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LETTER

Sputum ANA serves as a biomarker for severe asthma

To the Editor,

Autoimmune diseases are characterized by persistent self-reactivity and tissue damage mediated by autoantibodies against self-antigens in which antinuclear antibody (ANA) is a major autoantibody.[1,2] A recent study demonstrated the evidence of autoimmune response with localizing autoantibodies in the airway secretion of asthmatics and that increased IgG to eosinophil peroxide and/or ANA was associated with increased type 2 airway inflammation and eosinophilic degranulation, especially in patients with severe asthma (SA).[1,2] In addition, increased levels of C3c and deposition of C1q-bound/IL-5-bound IgG were noted in mepolizumab-resistant asthmatics, suggesting that the presence of airway autoimmunity may be associated with asthma exacerbation and treatment response.[3] In the present study, we evaluated ANA levels in the sputum of 46 adult asthmatics associated with clinical (including lung function, blood eosinophils, total IgE, and sputum eosinophil/neutrophil counts) and inflammatory parameters in sputum, and compared between the SA and nonsevere asthma (non-SA) groups. Levels of myeloperoxidase (MPO), eosinophil-derived neurotoxin (EDN), matrix metalloproteinase (MMP)-9, and tissue inhibitor of metalloproteinase-1 (TIMP-1) as well as ANA in sputum were measured using ELISA. Detailed information on the study subjects and methods is described in the online Appendix.

The SA group had significantly higher ANA levels and MMP-9/TIMP-1 levels in sputum than the non-SA group, while lung FEV_1 (%), FVC (%), FEV_1/FVC, and MMEF (%) were significantly lower in the SA group than in the non-SA group ($p < .050$ for all) (Table 1, Figure 1A). When compared between eosinophilic (higher than 300 peripheral eosinophils) and noneosinophilic type (less than 300 eosinophils) in severe asthmatics, the SA group with eosinophilic

TABLE 1 Comparison of clinical and inflammatory parameters between the severe and nonsevere asthma groups

Variables	Nonsevere asthma ($n = 29$)	Severe asthma ($n = 17$)	p value	Severe asthma		
				Noneosinophilic asthma ($n = 7$)	Eosinophilic asthma ($n = 10$)	p value
Age (years)	54.7 ± 13.6	55.7 ± 11.3	.991	59.1 ± 16.7	53.3 ± 5.1	.063
Female sex (%)	62.1	47.1	.369	28.5	60.0	.335
Atopy (%)	31.0	66.7	.030	57.1	75.0	.608
Baseline FEV_1 (%)	95.3 ± 16.2	74.5 ± 22.8	.001	78.6 ± 19.1	71.3 ± 25.9	.560
Baseline FVC (%)	93.4 ± 15.0	79.2 ± 19.9	.019	81.6 ± 17.3	77.3 ± 22.5	.791
FEV1/FVC ratio	84.4 ± 38.3	76.1 ± 9.1	.002	76.6 ± 10.1	75.7 ± 8.9	.874
MMEF (%)	75.9 ± 24.0	51.8 ± 24.6	.004	59.11 ± 25.4	46.0 ± 23.7	.315
Serum total IgE (kU/L)	242.0 ± 259.2	410.0 ± 444.3	.218	152.3 ± 113.7	555.0 ± 478.8	.005
Total eosinophil count (cells/μL)	317.2 ± 187.2	482.4 ± 340.0	.118	145.5 ± 86.3	1349.9 ± 1580.0	.001
Sputum eosinophil count (%)	19.6 ± 27.7	26.8 ± 37.2	.844	0.5 ± 0.8	46.5 ± 39.2	.032
Sputum neutrophil count (%)	67.9 ± 30.0	65.3 ± 37.6	.955	91.5 ± 4.1	45.6 ± 40.0	.039
Sputum ANA (U/ml)	86.0 ± 40.3	172.2 ± 107.1	.002	134.9 ± 121.8	164.5 ± 121.8	.922
Sputum EDN (ng/ml)	1031.0 ± 780.0	1467.2 ± 1152.0	.234	693.1 ± 779.8	2094.5 ± 1180.6	.039
Sputum MPO (pg/ml)	937.9 ± 1053.0	1262.0 ± 1076.0	.162	1304.4 ± 1052.5	1288.0 ± 1221.0	.906
Sputum MMP−9 (ng/ml)	437.0 ± 675.3	1181.0 ± 1365.0	.031	753.8 ± 693.0	1439.4 ± 1848.2	.922
Sputum TIMP−1 (ng/ml)	327.9 ± 414.7	953.3 ± 1054.0	.021	533.7 ± 390.4	713.2 ± 806.5	.770

Note: Values are given as n (%) for categorical variables and as mean ±SD for continuous variables. p values were applied by Pearson chi-square test for categorical variables and Mann-Whitney U test for continuous variables.

Abbreviations: ANA, antinuclear antibodies; EDN, eosinophil-derived neurotoxin; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; MMEF, maximal mid-expiratory flow; MMP-9, metalloproteinases-9; MPO, myeloperoxidase; TIMP-1, tissue inhibitor of metalloproteinase-1.

