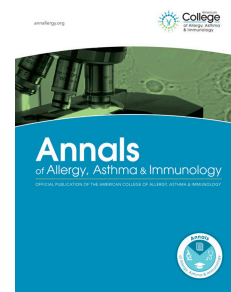


Journal Pre-proof

Sputum vitamin D binding protein: a potential biomarker of airway vitamin D axis in asthma

Rundong Qin, MD, Renbin Huang, MD, Wanyi Fu, Ph.D, MuLin Feng, MD, Jing Li, MD



PII: S1081-1206(20)30401-4

DOI: <https://doi.org/10.1016/j.anai.2020.06.005>

Reference: ANAI 3274

To appear in: *Annals of Allergy, Asthma and Immunology*

Received Date: 25 March 2020

Revised Date: 22 May 2020

Accepted Date: 1 June 2020

Please cite this article as: Qin R, Huang R, Fu W, Feng M, Li J, Sputum vitamin D binding protein: a potential biomarker of airway vitamin D axis in asthma, *Annals of Allergy, Asthma and Immunology* (2020), doi: <https://doi.org/10.1016/j.anai.2020.06.005>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Sputum vitamin D binding protein: a potential biomarker of airway vitamin D axis in asthma

Running title: Sputum vitamin D binding protein in asthma

Rundong Qin^{1#}, MD, Renbin Huang^{1#}, MD, Wanyi, Fu, Ph.D, MuLin Feng¹, MD, Jing Li^{1*}, MD

¹Department of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

*Correspondence to Jing Li, MD

Department of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, 1 kangda Rd, Haizhu Qu, Guangzhou 510120, China

E-mail: manu1991710@126.com

#Rundong Qin, and Renbin Huang contributed equally to this paper.

Conflict of interest

There are no financial or other issues that might lead to conflict of interest.

Authors' contributions

RDQ and RBH set up and performed several assessments, interpreted the data, and drafted the manuscript. WYF conceived, designed, and oversaw the experiments and helped to draft the manuscript. RDQ and MLF oversaw the experimental assays and revised the draft of the manuscript. RBH prepared the samples, accomplished the database of the patients. MLF handled and prepared the samples. JL conceived the study and participated in its design and coordination and helped to draft the

manuscript. All authors read and approved the final manuscript.

Funding

This work is supported by a grant of The Precision Medicine Research of The National Key Research and Development Plan of China (grant No.2016YFC0905800), The Breeding Program of Major Research Plan (9154204) of The National Natural Science Foundation of China, and Key project of National natural science foundation of China- Guangdong united fund (U1801286) and special funding for science and technology development of Guangdong province (Frontier and key technology innovation direction - major science and technology special project) (2017B020226006).

Keywords: asthma; vitamin D; vitamin D binding protein; sputum

Abbreviations:

VitD: vitamin D

VDBP: vitamin D binding protein

BAL: bronchoalveolar lavage fluid

25-(OH)D₃: 25-hydroxyvitamin D₃

FEV₁%; predicted forced expiratory flow in 1 second;

FEF₂₅₋₇₅%; predicted forced expiratory flow between 25 and 75% of vital capacity

FeNO: fractional exhaled nitric oxide

GINA: Global Initiative for Asthma

Sp: sputum

Se: serum

Word count: 985

Figures: 1

Tables: 0

Journal Pre-proof

VitD-axis, which includes VitD, VitD-binding protein (VDBP), and the VitD receptor, has been found to regulate immunomodulatory functions that may be of particular relevance in asthma.[1] Serum (Se) VitD is the most commonly used surrogate of the VitD-axis to explore the impact of the axis on clinical asthma expression. However, the findings are controversial.[2]

Currently, Gupta et al. demonstrated that VDBP concentration in bronchoalveolar lavage fluid (BAL), but not Se, was significantly higher in severe therapy-resistant asthma children patients and was inversely correlated with asthma severity.[3] Little is known whether Se-surrogates of VitD-axis levels could accurately represent their airway counterparts to reflect the local immunomodulatory effects in the lung. In the current study, we measured VitD and VDBP levels in matched Se and sputum supernatant (Sp) in adult asthmatic patients and compared the difference in clinical relevance. Although BAL provides insight into the pathophysiology of asthma, a major disadvantage is the invasiveness of the procedure, precluding frequent sampling in the same subject. Induced produced sputum has been proposed as a noninvasive alternative to bronchoscopy for investigating local airway inflammation at follow-ups.

