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Airway and serum biochemical correlates of refractory neutrophilic asthma

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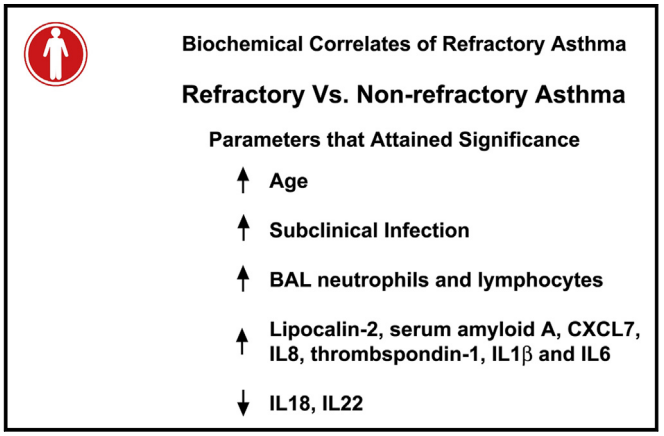
GRAPHICAL ABSTRACT



Background: Despite progress in the diagnosis and management of asthma, many patients have poorly controlled or refractory asthma (RA). The mechanism of this RA is not well understood.
Objective: We sought to explore the relationship between neutrophils and other biomarkers of RA.
Method: Sixty patients with RA, 30 patients with nonrefractory asthma (NRA), and 20 healthy subjects were enrolled. We performed a comprehensive characterization of these study subjects, which included laboratory and pulmonary function studies, chest computed tomography, and bronchoscopy with bronchoalveolar lavage (BAL). We analyzed BAL fluid and serum for a total of 244 biomolecules using a multiplex assay and correlated them with clinical and other laboratory parameters.
Results: RA was significantly different from NRA with regard to pulmonary function indices, bronchial basement membrane thickness, and BAL fluid neutrophil and lymphocyte counts but not eosinophil counts. BAL fluid neutrophil counts negatively and positively correlated with forced vital capacity and age, respectively. Of the 244 biomolecules studied, 52 and 14 biomolecules from BAL fluid and serum, respectively, were significantly different among the study groups. Thirteen of these 52 molecules correlated with BAL fluid neutrophil counts. BAL fluid from 40% of patients with RA was positive for a pathogenic microbe. Infection-negative neutrophilic RA was associated with an increase in levels of select biomarkers of inflammation in the serum, suggesting the presence of systemic inflammation.
Conclusions: RA was associated with increased numbers of neutrophils and proneutrophilic biomolecules in the airways. Subclinical infection was present in 40% of patients with RA, which likely contributed to neutrophilic inflammation.
A subgroup of patients with noninfected neutrophilic RA was associated with systemic inflammation. (J Allergy Clin Immunol 2017;140:1004-14.)

Key words: *Refractory asthma, neutrophilic asthma, bronchoalveolar lavage, cytokines, infection*

The majority of asthmatic patients respond well to controller medications, such as inhaled corticosteroids and long-acting β-agonists. However, a subset of patients does not respond to multiple controller medications, including omalizumab, and their asthma symptoms remain poorly controlled. This group of patients is labeled as having refractory asthma (RA) or severe asthma.[1-3] These patients experience a high level of morbidity because of the disease. Many of these patients require chronic systemic steroid therapy, which results in severe side effects and adds to the morbidity. Because of heavy use of health care services (emergency department and hospital), patients with RA account for a disproportionately higher health care cost.[2] It is estimated that about 3% to 10% of patients have severe RA.[2-5] The treatment options for RA are limited, primarily because of the lack of understanding of its mechanisms.

Asthma is a heterogeneous disease. Based on clinical and laboratory findings, asthma has been variably classified as atopic versus nonatopic asthma, eosinophilic versus neutrophilic versus noneosinophilic/nonneutrophilic asthma, obesity-associated asthma,[3] asthma/chronic obstructive pulmonary disease overlap syndrome,[6] T_H2-high asthma,[7] T_H2/T_H17-high asthma,[8] and T_H2-low asthma,[7,8] for example. Severe asthma and RA can occur within each category of patients. It is unclear whether a single or multiple mechanisms are operating in patients with severe RA. Previous studies have identified a number of factors in patients with severe asthma. Heightened activation of type 1 (T_H1, the interferon pathway),[9] type 2 (T_H2 cells, type 2 CD8 T cells),[7,10]

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Abbreviations used
BAL: Bronchoalveolar lavage
CT: Computed tomography
FENO: Fraction of exhaled nitric oxide
FVC: Forced vital capacity
GDF-15: Growth and differentiation factor 15
GO: Gene Ontology
MMP: Matrix metalloproteinase
NAP2: Neutrophil activating peptide-2
NRA: Nonrefractory asthma
OCS: Oral corticosteroid
RA: Refractory asthma
SAA: Serum amyloid A

innate response,[11] and airway neutrophilia[12] have all been implicated in patients with RA. Levels of the type 3 cytokines IL-17A and IL-17F are increased in asthmatic patients.[8,13] However, they were not associated with levels of T_H17 cytokines in human asthma nor were they associated with neutrophilic asthma,[13-15] which is unlike what has been reported in the mouse model of asthma.[16] Increased epithelial injury, smooth muscle hyperplasia and hypertrophy, and airway remodeling are some of the recognized intrinsic factors of RA.[1,2] The external factors associated with RA include heightened exposure to sensitizing allergens,[17,18] air pollution,[19,20] and infections.[21-23] Heightened levels of select cytokines/inflammatory mediators were associated with many of the foregoing subgroups of RA.[23,24]

Despite progress in the field, there is a need to examine the precise molecular mechanism of heterogeneity of RA that allows development of targeted therapeutic interventions. To address this matter, we studied 60 patients with RA[3] and compared them with 30 patients with nonrefractory asthma (NRA) and 20 healthy control subjects. Bronchoalveolar lavage (BAL) fluid and serum were analyzed for 244 biomolecules, levels of which were then correlated with the clinical and laboratory features of asthma. The latter included pulmonary function, PC_{20} for methacholine, allergic sensitivity, fraction of exhaled nitric oxide (FENO), body mass index, blood eosinophil counts, total IgE levels, BAL fluid inflammatory cell counts (eosinophil, neutrophil, lymphocyte, and macrophage counts), indices of airway tissue inflammation (quantitative histologic evaluation of bronchial tissue) and basement thickness, and the results of microbiological studies with BAL, tissue biopsy, and airway brushing. The focus of this article is on the neutrophilic phenotype of RA.

METHODS

Human subjects

Study subjects with RA were recruited from outpatient clinics of National Jewish Health. Patients with NRA and healthy control subjects were recruited from the community. The study protocol for bronchoscopy and BAL was approved by the institutional review board (HS#2639). Written informed consent was obtained from the study subjects. Patients were allowed to continue their routine medication. Asthmatic patients had a comprehensive phenotypical and laboratory work-up. The latter included full pulmonary function tests; methacholine tests (if FEV_1 was >60% of predicted value); chest computed tomography (CT); FENO values; blood test results for eosinophils, total IgE levels, and IgE antibody levels (when appropriate); bronchoscopy with BAL; airway brushing; and endobronchial biopsy (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org).

Bronchoscopy, BAL, brushing, biopsy, and other laboratory work-up

The foregoing procedures were performed, as described previously.[25] BAL fluid was processed within an hour. Cells were isolated by means of centrifugation. Supernatant fluid was aliquoted into small samples and frozen at −80°C. Total BAL fluid cells and differential counts; a PCR-based respiratory viral panel (12 different respiratory viruses) with BAL cells and bronchial brushing; a PCR-based test for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* with airway biopsy specimens, brushings, and BAL cells; BAL culture in triplicates for bacteria, *Mycobacterium tuberculosis*, and nontuberculous mycobacteria; and a quantitative analysis of airway tissue inflammatory cells, including $CD117^+$ mast cells (by using immunohistochemical staining) were performed in the National Jewish Health clinical laboratory (ADx-Advanced Diagnostic Lab, National Jewish Health).

Multiplex biomolecule analyses of BAL fluid and serum

Aliquots of BAL fluid and serum were analyzed for a total 244 biomolecules (Human Discovery MAP 250+, v2) by Myriad RBM (Austin, Tex). A list of the biomolecules analyzed by using this method is presented in Table E1 in this article's Online Repository at www.jacionline.org.

Pathway analyses

We performed pathway analyses for the 36 biomolecules that distinguished RA from NRA. We used multiple software at the Enrichnet.org Web site: Gene Ontology (GO) for biological process and molecular function, Reactome, and the Kyoto Encyclopedia of Genes and Genomes.

Statistical analyses

Comparison between study groups was done with the Mann-Whitney *U* test. Comparison among multiple study groups was performed with the Kruskal-Wallis test followed by the Dunn test to correct for multiple comparisons. In addition, we performed the Benjamini-Hochberg test for multiple comparisons, which corrects for false discovery. The false discovery rate for this test was set at 0.1. The Pearson correlation coefficient was used to calculate correlation coefficient. Statistical analyses were performed with Prism software (GraphPad Software, La Jolla, Calif).

RESULTS

Study populations

We recruited 20 healthy control subjects, 30 patients with well-controlled asthma, and 60 patients with RA, as defined by American Thoracic Society definitions.[26] The patients with NRA did not meet the criteria for RA because their asthma symptoms were well controlled by inhaled corticosteroids and other controllers. Thirty-six percent of patients with RA (22/60) were taking systemic steroids (10-60 mg/d). Asthmatic patients with a history of current or past smoking were excluded from the study. The demographic details of the study population are shown in Table I.