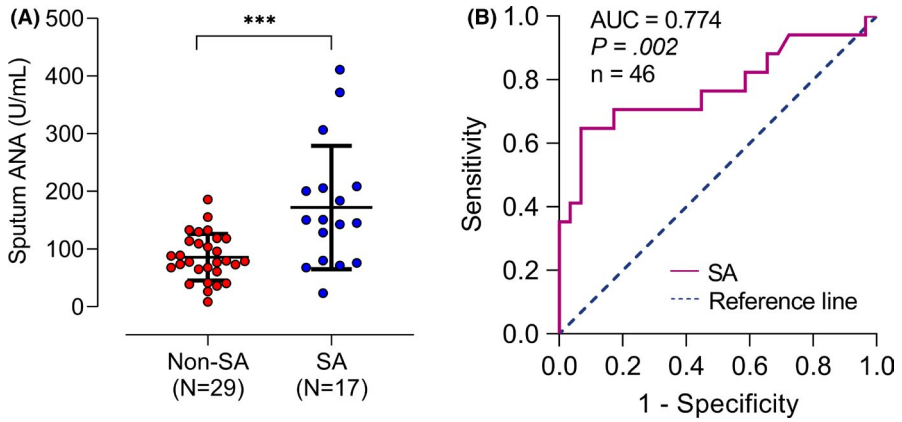

FIGURE 1 Sputum ANA levels for discriminating severe asthma (SA) group from the nonsevere asthma (non-SA) groups. Comparisons of sputum ANA levels between the two groups (A). Receiver operating characteristic curve of sputum ANA levels for discriminating the SA group from the non-SA group (B). The data are represented as mean values. *p* value was obtained by Mann-Whitey U test. ANA, antinuclear antibody; AUC, area under the curve

asthma had higher sputum eosinophils/EDN levels than that with noneosinophilic asthma ($p = .032$, $p = .039$, respectively), while no significant differences were noted in sputum ANA levels ($p = .922$; Table 1). The cutoff value of sputum ANA level predicting the SA group is 111.8 U/ml with a statistical significance ($p = .002$, 70.6% sensitivity, 72.4% specificity; Figure 1B). No differences were noted in serum ANA levels (data not shown) or sputum EDN/MPO levels between the 2 groups. When asthmatics were classified into the ANAhigh and ANAlow groups according to their cutoff value, the ANAhigh group had a significantly higher prevalence of SA, and higher sputum MMP-9/MPO levels than the ANAlow group ($p < .050$ for all, Table S1). Significantly positive correlations were noted between sputum MPO and MMP-9/TIMP-1 levels ($r = 0.815$, $p < .001$; $r = 0.770$, $p < .001$); significantly negative correlations were found between FEV_1 (%) and sputum MMP-9 /TIMP-1 levels ($r = -0.341$, $p < .025$; $r = -0.489$, $p < .001$; Figure S1), indicating that activated neutrophils may involve ANA-associated inflammation in the asthmatic airway, where MMP-9/TIMP-1 are associated with airway remodeling. Persistently higher blood neutrophil counts were noted in severe asthmatics even with anti-asthmatic medications.[4] Severe asthmatics had a higher count of neutrophil DNA extracellular trap (NET)-forming neutrophils than nonsevere asthmatics. NETs (derived from severe asthmatics) enhanced the expression of epithelial autoantigens and induced epithelial activation/dysfunction which was mediated by reactive oxygen species.[5] MPO is a component of NETs. Among asthmatics, the levels of MMP-9 and TIMP-1 (airway remodeling-related cytokines) were higher in uncontrolled status than in controlled status.[6] The present study demonstrated a significant association between sputum MPO (not EDN) and ANA levels. The levels of MMP-9 and TIMP-1 were higher in the sputum of the SA group than in the non-SA group with a negative correlation with FEV_1 (%), but a positive correlation with sputum MPO levels. Taken together, activated neutrophils (especially NET-induced inflammation) may increase MMP-9/TIMP-1 production in the asthmatic airway, contributing to airway remodeling and progression to SA.

This study has a limitation. This is a single-center observational study; however, we demonstrate a significant association of sputum ANA levels with lung function and inflammatory parameters (including MPO) according to the severity of asthma.

In conclusion, neutrophil/NET-induced inflammation is associated with ANA-associated autoimmune response in the asthmatic airway, contributing to asthma severity and lung function declines in which MMP-9/TIMP-1 are involved. Further studies are needed to evaluate whether epithelial biologics (anti-TSLP or IL-33 antibodies) could overcome this autoimmune cascade.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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REFERENCES

1. Mukherjee M, Bulir DC, Radford K, et al. Sputum autoantibodies in patients with severe eosinophilic asthma. *J Allergy Clin Immunol*. 2018;141(4):1269-1279.
2. Mukherjee M, Nair P. Autoimmune responses in severe asthma. *Allergy Asthma Immunol Res*. 2018;10(5):428-447.
3. Mukherjee M, Forero DF, Tran S, et al. Suboptimal treatment response to anti-IL-5 monoclonal antibodies in severe eosinophilic asthmatics with airway autoimmune phenomena. *Eur Respir J*. 2020;56(4):2000117.
4. Lee Y, Park Y, Kim C, et al. Longitudinal outcomes of severe asthma: real-world evidence of multidimensional analyses. *J Allergy Clin Immunol Pract*. 2021;9(3):1285-1294.e1286.
5. Choi Y, Pham DL, Lee DH, et al. Neutrophil extracellular DNA traps induce autoantigen production by airway epithelial cells. *Mediators Inflamm*. 2017;2017:5675029.
6. Hassan N, Mohamed Hussein A, Mohammed E, Mohamed O, Mohamed H, Mahmoud M. Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) as non-invasive biomarkers of remodelling in asthma. *Int J Respir Pulm Med*. 2015;46(3).

SUPPORTING INFORMATION

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