A total of 203 asthmatic patients were recruited for our study from the Department of Allergy and Clinical Immunology at The First Affiliated Hospital of Guangzhou Medical University from December 2017 to February 2019. All the subjects underwent a standardized clinical assessment, including pulmonary function test, sputum inflammatory cell differentiation, allergy status (Se-specific-IgE

measurement), fractional exhaled nitric oxide measurement, and complete blood count. Sputum induction was performed successfully for 124 subjects, of whom 96 had an adequate volume of sputum supernatant after sample processing for further measurement. The processing of sputum induction and sputum sample normalization were described in our previous study.[4] All of the subjects included in the current study had received treatment recommended by GINA and did not take VitD supplements for at least six months before recruitment. The study was approved by the Ethics Review Board of the First Affiliated Hospital of Guangzhou Medical University [Medical Ethics Year 2017(No 25)]. All participants provided written informed consent. The VDBP levels in Se and Sp were measured by using ELISA(R&D Systems, MN, USA), and VitD (25-hydroxyvitamin D₃(25-(OH)D₃)) levels were measured by our hospital laboratory department using Roche Modular E170.

Sp-VDBP concentrations were significantly lower in mild asthmatic patients compared to those either in moderate and severe asthmatic subjects ($p < 0.001$, and $p < 0.001$), and there was no difference among the groups with differing asthma severity regarding the concentrations of either Se-25-(OH)D₃ or Se-VDBP. In addition, the Sp-VDBP concentrations of subjects with airway eosinophilia were significantly higher compared to those in subjects without airway eosinophilia($p = 0.028$). 25-(OH)D₃ was not detectable in our Sp samples. Sp-VDBP negatively correlated with lung function indices including FEV₁% ($r = -0.341$, $p = 0.002$) and FEF₂₅₋₇₅% ($r = -0.286$, $p = 0.011$), and positively correlated with sputum eosinophil count ($r =$

0.280, $p = 0.013$). However, we did not observe a correlation between Se-VDBP or Se-VitD with any clinical parameter. There was no association between Sp-VDBP and Se-VDBP ($r = 0.095$, $p = 0.565$) or between Sp-VDBP and Se-VitD ($r = 0.161$, $p = 0.321$), and Se-VDBP and Se-VitD ($r = 0.220$, $p = 0.381$) (Figure 1).

Our study is the first to compare the clinical relevance of the surrogates of the VitD-axis in matched Se and Sp samples. Although Sp-25(OH)D₃ was not detectable, we found that patients with mild asthma presented lower Sp-VDBP levels as compared to moderate and severe asthmatic patients. Additionally, Sp-VDBP concentrations were significantly correlated with lung function indices and airway eosinophilia. A similar degree of clinical relevance was absent for both Se-25(OH)D₃ and Se-VDBP. This data revealed that Sp-surrogates of the VitD-axis might be more relevant in asthma characteristics than their Se counterparts.

VDBP is a critical component of the VitD-axis and has immunomodulatory functions relevant in a range of lung diseases, including chronic obstructive pulmonary disease, tuberculosis, and asthma, predominantly relating to macrophage activation and neutrophil chemotaxis.[5] K. Bratke et al. reported that a significant increase in VDBP in human BAL 24 hours after allergen challenge and the increased VDBP levels were significantly correlated with eosinophils in BAL, suggesting a role of VDBP in the asthmatic late-phase.[6] Our findings show that increased Sp-VDBP concentrations might be a risk factor of asthma severity through contributing to airway eosinophilia and decreased lung function, which are two dominant features of asthma. One possible interpretation of the increased production of airway VDBP in