Clinical and phenotypical characteristics

Healthy control subjects and patients with NRA were younger than patients with RA (Table I). Body mass index was not significantly different among the study groups. Patients with RA were more atopic (88%) than patients with NRA (66%) and healthy control subjects (55%), as judged by skin test positivity or the presence of IgE antibody against an environmental allergen. Pulmonary function indices (forced vital capacity

TABLE I. Demographic, clinical, and laboratory features of the study groups

Variable	Healthy Control subjects (n = 20)		Patients with NRA (n = 30)		Patients with RA (n = 60)		*P* value, all groups	*P* value, NRA vs RA
	Mean	Median	Mean	Median	Mean	Median		
Age (y)	33.6 ± 2	31	38 ± 2	35.5	54.5 ± 1	57	<.0001	<.0001
Female/male	13/7		19/11		36/24			
BMI (kg/m^2)	29.7 ± 1.2	29.8	28 ± 1.1	27.4	29.7 ± 0.7	29.4	.12	.051
Atopy	50%		66%		88%			
FVC (L)	4.1 ± 0.1	4.3	3.6 ± 0.1	3.5	2.7 ± 0.1	2.5	<.0001	<.0001
FEV_1 (L)	3.3 ± 0.1	3.4	2.6 ± 0.1	2.7	1.8 ± 0.09	1.6	<.0001	<.0001
FVC (%)	101 ± 2.9	97.5	92 ± 2.5	88	71 ± 2	71	<.0001	<.0001
FEV_1 (%)	96.5 ± 2.6	95	78 ± 3	74	60 ± 2.5	55	<.0001	<.0001
Reversibility (%)	5.2 ± 0.5	5.5	17.6 ± 2.1	17	22 ± 2.5	16	<.0001	<.0001
PC_{20} for methacholine	25 ± 0	25	2.5 ± 0.5 (n = 29/30)	1	3.4 ± 1.1 (n = 23/60)	1.5	<.0001	.19
FENO	23 ± 3	21	35 ± 3	28.7	43.8 ± 6	22.5	.17	.55
IgE (kU/L)	47 ± 9	33	333 ± 199	106	406 ± 149	116	<.001	.6
Blood eosinophils/μL	150 ± 21	100	283 ± 28	300	352 ± 43	300	<.0001	.4
Blood PMNs (1000/μL)	4.0 ± 0.2	3.6	3.6 ± 0.3	3.2	6.5 ± 0.4	5.1	.0001	.0001
BAL WBCs (× 10^6)	10.2 ± 0.8	9.4	8.7 ± 0.7	8.7	15.8 ± 2.7	8	.6	.5
BAL eosinophils (% [total × 10^6])	0.48 ± 0.1 (0.04 ± 0.01)	0.2 (0.022)	1.4 ± 0.2 (0.11 ± 0.02)	1 (0.079)	2.6 ± 0.6 (0.32 ± 0.09)	1 (0.042)	.07 (.19)	.3 (.6)
BAL PMNs (% [total × 10^6])	1.9 ± 0.2 (0.19 ± 0.02)	1.8 (0.16)	2.5 ± 0.4 (0.22 ± 0.05)	2 (0.15)	10.9 ± 2.4 (3.12 ± 1.8)	4.5 (0.40)	.002 (.003)	.01 (.004)
BAL lymphocytes (% [total × 10^6])	6 ± 0.7 (0.61 ± 0.08)	5.2 (0.45)	6.4 ± 1.1 (0.55 ± 0.12)	4.6 (0.30)	11.3 ± 1.3 (1.43 ± 0.27)	8 (0.60)	.08 (.2)	.03 (.1)
BAL macrophages (% [total × 10^6])	91.5 ± 0.9 (9.42 ± 0.7)	92.8 (8.8)	89.7 ± 1.2 (7.87 ± 0.6)	91.8 (7.6)	75.2 ± 2.8 (10.8 ± 1.7)	82.5 (6.3)	.001 (.3)	.006 (0.7)
Biopsy eosinophils/HPF	1.2 ± 0.6	0	6.1 ± 4.3	0	10.2 ± 3.4	0	.6	.4
Biopsy PMNs/HPF	0.7 ± 0.1	0.5	0.4 ± 0.2	0	2.3 ± 2	0	.0001	.09
Biopsy CD117$^+$ cells	3.8 ± 1.3	1	4.9 ± 1	3	1.5 ± 0.3	0	.002	.001
BM thickness	6.4 ± 0.6	6	7.5 ± 0.6	7.2	8.8 ± 0.4	8	.01	.04
Serum vitamin D	28.4 ± 3	26.4	37.5 ± 2.6	34.7	36.5 ± 2.8	34.6	.056	.4

BM, Basement membrane thickness; *BMI*, body mass index; *HPF*, high-powered field; *WBC*, white blood cells.

[FVC] and FEV_1) were significantly lower and reversibility was significantly higher in patients with RA compared with those with NRA. There was no difference in PC_{20} for methacholine between patients with RA and those with NRA. FENO values were not different among the 3 study groups. Blood eosinophil counts and total IgE levels were increased in asthmatic patients (both patients with RA and those with NRA) but were not different between patients with RA and those with NRA. Neither BAL fluid nor airway tissue eosinophil counts showed any significant difference between the NRA and RA groups. In contrast, BAL fluid and airway tissue neutrophil and BAL lymphocyte counts were increased in patients with RA. Surprisingly, numbers of tissue CD117$^+$ cells, which mostly represent mast cells, were decreased in patients with RA. Subepithelial basement membrane thickness, a sign of airway remodeling, was significantly increased in asthmatic patients, with the greatest thickness occurring in patients with RA.

Biochemical analyses of BAL fluid

Of the 244 biomolecules assayed with the Human DiscoveryMAPs v2 multiplex assay, 66 were less than the detection limit (see Fig E1, *B*). One hundred one biomolecules did not show any significant difference among the study groups: healthy control subjects, patients with NRA, and patients with RA. Fifty-two biomolecules were significantly different either in multigroup comparisons or in comparisons between patients with RA and those with NRA (Table II). Thirty-seven of these 52 biomolecules distinguished patients with RA from those with NRA (Table II and see Fig E1, *C*). Because BAL fluid neutrophils were one of the cell types that distinguished patients with RA from those with NRA, we focused on neutrophil-associated biomolecules for this study. We identified 17 neutrophil-associated biomolecules with levels that were significantly increased in patients with RA when compared with those in patients with NRA and healthy control subjects (Table III). Of these 17 biomolecules, 13 showed a significant positive correlation with the BAL fluid neutrophil count (Table IV). Four biomolecules did not show any significant correlation. It is noteworthy that levels of only one of these 13 biomolecules, CCL3 (macrophage inflammatory protein 1β), correlated with BAL fluid eosinophil counts. We classified these biomolecules based on their published cellular sources (see Table E2 in this article's Online Repository at www.jacionline.org). This classification suggests that innate immune and airway tissue cells are the primary source of RA-associated proneutrophilic biomolecules. This also implies that activation of innate immune and airway tissue cells contributes significantly to RA.

Biochemical analyses of serum

Of the 244 biomolecules assayed, 29 were undetectable. One hundred seventy-two biomolecules did not show any difference among the study groups. Fourteen biomolecules were significantly different among all 3 study groups and also between patients with NRA and those with RA (Table V). Of these 14

TABLE II. Biomolecules that are statistically different among the study groups

Biomolecules	Healthy subjects (n = 20)	Patients with NRA (n = 40)	*P* value vs healthy subjects	Patients with RA (n = 60)	*P* value vs healthy subjects	*P* value vs NRA	*P* value for multigroup analyses
1. α-1 Antitrypsin* (μg/mL)	0.4	0.7	NS	1.5	.009	.01	.005
2. Angiogenin (ng/mL)	0.63	0.6	NS	0.85	.002	.003	.007
3. Apolipoprotein C III (μg/mL)	0.01	0.018	NS	0.027	.02	NS	NS
4. Apolipoprotein E (ng/mL)	0.1	0.95	.008	1.5	.001	NS	.001
5. Axl receptor (ng/mL)	1.2	0.6	<.001	0.39	.001	NS	.004
6. C3 (ng/mL)	0.34	0.33	NS	0.66	.04	.03	.03
7. CCL3 (MIP1β) (pg/mL)	11	12	NS	23	.007	.002	.0001
8. CCL5 (RANTES [pg/mL])	6.8	7.2	NS	12	.009	.03	.01
9. CCL20 (MIP3α [pg/mL])	19	16	NS	31	NS	.02	NS
10. CCL21 (pg/mL)	3.8	4	NS	5.1	NS	.01	.02
11. CXCL9 (MIG [pg/mL])	69	79	NS	138	NS	.02	NS
12. EGF (pg/mL)	1.1	1.3	NS	2.6	NS	.01	.03
13. Ferritin (ng/mL)	6.1	5.5	NS	9.8	.01	.03	.01
14. Ficolin 3 (ng/mL)	8.2	6.5	NS	11	.03	.001	.002
15. Human epididymis protein 4 (pmol/L)	5000	3820	NS	4980	NS	.01	.03
16. IL-1β (pg/mL)	0	0	NS	0	.07	.01	.01
17. IL-6 (pg/mL)	0	0	.01	0	NS	.005	.003
18. IL-8 (pg/mL)	11	11	NS	24	.01	.002	.0005
19. IL-18 (pg/mL)	120	34	.03	14	.007	.003	.001
20. IL-22 (pg/mL)	170	100	.001	59	<.001	.001	<.001
21. Kallikrein 7 (pg/mL)	0	0	NS	145	NS	.006	.002
22. Lipocalin 2 (ng/mL)	65	82	NS	111	.05	.04	.04
23. MIF (pg/mL)	0.39	1.5	.0007	1.3	.0016	NS	.004
24. MMP3 (ng/mL)	0	0	NS	0.01	<.001	<.001	<.001
25. MMP7 (ng/mL)	0.9	0.6	.001	1.4	NS	.04	.04
26. MMP9, total (ng/mL)	2.9	4.3	NS	20	<.001	<.001	<.001
27. Myeloperoxidase (ng/mL)	51	46	NS	133	.0002	<.0001	<.0001
28. Myoglobin (ng/mL)	0.57	0.94	.03	0.91	.04	NS	NS
29. NAP2 (pg/mL)	0.41	0.79	NS	1.7	.01	.02	.001
30. Neuropilin 1 (pg/mL)	980	560	.001	580	<.001	NS	.001
31. Osteoprotegerin (pmol/L)	0	0	NS	0	NS	.006	.02
32. PAI-1 (pg/mL)	42	41	NS	11	<.001	<.001	<.001
33. Pepsinogen I (ng/mL)	0.064	0.06	NS	0.093	.007	<.001	.001
34. Pigment epithelium-derived factor (ng/mL)	3.2	6.3	NS	11	.02	.006	.005
35. Progranulin (ng/mL)	13	5.2	.01	4	.03	NS	.007
36. Prostasin (ng/mL)	520	309	.01	278	.001	NS	.005
37. RAGE	0.3	1	NS	6.5	<.001	<.001	<.001
38. Soluble CD25 (pg/mL)	69	39	NS	29	.003	.01	.001
39. Soluble CD40 (ng/mL)	0.093	0.056	.008	0.05	.002	NS	.03
40. SAA (ng/mL)	0.51	0.57	NS	2	.0002	.001	.003
41. Soluble ICAM (pg/mL)	30	19	.04	16	.004	NS	.02
42. Soluble IL-1 receptor (pg/mL)	65	26	.01	22	.003	NS	.008
43. Sortilin (ng/mL)	0	0.05	NS	0.12	.01	.01	.008
44. SP-D* (ng/mL)	50	39	NS	35.5	.01	NS	.04
45. Thrombospondin 1 (ng/mL)	0	1.4	NS	4.4	<.001	<.001	<.001
46. TIMP-1 (ng/mL)	1.3	1.8	NS	3.1	.004	.003	.001
47. TNFSF12 (pg/mL)	50	30	NS	20	.006	.01	.001
48. TNFSF13 (pg/mL)	18	4.3	.002	1.3	.001	NS	.001
49. Trefoil factor 3 (μg/mL)	0.1	0.22	NS	0.42	.004	.004	.006
50. VEGF (pg/mL)	364	292	NS	177	.001	NS	.008
51. Vitamin D binding protein* (μg/mL)	0.34	0.15	.002	0.2	.01	NS	.008
52. vWF (μg/mL)	0.004	0.008	NS	0.012	.003	.002	.009

EGF, Epidermal growth factor; *ICAM*, intercellular adhesion molecule; *MIF*, macrophage migration inhibitory factor; *MIG*, monokine induced by IFN-γ; *MIP*, macrophage inflammatory protein; *NS*, not significant; *PAI-I*, plasminogen activator inhibitor 1; *RAGE*, receptor for advanced glycosylation end products; *SP-D*, surfactant protein D; *TIMP-1*, tissue inhibitor of metalloproteinases 1; *TNFSF12 and 13*, tumor necrosis factor superfamily members 12 and 13; *VEGF*, vascular endothelial growth factor; *vWF*, von Willebrand factor.
*Steroid sensitive.

biomolecules, 6 of them were also detected in BAL fluid and were different among the study groups. They include ficolin 3, matrix metalloproteinase (MMP) 7, thrombospondin 1, apolipoprotein E, human epididymis protein 4, and neuropilin 1.

