the conclusion and the title are not supported by the results presented, for making an argument as a potential biomarker more analysis is required, eg ROC curves etc.

R4. The authors have changed as you suggest.

The title is changed to Annexin A1 in plasma from patients with bronchial asthma: its association with lung function

And added followings

Methods - Receiver operating characteristic (ROC) curve, the area under the curve (AUC) was calculated using significant predictors (as determined via multivariate regression) to derive best suitable cut-off values and to assess model discrimination and predictive accuracy. Determination of optimal cutoff points were conducted with SPSS statistical software package (ver. 14.0; SPSS Inc; Chicago, IL, USA).

Figure - Fig. 1 (B) ROC curves for Annexin-A1 ELISA in human plasma. The optimal cutoff value and AUC were 0.596 and 0.829, respectively. Diagonal line represents hypothetical curve corresponding to a test with no discriminatory power. AUC, area under the curve; ROC, Receiver operating characteristic.

C5. the statistical tests used should be indicated in legends. For skewed data show median and IQR not mean and SD/SEM.

R5. The authors have changed to following.

Healthy control subjects (2.36 ± 0.59 ng/ml; Interquartile range, IQR: 0.0-0.6107), (p < 0.01) (Fig. 1A). Plasma ANXA1 levels were significantly lower in exacerbated patients (3.99 ± 0.45 ng/ml; IQR: 0.0375-0.8502) compared with stable patients (5.38 ± 0.43 ng/ml; IQR: 0.4474–0.8713), (p < 0.05) (Fig. 1A).

C6. for fig 1 show scattered not aligned dot plot. what else is shown? median and IQR?

R6. A box plot graph was added. Data are box plots with median value and minimal and maximal distribution.

C7. the fact that healthy and exacerbated asthmatics have same annexin levels is counterintuitive (though explainable) and the discussion does not really address this well. For instance the sentence on page 15 really does not make sense to me.

R7. The authors have changed to following in the discussion session.

In this study plasma levels of ANXA1 were increased in asthmatic patients compared with healthy control subjects, which is similar to the findings of other studies [17-20], suggesting that ANXA1 has compensatory anti-inflammatory effects in asthma. But increased plasma
ANXA1 level in stable asthma is decreased in exacerbated asthmatics, indicating that circulating ANXA1 may be decreased due to ANXA1 increase in target inflammatory site in exacerbated state of asthma. To clarify the effect of steroids and particulate matter on ANXA1, we determined the effect of corticosteroids and particulate matter on ANXA1 expression. Dexamethasone (DEX) and particulate matter induced ANXA1 protein expression, indicating that particulate matter and corticosteroids can activate ANXA1.

C8. figure 1: are these the same asthmatics but different times (or at least some of them?), this would need to be addressed statistically (matched samples)

R8. Fifty asthmatic patients were recruited and followed for 6.6 ± 3.6 years, and plasma ANXA1 levels were determined during the stable and exacerbated states (Table 1).

C9. figure 2: numbers? asthmatics and controls? and exacerbated? totally unclear. correlation coefficients should be calculated for each group and use different symbole for each group [eg share, circle, triangle OR colour] (is this spearman? then write "healthy: rs=..., p=..., n=..."). there are many undetectable levels, different to figure 1 where there are not many with "0ng/ml". is lung function pre bronchodilator? do you have % change and can correlate this?

R9. The authors have changed as you suggest.

Methods - Different correlation coefficients were calculated the Spearman's correlation analysis.

Figure - Fig. 2. Relationship between plasma AXNA1 level and lung function. Scatter plots of measured values for (A) FEV1 % (spearman, r = -0.191, p = 0.033) (B) FEV1/FVC (spearman, r = -0.202, p = 0.024) against Annexin-A1 levels. Healthy controls (n = 25), stable state (n = 45), exacerbated asthma (n = 48). FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity.

C10. figure 3: need ahr etc, see above; the method of quantification is not described sufficiently

R10. The authors have changed as you suggest.

Measurements of airway hyperresponsiveness were conducted using an animal pulmonary instrument (OCP-3000) 1 min after each dose with 3 min between doses. The following day, BALF was obtained, centrifuged, and the supernatant stored (-20°C). The cell pellet was resuspended for cell counting, and cytospin slides were prepared for stained with modified Diff-Quick stain. Differential cell counting was performed on at least 500 cells in each slide using standard morphological criteria under a light microscope.

C11. figure 7: legend does not make sense, eg "compared to control subjects"; I assume this were cells from healthy cultured in different conditions? explain exactly the experiment. what does "3" really relate to? 3x western blot of the same supernatant? 3xculture of same cell? 3xcells? it would not be appropriate to just perform a densitometry of the same plot 3x, repeat experiments needed.
Methods - Additionally, control cell lines are not exposed to DEX and TiO2. Cells were treated with different doses time (4, 8, 24 h).

Results - (p < 0.01, controls vs. DEX or DEX+TiO₂ or TiO₂).

Figure legends - The experiment was repeated three times and densitometry was determined with normalized to β-actin.

C12. throughout English and spelling and grammar needs to improve.

R12. English was checked and reviewed by native speaker.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see:

http://www.textcheck.com/certificate/6Xq0Oo

(1) We recommend that you include the above statement in the end of your document to inform reviewers that the English has been professionally checked. If you do not, then you may receive the comment "English needs revision". Some reviewers who are not native speakers of English seem to add the comment "English needs revision" to EVERY paper they review by non-US authors... (The above text can be deleted later).