asthma might relate to chronic airway inflammation leading to immune cell recruitment and induction of inflammatory cytokines, which are known to stimulate hepatic transcription of VDBP, and this may also occur locally in the lung.[7] VDBP is expressed in many tissues and by neutrophils. However, there is no evidence showed that VDBP is expressed on eosinophils.[5] VDBP might play a function in driving alveolar macrophages into a more inflammatory state that causes macrophage dysfunction, which subsequently contributes to airway eosinophilia.[8] Furthermore, a vitro experiment showed that the addition of VDBP to monocyte cultures impairs VitD responsiveness, presumably by limiting the free diffusion of unbound VitD into the cells,[9] which may represent another mechanism by which excessive VDBP in the lung may limit VitD to perform immune functions in airway inflammatory or epithelial cells, leading to asthma development.

The mechanism of VitD-axis relating to asthma is very complex, and it implicates VitD supplement, VDBP production, VitD receptor expression, and polymorphisms. Abnormality of each of these significant components of the VitD-axis might contribute to the development of asthma. In the current study, through comparison of Se and Sp surrogate of VitD-axis with clinical asthma features, we found that rather than Se-VitD or Se-VDBP, Sp-VDBP presented strong clinical relevance relating airway eosinophilia and decreased lung function. The findings offer a new perspective that Sp-VDBP might serve as an alternative of VitD to explore the immunomodulatory role of the VitD-axis locally in airways in asthma.

References:

1. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, Hormone, and Immunomodulator. *Nutrients*. 2018; 11.
2. Whitehead L. Vitamin D for the Management of Asthma: A Cochrane Review Summary. *Int J Nurs Stud*. 2017; 105-6.
3. Gupta A, Dimeloe S, Richards DF, Bush A, Saglani S, Hawrylowicz CM. Vitamin D Binding Protein and Asthma Severity in Children. *J Allergy Clin Immunol*. 2012; 6: 1669-71.
4. Qin R, Long F, Xiao X et al. Sputum Autoantibodies are More Relevant in Autoimmune Responses in Asthma than are Serum Autoantibodies. *Allergy Asthma Immunol Res*. 2019; 3: 406-21.
5. Chishimba L, Thickett DR, Stockley RA, Wood AM. The Vitamin D Axis in the Lung: A Key Role for Vitamin D-binding Protein. *Thorax*. 2010; 5: 456-62.
6. Kai B, Wendt A, Garbe K, Kuepper M, Virchow JC. Vitamin D Binding Protein and Vitamin D in Human Allergen-Induced Endobronchial Inflammation. *Clinical & Experimental Immunology*. 2014; 1: 366-72.
7. Guha C, Osawa M, Werner PA, Galbraith RM, Paddock GV. Regulation of Human Gc (Vitamin D-binding) Protein Levels: Hormonal and Cytokine Control of Gene Expression in Vitro. *Hepatology*. 1995.
8. Fricker M, Gibson PG. Macrophage Dysfunction in the Pathogenesis and Treatment of Asthma. *Eur Respir J*. 2017; 3: 1700196.
9. Chun RF, Lauridsen AL, Lizabeth S et al. Vitamin D-Binding Protein Directs Monocyte Responses to 25-Hydroxy- and 1,25-Dihydroxyvitamin D. *J Clin Endocr Metab*. 2010; 7: 7.

Acknowledgement

We thank for Dr. Wanjun Wang, Dr. Yukai Liu and Dr. Tian Luo for recruiting the patients and helping perform several assessments, Dr. Lin Xinliu and Dr. Li He for inducing sputum and performing FeNO and help preparing the samples and Dr. An Jiayin for performing pulmonary function tests.