Next, we examined the correlation of these serum biomolecules with BAL fluid inflammatory cells (see Table E3 in this article's Online Repository at www.jacionline.org). Five biomolecules correlated with increased BAL fluid neutrophils: growth and

TABLE III. Neutrophil-associated biomolecules that are statistically different among the study groups

Biomolecules	Healthy subjects (n = 20)	Patients with NRA (n = 40)	*P* value vs healthy subjects	Patients with RA (n = 60)	*P* value vs healthy subjects	*P* value vs NRA	*P* value for multigroup analyses
1. C3 (ng/mL)	0.34	0.33	NS	0.66	.06	.03	.03
2. CCL3 (MIP1β [pg/mL])	11	12	NS	23	.007	.002	.0001
3. CCL20 (MIP3α [pg/mL])	19	16	NS	31	NS	.02	.05
4. EGF (pg/mL)	1.1	1.3	NS	2.6	NS	.01	.03
5. Ficolin 3 (ng/mL)	8.2	6.5	NS	11	.03	.001	.002
6. IL-1β (pg/mL)	0	0	NS	0	.07	.01	.01
7. IL-6 (pg/mL)	0	0	.01	0	NS	.005	.003
8. IL-8 (pg/mL)	11	11	NS	24	.01	.002	.0005
9. Lipocalin 2 (ng/mL)	65	82	NS	111	.05	.04	.04
10. MMP7 (ng/mL)	0.68	0.6	NS	1.6	.002	.0003	.0003
11. MMP9, total (ng/mL)	2.9	4.3	NS	20	<.001	<.001	<.001
12. Myeloperoxidase (ng/mL)	51	46	NS	133	.0002	<.0001	<.0001
13. NAP2 (pg/mL)	0.41	0.79	NS	1.7	.01	.02	.001
14. RAGE	0.3	1	NS	6.5	<.001	<.001	<.001
15. SAA (ng/mL)	0.51	0.57	NS	2	.0002	.001	.003
16. Thrombospondin 1 (ng/mL)	0	1.4	NS	4.4	<.001	<.001	<.001
17. TIMP-1 (ng/mL)	1.3	1.8	NS	3.1	.004	.003	.001

EGF, Epidermal growth factor; *MIP*, macrophage inflammatory protein; *NS*, not significant; *RAGE*, receptor for advanced glycosylation end products; *TIMP-1*, tissue inhibitor of metalloproteinases 1.

TABLE IV. Correlation of BAL fluid biomolecule level with inflammatory and other biological processes

Biomolecules	Correlation with BAL fluid neutrophils *(r)*	*P* value	Correlation with BAL fluid eosinophils *(r)*	*P* value	Correlation with BAL fluid lymphocytes *(r)*	*P* value
Group 1. Biomolecules correlated with neutrophils						
1. Lipocalin 2	0.86	<.001	0.13	NS	−0.15	NS
2. SAA	0.70	<.001	0.25	NS	0.002	NS
3. CXCL7 (NAP2)	0.65	<.001	0.07	NS	0.002	NS
4. IL-8	0.62	<.001	−0.04	NS	0.06	NS
5. Thrombospondin 1	0.60	<.001	0.06	NS	0.15	NS
6. IL-1β	0.58	<.001	0.05	NS	0.03	NS
7. Ficolin 3	0.57	.001	0.18	NS	0.32	.03
8. IL-6	0.52	<.001	0.07	NS	0.17	NS
9. PAI	0.46	.003	−0.02	NS	−0.02	NS
10. MMP7	0.41	.01	0.12	NS	0.16	NS
11. MMP9, total	0.37	.003	0.05	NS	−0.07	NS
Group 2. Biomolecules correlated with multiple inflammatory cells						
1. C3	0.48	<.001	0.11	NS	0.45	.002
2. CCL3 (MIP1β)	0.36	.006	0.41	.001	0.19	NS
Group 3. Known proneutrophilic biomolecules not correlated with neutrophils						
1. CCL20	0.12	NS	0.09	NS	0.05	NS
2. EGF	−0.01	NS	0.13	NS	0.01	NS
3. RAGE	−0.01	NS	0.13	NS	0.08	NS
4. TIMP-1	0.21	NS	0.23	NS	0.36	NS

Italicized biomolecules are also changed in the serum.
EGF, Epidermal growth factor; *MIP*, macrophage inflammatory protein; *PAI*, plasminogen activator inhibitor; *RAGE*, receptor for advanced glycosylation end products; *TIMP-1*, tissue inhibitor of metalloproteinases 1.

differentiation factor 15 (GDF-15), human epididymis protein 4, MMP7, tetranectin, and von Willebrand factor. None of these biomolecules correlated with BAL fluid eosinophil or lymphocyte counts.

Microbial analyses of BAL fluid

BAL fluid from each patient was cultured in triplicate to detect pathogenic microbial organisms. The results were presented as negative or normal respiratory flora or positive for a pathogenic strain. A total of 24 of 60 patients with RA had positive results for bacteria, a virus, or both. The following bacterial strains were identified in 22 patients with RA: methicillin-sensitive *Staphylococcus aureus* (7 patients), methicillin-resistant *Staphylococcus aureus* (1 patient), *Pseudomonas aeruginosa* (5 patients), *Haemophilus influenzae* (1 patient), *Haemophilus* species but not *influenzae* (subspecies not identified, 2 patients), *Haemophilus parainfluenzae* (1 patient), β-hemolytic Streptococci (not group A) (1 patient), α-hemolytic streptococci (1 patient), *Streptococcus pneumoniae* (1 patient), *Moraxella catarrhalis* (1 patient), *Serratia marcescens* (1 patient), and *Capnacytophaga* species (1 patient). Note that some patients

TABLE V. Serum biomolecules with levels that are statistically different among the study groups

Biomolecules (total n = 14)	Healthy subjects (n = 20)	Patients with NRA (n = 40)	*P* values vs healthy subjects	Patients with RA (n = 60)	*P* values vs healthy subjects	*P* value vs NRA	*P* values for multigroup analyses
Proinflammatory							
1. CCL18 (PARC [ng/mL])	105	97	NS	146	.006	.0006	.0006
2. Cathepsin D	569	480	.02	585	NS	<.0001	.004
3. Ficolin 3 (μg/mL)	21.5	21	NS	29	NS	.002	.002
4. GDF-15 (ng/mL)	0.33	0.32	NS	0.40	NS	.02	.03
5. Thrombin-activatable fibrinolysis (μg/mL)	11.8	8.6	.008	11	NS	.005	.005
6. von Willebrand factor (μg/mL)	109.5	61	.08	120	NS	<.0001	.0002
7. YKL-40 (ng/mL)	40	27	NS	42	NS	.003	.008
Remodeling/regeneration/angiogenesis							
1. MMP7 (ng/mL)	5.7	4.1	.03	5.1	NS	.002	.009
2. Tenascin C (ng/mL)	493	400	NS	733	.009	.003	.002
3. Tetranectin (μg/mL)	22	20	NS	18	.002	.02	.005
4. Thrombospondin 1 (μg/mL)	14.7	12	.01	14	NS	.001	.004
Anti-inflammatory							
1. Apolipoprotein E (ng/mL)	65	56	NS	65	NS	.01	.07
2. Human epididymis protein 4 (pmol/L)	690	555	.07	787	NS	.002	.005
3. Neuropilin 1 (pg/mL)	248	177	.005	214	.003	.006	.003

Italicized variables were also altered in BAL fluid samples.
NS, Not significant; *PARC*, pulmonary and activation-regulated chemokine.

had more than 1 strain of bacteria. Two patients had positive result for viruses only (1 with respiratory syncytial virus and 1 with coronavirus OC43). Four patients with a bacterial isolate also had positive results for viruses (3 with rhinovirus and 1 with parainfluenza virus 3). It should be noted that these infections were subclinical in nature because the patients did not report symptoms of acute infection (fever, coryza, pharyngitis, or myalgia). All patients had chest CT. A summary of chest CT findings from patients with positive results for infection is shown in Table E4 in this article's Online Repository at www.jacionline.org. One patient with *Pseudomonas* species had consolidation; 1 patient with *Serratia marcescens* had infiltrates, and 3 patients had ground-glass opacity. The rest of the patients did not have any sign of acute infection.

Effect of infection on clinical and laboratory parameters of RA

Next, we examined the effect of infection on cellular and molecular parameters in patients with RA. As anticipated, infection increased the frequency of neutrophils more than that of eosinophils in the airways when compared with that seen in patients with NRA (see Fig E1, *B*). This was associated with a dramatic increase in levels of the neutrophilic granular protein myeloperoxidase (see Fig E1, *A*) and a number of proneutrophilic biomolecules, especially MMP9, thrombospondin 1, IL-8, IL-1β, serum amyloid A (SAA), IL-6, lipocalin 2, neutrophil activating peptide-2 (NAP2), and 6 conserved cysteine containing CC-chemokine (see Fig E1, *B*). Although the foregoing changes were statistically significant for the groups, not all infection-positive patients with RA had increased BAL fluid neutrophil counts. Of the 24 infection-positive patients with RA, 15 had increased neutrophil frequencies (≥3%, threshold based on the normal range for the clinical laboratory) in BAL fluid, and 9 did not. Ten of these 15 infection-positive neutrophilic asthmatic patients had increased (≥1%) eosinophil frequencies in BAL fluid. Conversely, 4 of the 9 infection-positive but nonneutrophilic patients had increased eosinophil counts in BAL fluid.

We compared BAL fluid neutrophil frequencies (as a percentage) and absolute neutrophil counts in blood among infection-positive and infection-negative patients with RA and those with NRA. A total of 22 patients with RA (10 with infection-positive RA and 12 with infection-negative RA) were receiving systemic steroids, which could have a direct effect on blood and BAL fluid neutrophil counts. For this reason, we analyzed data with and without inclusion of patients receiving oral corticosteroids (OCSs). BAL fluid neutrophil counts were increased in both infection-positive and infection-negative patients with RA regardless of their OCS use compared with healthy control subjects (Fig 1, *A*). Blood neutrophil counts were also increased in both infection-positive and infection-negative patients with RA when the data were analyzed for the entire group (Fig 1, *B*). However, this increase was lost in the blood but not BAL fluid (Fig 1, *C* and *D*) in the infection-negative RA group when the patients receiving OCSs were excluded from analysis. The results suggest that blood neutrophilia in infection-negative patients with RA was caused by OCSs. Strikingly, the absolute neutrophil number remained increased in the infection-positive RA group, even when the patients receiving OCSs were excluded from the analysis. The results indicate that an increase in absolute neutrophil counts could serve as a blood biomarker for airway infection in patients with RA. The results also suggest that blood neutrophilia in this subgroup was due to airway infection (Fig 1, *C*).

Asthma is a type 2 immune response disease, and eosinophils play an important role. As noted earlier, the median BAL fluid eosinophil number did not increase significantly in patients with RA compared with those with NRA. Because infection contributed to RA in a significant number of patients, we reasoned that the former might have affected the eosinophilic influx by

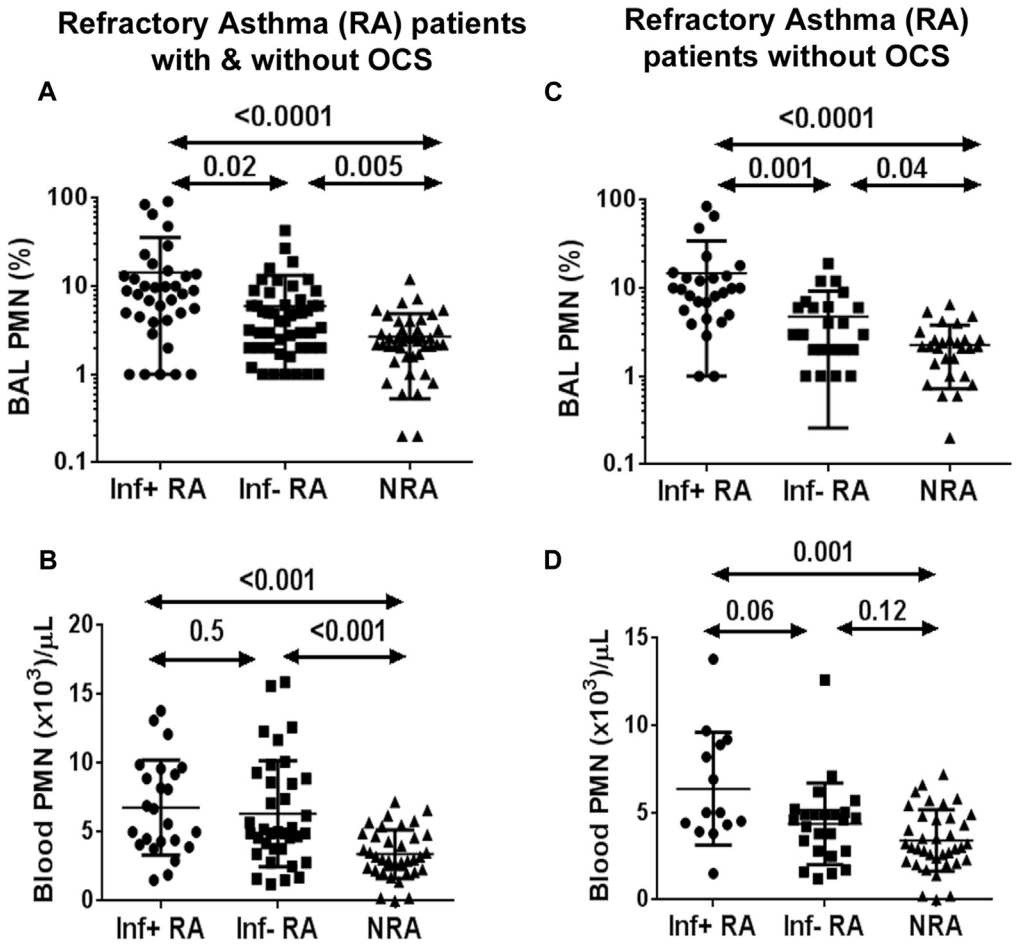

FIG 1. A-D, Comparison of blood and BAL fluid neutrophil counts among infection-positive *(Inf+)* and infection-negative *(Inf−)* patients with RA and patients with NRA, including and excluding patients with RA taking OCSs.

favoring neutrophilic influx. For this reason, we analyzed BAL fluid eosinophils and neutrophils separately in infection-positive and infection-negative patients with RA and compared them with counts in patients with NRA. Median BAL eosinophil counts were 1, 1, and 1 in patients with NRA, infection-positive patients with RA, and patients with infection-negative RA, respectively. On the other hand, median BAL fluid neutrophil counts were 2.2, 8, and 3.2 in patients with NRA, infection-positive patients with RA, and infection-negative patients with RA, respectively, and the difference among the groups was statistically significant ($P = .02$).

Serum biomolecules that identify neutrophilic asthma in noninfected patients

Subclinical infection was the most plausible explanation for increased airway neutrophil counts in our infection-positive patients with RA. The cause of neutrophilic asthma in infection-negative patients with RA was of major interest to us. To this goal, we compared serum proneutrophilic biomolecule levels between infection-negative and infection-positive patients with RA (Fig 2). IL-1β, IL-6, IL-8, CXCL1, and CCL3 levels were increased in serum from infection-negative patients with RA compared with those in infection-positive patients with RA and NRA. Interestingly, the BAL fluid concentrations of these molecules were not increased in the infection-negative patients with RA when compared with those in infection-positive patients with RA. The results raised the possibility of a systemic inflammatory process contributing to airway neutrophilia in infection-negative patients with RA.

Next, we compared levels of C-reactive protein, soluble ST2 (serum stimulation 2, also known as suppressor of tumorigenicity 2), and GDF-15, 3 molecules associated with atherosclerosis, cardiovascular diseases, and systemic inflammatory diseases.[27,28] Indeed, levels of all 3 molecules were increased in infection-negative but not infection-positive patients with neutrophilic asthma (Fig 3). The erythrocyte sedimentation rate was similar in both groups. We were concerned about a potential role for inhaled corticosteroids in airway neutrophilia. To this goal, we compared inhaled corticosteroid treatment of patients with NRA and those with RA (see Table E5 in this article's Online Repository at www.jacionline.org). All patients with RA were receiving high-dose inhaled steroids and long-acting β-agonists. In contrast, some patients with NRA were receiving low- to medium-dose inhaled steroids. Thus it is possible that this difference in the dose of inhaled steroids contributed to airway neutrophilia.

Clinical correlations

Next, we examined the clinical correlation of airway inflammatory cells and biomolecules. There was a positive

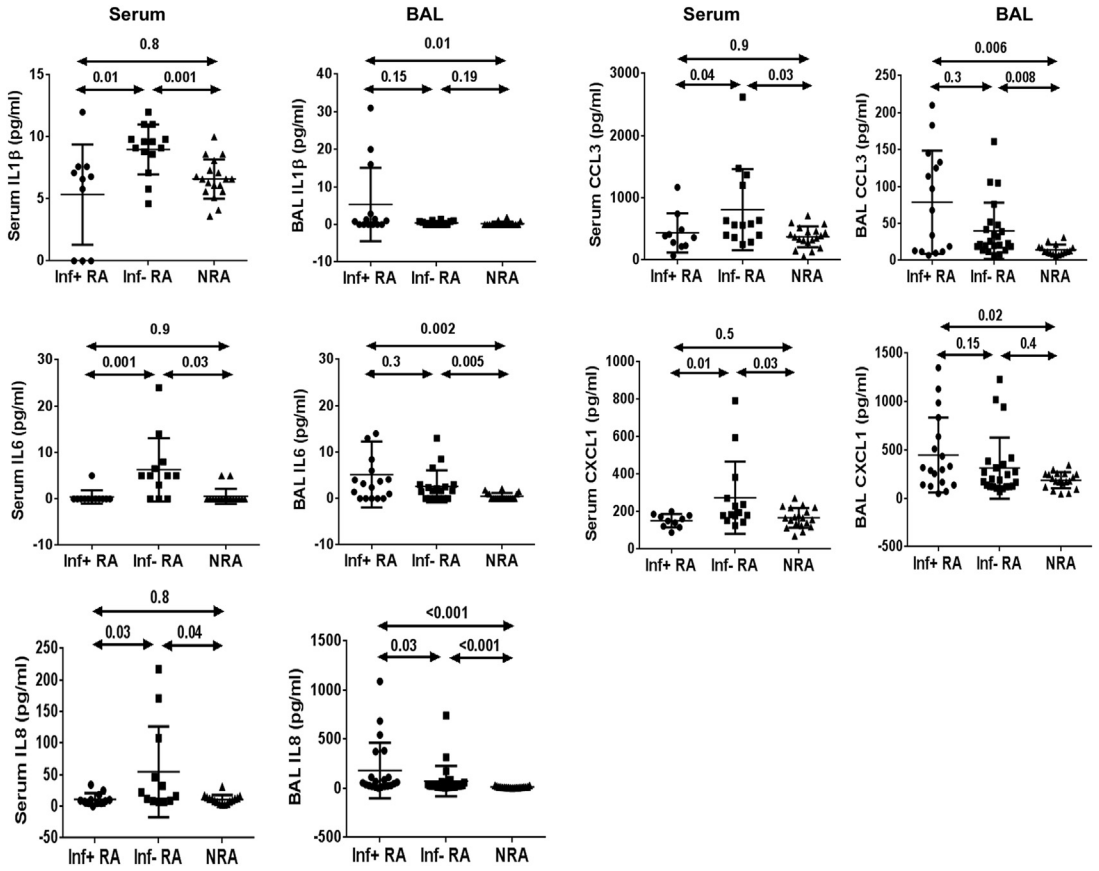

FIG 2. Comparison of biomolecule levels between serum and BAL fluid among infection-positive *(Inf+)* patients with RA, infection-negative *(Inf−)* patients with RA, and patients with NRA.