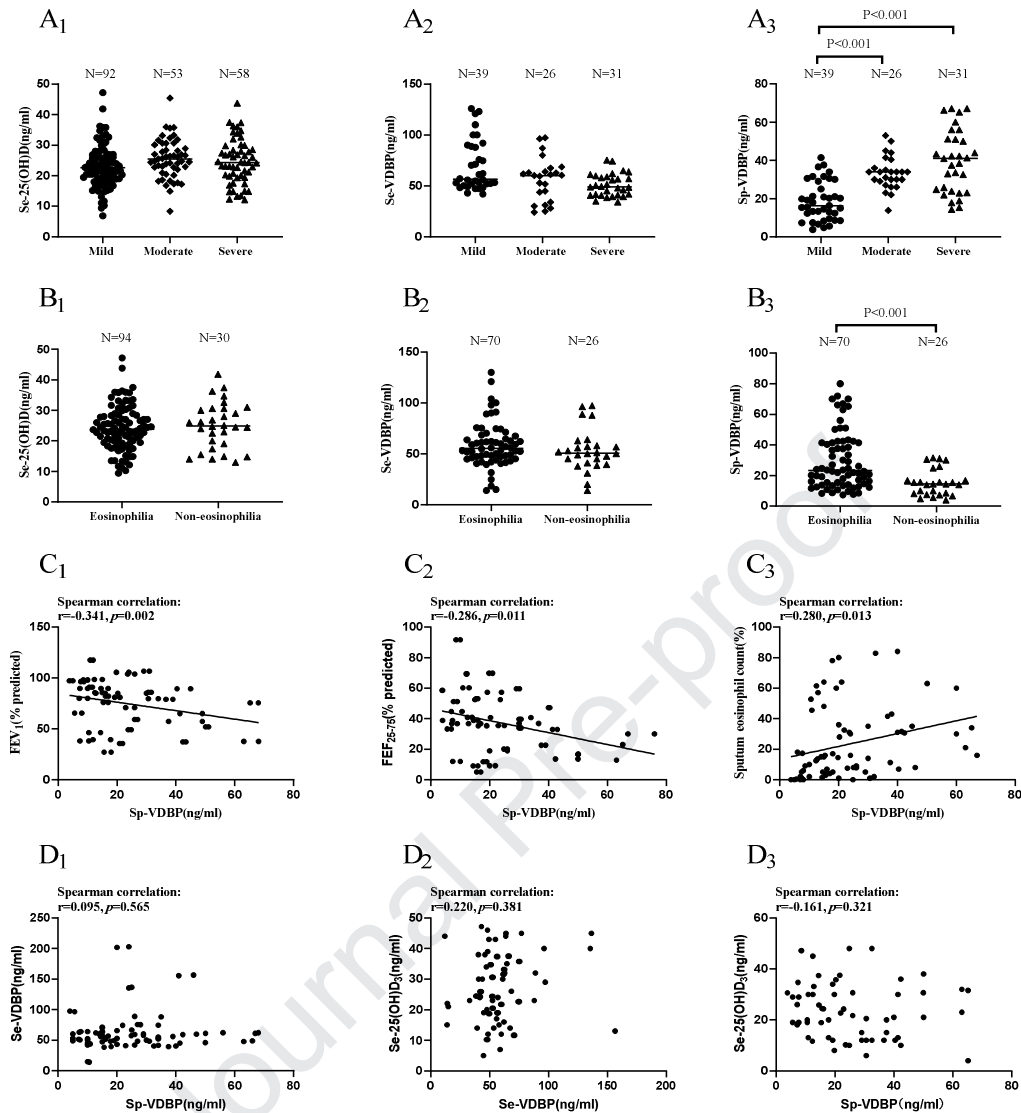


Figure 1. (A₁-A₃) Se-25(OH)D₃, Se-VDBP, and Sp-VDBP concentrations in mild, moderate, and severe asthma groups; (B₁-B₃) Se-25(OH)D, Se-VDBP, and Sp-VDBP concentrations in airway eosinophilia and non-eosinophilia groups; (C₁-C₃) Spearman correlation between Sp-VDBP with FEV₁%, FEF₂₅₋₇₅%, and sputum eosinophil count, respectively; (D₁-D₃) No association between Sp-VDBP and Se-VDBP and Sp-VDBP, and Se-VDBP and Se-VitD.