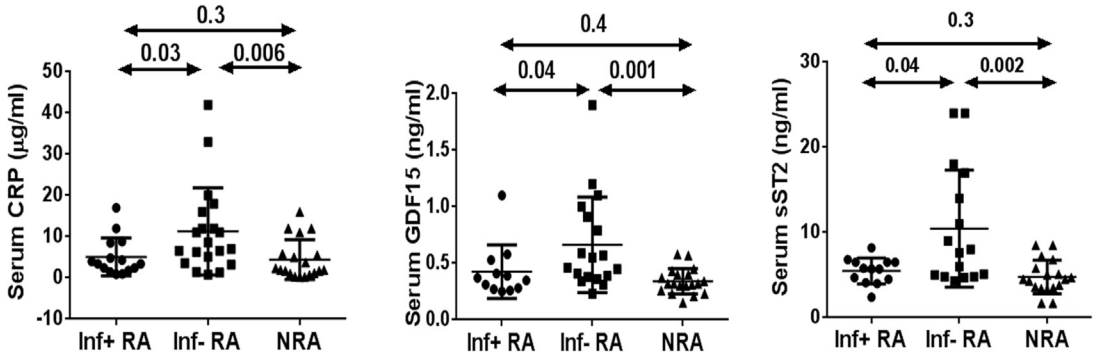

FIG 3. Comparison of systemic inflammatory markers in serum from infection-positive *(Inf+)* patients with RA, infection-negative *(Inf−)* patients with RA, and patients with NRA.

correlation ($r = 0.49$, $P < .001$) between the age of the patient and the BAL PMN count. Age also positively correlated with FVC ($r = 0.52$, $P < .05$) and BAL fluid IL-1β levels ($r = 0.67$, $P < .001$) but not with levels of other proneutrophilic biomolecules, including IL-6 ($r = 0.40$) and IL-8 ($r = 0.18$). BAL fluid PMN counts but not BAL fluid eosinophil, lymphocyte, or blood eosinophil counts negatively correlated with FVC percentages (see Table E6 in this article's Online Repository at www.jacionline.org). $CD117^+$ cells, which largely but not exclusively represent mast cells, did not correlate with levels of any BAL biomolecules. Levels of 2 BAL fluid biomolecules correlated with 2 pulmonary function indices. Myoglobin levels negatively correlated with FVC percentage, whereas prostasin levels negatively correlated with reversibility and positively with FEV_1 percentage. We performed pathway analyses with the 36 BAL fluid biomolecules that distinguished patients with RA from those with NRA. We compared pathway results by using GO for biological process and molecular function, Reactome, and Kyoto Encyclopedia of Genes and Genomes (data not shown). The GO analysis identified the most meaningful pathways. The top pathways included neutrophil chemotaxis, chemokine receptor signaling, fibroblast growth factor stimulation, and cellular response to IL-1 (see Table E7 in this article's Online Repository at www.jacionline.org).

DISCUSSION

We showed that RA was associated with an increase in neutrophil and lymphocyte counts in the airways. As mentioned previously, we specifically focused on neutrophilic asthma in this article. There was a concurrent increase in levels of a multitude of biomolecules known to be associated with innate immune and airway tissue responses. We identified 51 biomolecules with increased levels in asthmatic patients, and 36 of them distinguished RA from NRA. Sixteen biomolecules correlated with neutrophilic asthma. The vast majority of these biomolecules are known to be produced by macrophages and airway epithelial cells, which indirectly points to their role in patients with neutrophilic asthma. A pathway analysis identified neutrophilic influx as an important biological process in RA. Multiple previous studies demonstrated a similar role for neutrophilic inflammation in severe asthma. Our RA group resembles cluster 5 (neutrophilic) of the SARP cohort.[29] In a subsequent study of SARP patients, increased neutrophil and mixed neutrophil and eosinophil counts in the sputum (clusters C and D) were associated with asthma severity.[30] Our patients with RA are similar to those of cluster H (mixed neutrophilic/eosinophilic) of the multinational study by Hinks et al.[31] This cluster had severe asthma, which was associated with increased IL-6, IL-8, MMP3, and MMP8 levels in sputum.

One of the weaknesses of this study is that patients with RA were significantly older than patients with NRA. RA is more prevalent in older patients,[4,5] which led to this unintended recruitment outcome. Increased age is associated with sputum neutrophilia, even in nonasthmatic subjects.[32] Thus increased age could affect the quality and quantity of the mediators involved in neutrophilic asthma.

We used a commercially available multiplex assay service for measurement of biomolecules. Multiplex assays are less sensitive than other highly sensitive assays (eg, ELISA). This perhaps explains why we did not detect the typical type 2 (eg, IL-4, IL-5, and IL-13) or type 3 (IL-17) cytokines. However, the scope of biomolecules detected by using this assay is an advantage.

One outstanding issue related to the heightened production of proneutrophilic biomolecules is what induces their production in patients with RA. We observed subclinical infection (both bacterial and viral) in one third of the patients with RA. This subclinical infection could stimulate production of proneutrophilic biomolecules. We used standard microbiological cultures to detect bacteria. The sensitivity of these cultures is relatively low when compared with that of DNA-based technologies. It is possible that new technologies will identify additional patients with subclinical infections, which could explain increased airway neutrophil counts in the currently classified infection-negative patients.

The persistence of infection at a subclinical level in a subgroup of patients raises concerns for a defective immune response. This inability to clear infection could be primary or secondary to ongoing treatment with steroids (inhaled steroids in all patients and systemic steroids in 36% of patients with RA). The potential role of inhaled steroids in patients with subclinical infection cannot be addressed at this time because their discontinuation in patients with RA will be considered unethical.

We detected decreased IL-18 and IL-22 levels in patients with RA. Low IL-18 levels were previously reported in sputum from asthmatic patients and in patients during rhinovirus infection.[33,34] IL-18 and IL-22 are important for defense against infection.[35,36] Their decrease could impair clearance of infection. Macrophage scavenging of infected and dead cells is important for resolution of inflammation.

Axl receptor tyrosine kinase is specifically expressed on airway macrophages.[37] We observed decreased expression of Axl receptor in BAL, which suggested a decreased expression on macrophages and, consequently, an impaired scavenger function. The latter could promote persistence of infection.

Thrombospondin 1 is a multifunctional and pleiotropic protein, levels of which are increased in patients with RA. It was recently reported to inhibit bactericidal activity of neutrophils and delay the clearance of lung infection.[38] Thus we have identified a number of biomolecules that could promote persistence of subclinical infection in patients with RA.

Systemic glucocorticoids are known to induce blood and tissue neutrophilia.[39] Thirty-six percent of patients with RA were taking systemic steroids. Our analysis of patients who were not taking OCSs suggested that systemic steroids contributed to blood but not airway neutrophilia in infection-negative patients with RA. Our understanding of the role of inhaled steroids in patients with airway neutrophilia is incomplete. A previous study showed that systemic but not inhaled steroids were associated with tissue neutrophilia.[39] However, this study was done with a small number of patients, and the result showed a trend for increased airway neutrophil counts with inhaled steroids. Both patients with NRA and those with RA were receiving inhaled steroids in our study. However, unlike patients with RA, not all patients with NRA received maximal doses of inhaled steroids. Thus it is possible that higher doses of inhaled steroids in patients with RA contributed to some extent to airway neutrophilia. Environmental toxicants frequently induce cytokines/chemokines that can elicit low-level neutrophilic inflammation.[40] There could be a cumulative effect during aging. The T_H17 immune response induces neutrophilic inflammation in animal models.[16] Our assay did not detect IL-17, likely because of the low sensitivity of the assay. However, the assay detected increased levels of multiple proneutrophilic biomolecules, including SAA, NAP2, thrombospondin 1, IL-6, IL-8, C3, CCL3, CCL20, epidermal growth factor, and receptor for advanced glycosylation end products. All except the last 3 significantly correlated with BAL fluid neutrophil counts. SAA induces granulocyte colony-stimulating factor and stimulates neutrophil generation.[41] It also induces T_H17.[42] IL-6 is an inducer of T_H17[43] and a marker of systemic inflammation.[44] NAP2, IL-8, C3, and CCL3 directly induce neutrophilic inflammation.[45-48] C3 also induces IL-1, IL-8, and other cytokines/chemokines, which could indirectly affect neutrophilic inflammation.[48] Some of our findings on biomolecules are in agreement with those of many previous studies. Similar to our finding, IL-1β and IL-6 discriminated severe from moderate asthma in a SARP study.[25] IL-8 levels have previously been reported to be increased in sputum[49,50] and BAL fluid[51] from patients with RA and neutrophilic asthma.

Although the existence of neutrophilic asthma in the absence of infection has been known for many years, its mechanism is poorly understood. Our study identified a unique subgroup of infection-negative patients with neutrophilic RA, which was selectively associated with increased markers of inflammation in serum. These markers included IL-1β, IL-6, IL-8, CXCL1, and CCL3. The concomitant increase in C-reactive protein levels in

the same patients suggested a systemic inflammatory process akin to what has been observed in patients with atherosclerotic cardiovascular[52] and autoinflammatory[53] diseases. Remarkably, this subgroup of patients also had increases in 2 additional cardiovascular risk factors: GDF-15[54] and soluble ST2 (IL-33 receptor).[52] We recognize that the latter is a nonspecific biomarker of systemic inflammation. Four (11%) of 36 (11%) infection-negative patients with neutrophilic RA and 5 (21%) of 24 infection-positive patients with neutrophilic RA had a history of cardiovascular disease. Therefore the frequency of existing cardiovascular disease did not explain the systemic (serum) increase in levels of these inflammatory cytokines. We also did not find any correlation of these inflammatory cytokines with other common comorbidities: diabetes and autoimmune disorders.

There are multiple known mechanisms for induction of IL-1, IL-6, IL-8, CXCL1, and CCL3. This constellation of inflammatory cytokines also constitutes the senescence-associated secretory product.[55] Senescence is a biological process that defends against DNA damage–induced carcinogenesis but, by producing multiple cytokines, contributes to systemic inflammation.[56] Recently, GDF-15 has been shown to induce cellular senescence.[57] The correlation of neutrophilic asthma with age also raises the possibility that senescence contributed to the increased proneutrophilic cytokine profile in the serum. Additional studies will be needed to establish the relationship between cellular senescence and predisposition to neutrophilic RA.

It should be noted that 37% of infection-positive patients with RA did not have neutrophilia in BAL fluid, which further underscores the notion that this was a subclinical infection. Nearly half of them had increased eosinophil counts in BAL fluid, suggesting type 2 immunodominant asthma. Type 2 cytokines inhibit IL-8 and other proneutrophilic cytokines,[58] which could explain the lack of neutrophilia. However, the absence of neutrophilia in noneosinophilic infection-positive patients with RA suggests a defective immune response or perhaps an active immune evasion by the microbial organism.

Of the 14 serum biomolecules that were different among the study groups, only 5 biomolecules overlapped with those from the BAL fluid. However, the pattern of change was different from that in BAL fluid. Four of these biomolecules showed reduced expression in patients with NRA but not in those with RA when compared with expression in healthy control subjects. The reason for this reduced expression in patients with NRA but not those with RA is not clear. Ficolin 3 is the only biomolecule in the serum that distinguished RA from NRA. Ficolin 3 is a recognition molecule of the lectin pathway of the complement system. Its deficiency is associated with increased infection.[59]

If subclinical infection is one of the mechanisms of RA in a subgroup of patients, its importance can be tested through targeted and bacterial strain–specific antibiotic therapy. We are aware that antibiotic therapy of patients with RA produced mixed results in the past.[60,61] The failure of antibiotic therapy in some studies could be due to inappropriate selection of study patients (eg, inclusion of patients who did not have any infection) and use of an inappropriate antibiotic.[3] Note that we detected both gram-positive and gram-negative bacteria, as well as viruses, in patients with RA. The results underscore the importance of the selection of an appropriate antibiotic (targeting gram-positive vs gram-negative bacteria) and its application to a properly identified target patient population. If low-level systemic inflammation is indeed the cause of airway neutrophilia in infection-negative patients with RA, this hypothesis can be tested by using therapeutic agents targeting molecules, such as IL-1, IL-6, and IL-8. Some of these therapeutic agents are already in use in patients with rheumatologic conditions.

The number of $CD117^{+}$ cells representing mostly mast cells was reduced in patients with RA. Reduced numbers of tryptase-positive cells representing mast cells in patients with severe asthma was previously reported in a SARP study.[62] This was thought to be due to the loss of tryptase as a consequence of mast cell degranulation. However, unlike tryptase, CD117 is a cell-surface marker and not a granular protein and is not expected to be affected by mast cell degranulation. It is possible that the reduced $CD117^{+}$ cell number is a consequence of the underlying disease process.

We acknowledge the excellent technical assistance of Christena Kolakowski and Allen Stevens.

Clinical implications: This study identifies airway neutrophils and proneutrophilic biomolecules as significant contributors to RA. A subgroup of neutrophilic asthma was associated with a subclinical infection. This group is likely to benefit from specific antimicrobial therapy. Neutrophilic asthma without a subclinical infection showed signs of systemic inflammation. Therapies targeting proneutrophilic biomolecules, autoinflammation, or both are likely to control asthma in this subgroup.

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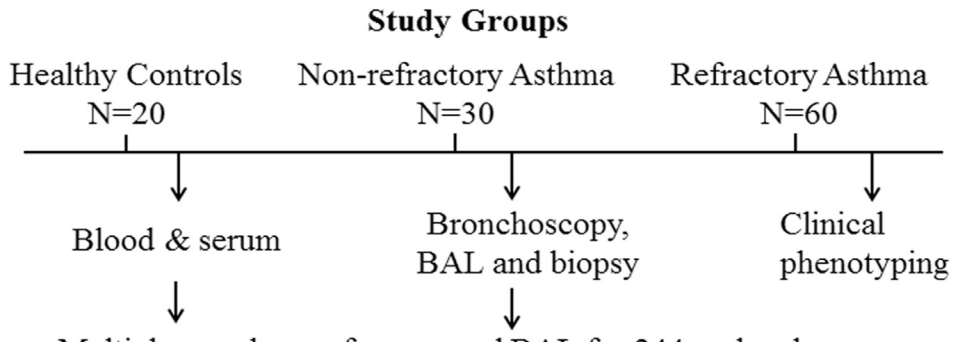
A



B

Sample	Molecules studied	Not detected	Changed	Unchanged
BAL	244	91	52	101
Serum	244	54	18	172

C

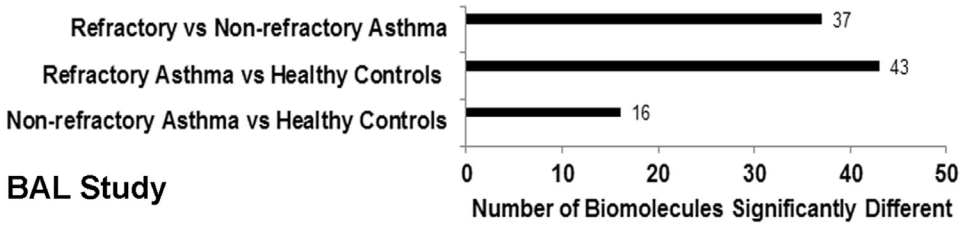


FIG E1. Study design and overview of the results. **A,** Schematic presentation of study design. **B,** Inserted table summarizes the number of biomolecules studied, not detected, changed, and unchanged in BAL fluid and serum from the study subjects. **C,** Number of biomolecules in BAL fluid that were significantly ($P < .05$) different among study populations.

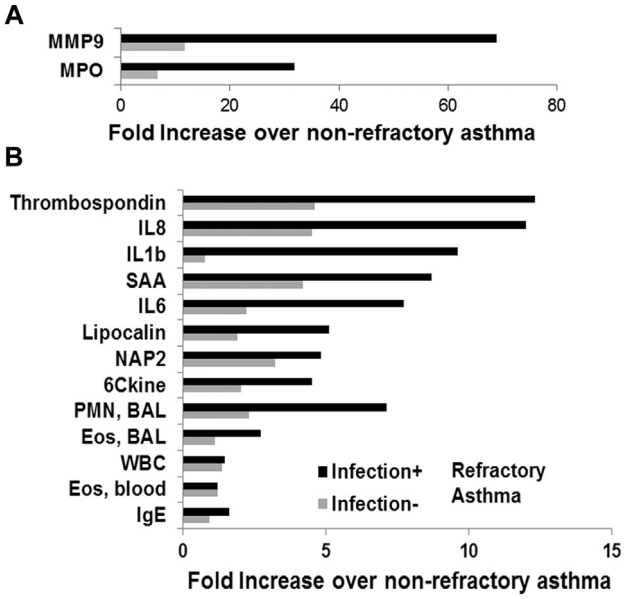


FIG E2. Fold increase in biomolecule level in patients with RA compared with those with NRA (**A** and **B**). Patients with RA were subgrouped as having infection (infection positive, *black bars*) or no infection (infection negative, *gray bars*). Results in Fig E2, *A*, were separated from results in Fig E2, *B*, because of the higher level of increase (see labels on *x-axis*).

TABLE E1. List of biomolecules

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
Adiponectin	μg/mL	0.000086	0.00030
Agouti-related protein (AgRP)	pg/mL	24	23
Aldose reductase	ng/mL	0.41	0.67
Alpha-1-antichymotrypsin (AACT)	μg/mL	0.70	1.4
Alpha-1-antitrypsin (AAT)	mg/mL	0.000000038	0.000000082
Alpha-1-microglobulin (A1Micro)	μg/mL	0.000014	0.000025
Alpha-2-macroglobulin (A2Macro)	mg/mL	0.000098	0.000087
Alpha-fetoprotein (AFP)	ng/mL	0.014	0.11
Amphiregulin (AR)	pg/mL	131	199
Angiogenin	ng/mL	0.016	0.0081
Angiopoietin 2 (ANG-2)	ng/mL	0.048	0.048
Angiotensin-converting enzyme (ACE)	ng/mL	0.019	0.035
Angiotensinogen	ng/mL	0.051	0.092
Apolipoprotein(a) (Lp[a])	μg/mL	0.00020	0.00091
Apolipoprotein A-I (Apo A-I)	mg/mL	0.000000016	0.00000006
Apolipoprotein A-II (Apo A-II)	ng/mL	0.000011	0.000014
Apolipoprotein A-IV (Apo A-IV)	μg/mL	0.075	0.16
Apolipoprotein B (Apo B)	μg/mL	0.00044	0.0021
Apolipoprotein C-I (Apo C-I)	ng/mL	0.0000062	0.000022
Apolipoprotein C-III (Apo C-III)	μg/mL	0.0000024	0.000011
Apolipoprotein D (Apo D)	μg/mL	0.020	0.037
Apolipoprotein E (Apo E)	μg/mL	0.00089	0.0024
Apolipoprotein H (Apo H)	μg/mL	0.000015	0.000062
AXL receptor tyrosine kinase (AXL)	ng/mL	0.0058	0.010
B cell-activating factor (BAFF)	pg/mL	1.5	3.7
B-lymphocyte chemoattractant (BLC)	pg/mL	9.8	12
Beta-2-microglobulin (B2M)	μg/mL	0.000045	0.000051
Betacellulin (BTC)	pg/mL	19	27
Brain-derived neurotrophic factor (BDNF)	ng/mL	0.0046	0.0072
6 Conserved cysteine containing CC-chemokine	pg/mL	8.2	15
C-peptide	ng/mL	0.0041	0.0030
C-reactive protein (CRP)	μg/mL	0.0000034	0.0000078
Calbindin	ng/mL	0.53	0.90
Cancer antigen 125 (CA-125)	U/mL	0.45	0.76
Cancer antigen 15-3 (CA-15-3)	U/mL	0.055	0.090
Cancer antigen 19-9 (CA-19-9)	U/mL	2.1	1.4
Cancer antigen 72-4 (CA 72-4)	U/mL	6.5	4.0
Carcinoembryonic antigen (CEA)	ng/mL	0.028	0.037
Cathepsin D	ng/mL	0.23	0.18
CD5 antigen-like (CD5L)	ng/mL	0.0056	0.0081
CD40 antigen (CD40)	ng/mL	0.0036	0.0031
CD40 ligand (CD40L)	ng/mL	0.0037	0.0033
Cellular fibronectin (cFib)	μg/mL	0.0089	0.042
Chemokine CC4 (HCC-4)	ng/mL	0.0066	0.0095
Chromogranin-A (CgA)	ng/mL	5.0	2.6
Ciliary neurotrophic factor (CNTF)	pg/mL	4.5	19
Clusterin (CLU)	μg/mL	0.0013	0.0024
Collagen IV	ng/mL	1.2	1.9
Complement C3 (C3)	mg/mL	0.000000016	0.0000001
Complement factor H–related protein 1 (CFHR1)	μg/mL	0.00076	0.0011
Cortisol (Cortisol)	ng/mL	1.2	2.0
Creatine kinase-MB (CK-MB)	ng/mL	0.035	0.070
Cystatin-C	ng/mL	0.011	0.0075
E-selectin	ng/mL	0.033	0.041
EN-RAGE	ng/mL	0.0036	0.0076
Endoglin	ng/mL	0.0023	0.0041
Endostatin	ng/mL	0.014	0.054
Eotaxin-1	pg/mL	1.9	29
Eotaxin-2	pg/mL	4.7	7.6
Eotaxin-3	pg/mL	16	31
Epidermal growth factor (EGF)	pg/mL	0.78	5.8
Epidermal growth factor receptor (EGFR)	ng/mL	0.031	0.058

(Continued)

TABLE E1. *(Continued)*

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
Epiregulin (EPR)	pg/mL	3.7	14
Epithelial cell adhesion molecule (EpCam)	pg/mL	16	80
Epithelial-derived neutrophil-activating protein 78 (ENA-78)	ng/mL	0.0030	0.017
Ezrin	ng/mL	2.5	3.6
Factor VII	ng/mL	1.2	0.77
Fas ligand (FasL)	pg/mL	2.6	5.3
FASLG receptor (FAS)	ng/mL	0.47	1.2
Fatty acid–binding protein, adipocyte (FABP, adipocyte)	ng/mL	0.023	0.049
Fatty acid–binding protein, heart (FABP, heart)	ng/mL	1.0	3.0
Fatty acid–binding protein, liver (FABP, liver)	ng/mL	2.8	4.8
Ferritin (FRTN)	ng/mL	0.013	0.021
Fetuin-A	μg/mL	0.00034	0.00062
Fibrinogen	mg/mL	0.00000022	0.00000024
Fibroblast growth factor 4 (FGF-4)	pg/mL	123	87
Fibroblast growth factor basic (FGF-basic)	pg/mL	5.9	6.9
Fibulin-1C (Fib-1C)	μg/mL	0.00015	0.00015
Follicle-stimulating hormone (FSH)	mIU/mL	0.11	0.13
Galectin-3	ng/mL	0.071	0.074
Gelsolin	μg/mL	0.00034	0.00072
Glucagon	pg/mL	31	64
Glucagon-like peptide 1, active (GLP-1 active)	pg/mL	1.4	2.6
Glucagon-like peptide 1, total (GLP-1 total)	pg/mL	0.89	1.1
Glucose-6-phosphate isomerase (G6PI)	ng/mL	0.062	0.12
Glutathione S-transferase α (GST-α)	ng/mL	0.29	0.59
Glutathione S-transferase Mu 1 (GST-M1)	ng/mL	0.58	1.3
Granulocyte colony-stimulating factor (G-CSF)	pg/mL	0.73	1.1
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	pg/mL	3.2	18
Growth hormone (GH)	ng/mL	0.0054	0.030
Growth-regulated α protein (GRO-α)	pg/mL	0.36	0.94
Haptoglobin	mg/mL	0.00000012	0.00000032
HE4	pmol/L	16	14
Heat shock protein 60 (HSP-60)	ng/mL	5.3	7.4
Heparin-binding EGF-like growth factor (HB-EGF)	pg/mL	9.2	43
Hepatocyte growth factor (HGF)	ng/mL	0.33	0.20
Hepatocyte growth factor receptor (HGF receptor)	ng/mL	0.055	0.079
Hepsin	pg/mL	15	14
Human chorionic gonadotropin β (hCG)	mIU/mL	0.24	0.45
Human epidermal growth factor receptor 2 (HER-2)	ng/mL	0.0029	0.0060
IgA	mg/mL	0.000000035	0.00000028
IgE	μ/mL	0.43	3.6
IgM	mg/mL	0.00000036	0.00000047
Insulin	mg/mL	0.0035	0.043
Insulin-like growth factor-binding protein 1 (IGFBP-1)	ng/mL	0.11	0.19
Insulin-like growth factor-binding protein 2 (IGFBP-2)	ng/mL	0.57	0.48
Insulin-like growth factor-binding protein 3 (IGFBP-3)	ng/mL	1.2	3.2
Insulin-like growth factor binding protein 4 (IGFBP4)	ng/mL	0.19	0.30
Insulin-like growth factor binding protein 5 (IGFBP5)	ng/mL	1.3	1.9
Insulin-like growth factor binding protein 6 (IGFBP6)	ng/mL	0.65	0.77
Intercellular adhesion molecule 1 (ICAM-1)	ng/mL	0.35	0.26
IFN-γ	pg/mL	0.49	0.30
IFN-γ induced protein 10 (IP-10)	pg/mL	2.9	25
Interferon-inducible T-cell α chemoattractant (ITAC)	pg/mL	4.0	4.2
IL-1α	ng/mL	0.00030	0.00040
IL-1β	pg/mL	0.30	0.57
IL-1 receptor antagonist (IL-1ra)	pg/mL	9.9	19
IL-2	pg/mL	1.0	1.7
IL-2 receptor α (IL-2 receptor α)	pg/mL	25	18
IL-3	ng/mL	0.0011	0.0032
IL-4	pg/mL	4.4	5.9
IL-5	pg/mL	1.0	2.6
IL-6	pg/mL	0.84	2.2
IL-6 receptor (IL-6r)	ng/mL	0.0027	0.0054

(Continued)

TABLE E1. *(Continued)*

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
IL-6 receptor subunit β (IL-6Rβ)	ng/mL	0.023	0.049
IL-7	pg/mL	2.1	1.8
IL-8	pg/mL	0.63	0.79
IL-10	pg/mL	1.1	1.4
IL-12 subunit p40 (IL-12p40)	ng/mL	0.016	0.055
IL-12 subunit p70 (IL-12p70)	pg/mL	5.9	9.7
IL-13	pg/mL	0.63	1.2
IL-15	ng/mL	0.12	0.079
IL-16	pg/mL	4.3	17
IL-17	pg/mL	1.2	0.99
IL-18	pg/mL	3.7	8.3
IL-23	ng/mL	0.12	0.080
Kallikrein 5	ng/mL	0.045	0.14
Kallikrein-7 (KLK-7)	pg/mL	90	67
Kidney injury molecule-1 (KIM-1)	ng/mL	0.0071	0.0030
Lactoylglutathione lyase (LGL)	ng/mL	0.039	0.18
Latency-associated peptide of transforming growth factor β1 (LAP TGF-β1)	ng/mL	0.0092	0.015
Lectin-like oxidized LDL receptor 1 (LOX-1)	ng/mL	0.16	0.18
Leptin	ng/mL	0.011	0.027
Luteinizing hormone (LH)	mIU/mL	0.042	0.15
Macrophage colony-stimulating factor 1 (M-CSF)	ng/mL	0.035	0.053
Macrophage-derived chemokine (MDC)	pg/mL	1.2	3.7
Macrophage inflammatory protein-1 α (MIP1α)	pg/mL	8.2	8.4
Macrophage inflammatory protein-1 β (MIP1β)	pg/mL	4.0	6.2
Macrophage inflammatory protein-3 γ (MIP3α)	pg/mL	2.5	6.0
Macrophage inflammatory protein 3β (MIP3β)	pg/mL	3.2	7.8
Macrophage migration inhibitory factor (MIF)	ng/mL	0.0015	0.0020
Macrophage-stimulating protein (MSP)	ng/mL	0.037	0.051
Malondialdehyde-modified low-density lipoprotein (MDA-LDL)	ng/mL	25	49
Maspin	pg/mL	466	353
Matrix metalloproteinase-1 (MMP-1)	ng/mL	0.12	0.18
Matrix metalloproteinase-2 (MMP-2)	ng/mL	1.3	0.87
Matrix metalloproteinase-3 (MMP-3)	ng/mL	0.0066	0.012
Matrix metalloproteinase-7 (MMP-7)	ng/mL	0.017	0.0088
Matrix metalloproteinase-9 (MMP-9)	ng/mL	5.0	7.9
Matrix metalloproteinase-9, total (MMP-9, total)	ng/mL	0.26	0.34
Matrix metalloproteinase-10 (MMP-10)	ng/mL	0.0027	0.0054
Mesothelin (MSLN)	nmol/L	0.32	0.31
MHC class I chain–related protein A (MICA)	pg/mL	9.2	15
Monocyte chemotactic protein 1 (MCP-1)	pg/mL	4.6	9.0
Monocyte chemotactic protein 2 (MCP-2)	pg/mL	1.1	1.3
Monocyte chemotactic protein 3 (MCP-3)	pg/mL	0.45	1.9
Monocyte chemotactic protein 4 (MCP-4)	pg/mL	131	89
Monokine induced by IFN-γ (MIG)	pg/mL	10	22
Myeloid progenitor inhibitory factor 1 (MPIF-1)	ng/mL	0.0052	0.028
Myeloperoxidase (MPO)	ng/mL	3.2	3.7
Myoglobin	ng/mL	0.011	0.040
N-terminal prohormone of brain natriuretic peptide (NT proBNP)	pg/mL	11	15
Nerve growth factor β (NGF-β)	ng/mL	0.0099	0.016
Neuron-specific enolase (NSE)	ng/mL	0.030	0.035
Neuronal cell adhesion molecule (Nr-CAM)	ng/mL	0.044	0.040
Neuropilin-1	ng/mL	0.00077	0.0017
Neutrophil gelatinase-associated lipocalin (NGAL)	ng/mL	0.013	0.058
Osteopontin	ng/mL	0.30	0.62
Osteoprotegerin (OPG)	pmol/L	0.075	0.17
Pancreatic polypeptide (PPP)	pg/mL	0.11	0.96
Pepsinogen I (PGI)	ng/mL	0.029	0.029
Peptide YY (PYY)	pg/mL	31	26
Phosphoserine aminotransferase (PSAT)	ng/mL	0.067	0.12
Placenta growth factor (PLGF)	pg/mL	3.7	11
Plasminogen activator inhibitor 1 (PAI-1)	ng/mL	0.0079	0.016
Platelet-derived growth factor BB (PDGF-BB)	pg/mL	31	144

(Continued)

TABLE E1. *(Continued)*

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
Progesterone	ng/mL	0.45	0.65
Proinsulin, intact	pmol/L	1.4	0.73
Proinsulin, total	pmol/L	7.9	6.7
Prolactin (PRL)	ng/mL	0.013	0.017
Prostasin	ng/mL	0.28	0.33
Prostate-specific antigen, free (PSA-f)	ng/mL	0.00053	0.0027
Protein S100-A4 (S100-A4)	ng/mL	1.0	1.9
Pulmonary and activation-regulated chemokine (PARC)	ng/mL	1.2	1.2
Receptor for advanced glycosylation end products (RAGE)	ng/mL	0.034	0.069
Receptor tyrosine-protein kinase erbB-3 (ErbB3)	ng/mL	0.0086	0.0065
Resistin	ng/mL	0.0012	0.0052
S100 calcium-binding protein B (S100-B)	ng/mL	0.076	0.099
Serotransferrin (transferrin)	mg/dL	0.0000069	0.000047
Serum amyloid P-component (SAP)	μg/mL	0.000011	0.000014
Sex hormone-binding globulin (SHBG)	nmol/L	0.00038	0.0010
Sortilin	ng/mL	0.046	0.044
Squamous cell carcinoma antigen-1 (SCCA-1)	ng/mL	0.053	0.22
Stem cell factor (SCF)	pg/mL	18	23
Stromal cell–derived factor-1 (SDF-1)	pg/mL	8.7	13
Superoxide dismutase 1, soluble (SOD-1)	ng/mL	0.032	0.024
T cell–specific protein RANTES (RANTES)	ng/mL	0.00018	0.0010
T lymphocyte–secreted protein I-309 (I-309)	pg/mL	7.0	11
Tamm-Horsfall urinary glycoprotein (THP)	μg/mL	0.000045	0.00011
Tenascin-C (TN-C)	ng/mL	5.7	9.7
Testosterone, Total	ng/mL	0.061	0.10
Tetranectin	μg/mL	0.00093	0.0025
Thrombomodulin (TM)	ng/mL	0.017	0.049
Thrombospondin 1	ng/mL	0.12	0.35
Thyroglobulin (TG)	ng/mL	1.8	2.8
Thyroid-stimulating hormone (TSH)	uIU/mL	0.0036	0.0077
Thyroxine-binding globulin (TBG)	μg/mL	0.000019	0.000035
Tissue inhibitor of metalloproteinases 1 (TIMP-1)	ng/mL	0.010	0.016
Tissue-type plasminogen activator (tPA)	ng/mL	0.0075	0.089
TNF-related apoptosis-inducing ligand receptor 3 (TRAIL-R3)	ng/mL	0.034	0.19
TGF-α	pg/mL	4.6	17
TGF-β3	pg/mL	4.2	21
Transthyretin (TTR)	mg/dL	0.00000095	0.000004
Trefoil factor 3 (TFF3)	μg/mL	0.00005	0.00010
TNF-α	pg/mL	2.9	4.5
TNF-β	pg/mL	2.3	1.9
TNF receptor I (TNF RI)	pg/mL	5.9	7.6
TNF receptor 2 (TNFR2)	ng/mL	0.0011	0.0057
Tyrosine kinase with immunoglobulin and EGF homology domains 2 (TIE-2)	ng/mL	0.021	0.036
Urokinase-type plasminogen activator (uPA)	pg/mL	8.1	23
Urokinase-type plasminogen activator receptor (uPAR)	ng/mL	0.034	0.059
Vascular cell adhesion molecule 1 (VCAM-1)	ng/mL	0.0090	0.057
Vascular endothelial growth factor (VEGF)	pg/mL	12	6.8
Vascular endothelial growth factor B (VEGF-B)	ng/mL	2.7	2.3
Vascular endothelial growth factor C (VEGF-C)	ng/mL	0.19	1.1
Vascular endothelial growth factor D (VEGF-D)	pg/mL	100	100
Vascular endothelial growth factor receptor 1 (VEGFR-1)	pg/mL	29	91
Vascular endothelial growth factor receptor 2 (VEGFR-2)	ng/mL	0.0074	0.12
Vascular endothelial growth factor receptor 3 (VEGFR-3)	ng/mL	0.26	1.1
Vitamin D–binding protein (VDBP)	μg/mL	0.00002	0.000028
Vitamin K–dependent protein S (VKDPS)	μg/mL	0.000021	0.000022
Vitronectin	μg/mL	0.013	0.029
von Willebrand factor (vWF)	μg/mL	0.0016	0.0034
YKL-40	ng/mL	0.0040	0.0073

Least detectable dose *(LDD)* was determined as the mean + 3 SDs of 20 blank readings. Results less than the LDD will be more variable than results greater than the LDD. Lower limit of quantitation *(LLOQ)* was the lowest concentration of an analyte in a sample that can be reliably detected and at which the total error meets the laboratory's requirements for accuracy. Myriad RBM's requirement for accuracy is the concentration of an analyte at which the coefficient of variation of replicate standard samples is 30%.
MIP, Macrophage inflammatory protein; *ND*, not detected.

TABLE E2. Predominant cellular sources of biomolecules

Biomolecule	Cellular source	Biomolecule	Cellular source
Innate immune cell–derived biomolecules		Mixed cell–derived biomolecules	
1. C3	M, DC	1. IL-1β	Most cell types
2. CCL3 (MIP1β)	M	2. IL-6	M, DC, Ep
3. CXCL7 (NAP2)	P, M	3. IL-8	Ep, M, En, F, SM
4. Ficolin-3	M	4. Lipocalin 2	N, Ep
5. MMP9, total	N	5. MMP7	N, Ep
		6. PAI	M, Ma, Ep
Airway tissue–derived biomolecules		7. SAA	M, Ep
1. Thrombospondin 1	F		

DC, Dendritic cell; *En*, endothelial cell; *Ep*, epithelial cell; *F*, fibroblast; *M*, macrophage/monocyte; *Ma*, mast cell; *MIP*, macrophage inflammatory protein; *N*, neutrophil; *P*, platelet; *PAI*, plasminogen activator inhibitor; *SM*, smooth muscle.

TABLE E3. Correlation of serum biomolecule levels with inflammatory cell counts

Variables	Correlation with BAL fluid neutrophil counts *(r)*	*P* value	Correlation with BAL fluid eosinophil counts *(r)*	*P* value	Correlation with BAL fluid lymphocyte counts *(r)*	*P* value
Serum biomolecule levels associated with neutrophil counts						
1. GDF-15	0.44	.003	−0.1	NS	−0.18	NS
2. *Human Epididymis protein 4*	0.43	.004	0.29	.05	−0.05	NS
3. MMP7	0.35	.02	−0.06	NS	−0.25	NS
4. Tetranectin	−0.35	.02	0.02	NS	0.06	NS
5. von Willebrand factor	0.34	.02	0.08	NS	0.03	NS

NS, Not significant.

TABLE E4. Chest CT findings in infection-positive patients with RA

Identifier	Description of chest CT findings
001R	Bronchial wall thickening
003R	Bronchiolitis, consistent with aspiration, ground glass, consistent with asthma, consistent with reflux, esophageal thickening
004R	Bronchial wall thickening, consistent with asthma, bronchiectasis, infiltrates, hiatal hernia, esophageal thickening, consistent with reflux, air trapping
005R	Bronchial wall thickening, consistent with aspiration, consistent with asthma, tracheobronchomalacia, pulmonary hypertension, consistent with reflux, esophageal thickening, hiatal hernia
006R	Bronchial wall thickening, consistent with aspiration, consistent with asthma, dysmotility, consistent with reflux
008R	Tracheobronchomalacia, bronchial wall thickening, consistent with asthma, consistent with aspiration, dysmotility, consistent with reflux
011R	Bronchial wall thickening, bronchiectasis, air trapping, hiatal hernia, dysmotility
015R	Hiatal hernia, esophageal thickening, bronchial wall thickening, air trapping, consistent with reflux
012R	Bronchiectasis, bronchial wall thickening, consistent with asthma, scarring, hiatal hernia, dysmotility
020R	Bronchial wall thickening, ground glass, esophageal thickening, hiatal hernia, dysmotility, consistent with reflux
021R	Bronchial wall thickening, consistent with asthma, consistent with aspiration, hiatal hernia, consistent with reflux, esophageal thickening
030R	Bronchial wall thickening, hiatal hernia, esophageal thickening
033R	Hiatal hernia, bronchiectasis, consistent with aspiration, bronchial wall thickening, air trapping
035R	Bronchial wall thickening, consistent with asthma, esophageal thickening, dysmotility, consistent with reflux, air trapping, tracheobronchomalacia
018R	Air trapping, bronchial wall thickening, consolidation, hiatal hernia, esophageal thickening, bronchiectasis, consistent with reflux
047R	Tracheobronchomalacia, consistent with reflux, consistent with aspiration, consistent with asthma, bronchial wall thickening, air trapping, esophageal thickening
048R	Bronchial wall thickening, atelectasis
049R	Hiatal hernia, bronchial wall thickening, scarring, granuloma, air trapping, tracheobronchomalacia
053R	Bronchial wall thickening, atelectasis, air trapping, consistent with asthma
055R	Bronchial wall thickening, scarring, ground glass, consistent with reflux, hiatal hernia, consistent with asthma, consistent with aspiration
061R	Bronchial wall thickening, atelectasis, scarring, air trapping, consistent with asthma
062R	Esophageal thickening, bronchial wall thickening, atelectasis, scarring, air trapping, consistent with reflux, dysmotility, consistent with aspiration
063R	Esophageal thickening, bronchial wall thickening, air trapping, consistent with asthma
068R	Bronchial wall thickening, atelectasis, scarring, air trapping, tracheobronchomalacia, dysmotility

TABLE E5. List of medications

Patient ID	Asthma medications (ICS, LABA, oral steroid and SABA)	Oral steroid	Medication by pharmacologic class	Others
002NRA	Advair 250/50 bid, Xopenex prn	No		
003NRA	Symbicort 160/4.5 2 puffs bid, albuterol prn	No		
004NRA	Proventil prn, Advair HFA 230/12 bid	No		
005NRA	Alvesco 80 μg qd, Albuterol prn	No		
007NRA	Alvesco 80 μg 2 puffs qd, Albuterol MDI prn	No		
008NRA	Xopenex prn, Advair 100/50	No		
009NRA	Advair 250/50 bid	No		
010NRA	Flovent 220 bid, albuterol prn	No		
011NRA	Dulera 100/5 bid, albuterol prn	No		
012NRA	QVAR 80 bid, albuterol prn	No		
013NRA	Flovent 44 2 puffs bid	No		
014NRA	Alvesco 160; Xopenex prn	No		
015NRA	QVAR 80 2 puffs/d	No		
016NRA	QVAR 80 2 puffs/bid; albuterol prn	No		
017NRA	Flovent 220 bid	No		
018NRA	Flovent 220 bid, albuterol prn	No		
022NRA	Dulera 100/5 bid	No		
024NRA	QVAR 2 puffs bid	No		
026NRA	Advair 100/50 bid	No		
027NRA	Alvesco 160 2 puffs/d; Xopenex prn	No		
028NRA	Flovent 110 2 puffs/d, albuterol prn	No		
029NRA	Advair 250/50 bid; ProAir prn	No		
030NRA	Advair 250/50 bid; albuterol once/wk	No		
036NRA	QVAR 80 2 puffs/d; albuterol prn	No		
039NRA	Pulmicort 180 μg/d, ProAir prn	No		
040NRA	Dulera 100/50 2 puffs bid; Ventolin prn	No		
042NRA	QVAR 80 2 puffs/d; Proventil prn	No		
044NRA	Advair 100/50 bid	No		
046NRA	Advair 100/50, Proventil prn	No		
049NRA	QVAR 80 bid	No		
001R	Advair 500/50 bid, monthly prednisone tapers, QVAR 160 μg bid	Yes		
002R	Spiriva 18 μg/d, Symbicort 160/4.5 2× bid, albuterol prn, Singulair 10 mg/d, QVAR 80 μg 4× bid	No	LAMA, ICS, LTRA	
003R	Dulera 200/5.0 2× bid, fluticasone 220 2× bid, Xopenex prn	No	ICS	
004R	Prednisone 40 mg/d; Symbicort 160 2× bid; Combivent 2 puffs prn; DuoNeb prn	Yes	LAMA	
005R	Advair HFA 230/21 2 puffs bid; albuterol prn, prednisone, Singulair 10 mg qd, Proventil	Yes	LTRA	
006R	Dulera 200 two puffs bid; albuterol neb prn, Acapella device	No		
007R	40 mg prednisone daily, Advair 500/50 bid, Singulair 10 mg/d	Yes	LTRA	
008R	Symbicort 160/4.5 2 puffs bid; QVAR 4 puffs bid; Proventil prn	No	ICS	
009R	Advair 500/50 bid, QVAR 80 2× bid, Singulair 10 mg	No	ICS, LTRA	
010R	Advair 250/50 bid; Singulair 10 mg/d; ProAir prn	No	LTRA	
011R	Advair 250/50 bid, Ventolin 2× bid	No		
012R	Advair 500/50 bid, Singulair 10 mg qd, Spiriva once daily, Xopenex neb prn	No	LAMA, LTRA	
013R	Advair 500/50 bid, Flovent 110 twice bid, Singulair 10 mg/d, Ventolin prn	No	ICS, LTRA	
015R	Advair HFA 230/21 2 puffs bid, prednisone 60 mg qd, Ventolin prn, Combivent 18-103 prn	Yes	LAMA	
016R	Xolair, Symbicort 160/4.5 two puffs bid, Spiriva qd, theophylline 900 mg qd, albuterol neb prn	No	LAMA, theophylline, Xolair	
017R	Symbicort 160/4.5 2 puffs bid; Alvesco 160 2 puffs bid; Xopenex prn	No	ICS	
018R	Zyflo CR 1200 mg bid; Advair HFA 230/21 two puffs bid	No	Zileuton	
020R	Prednisone 60 mg qd; Singulair 10mg qd; Advair 500/50 bid	Yes	LTRA	
021R	Albuterol HFA prn; Asmanex 220 μg bid; prednisone 15 mg qd; Serevent bid; Zyflo 600 mg qd	Yes	Zileuton	
022R	Dulera 200/5.0 2 puffs bid, Pulmicort 180 μg 2 puffs bid, Singulair 10 mg qd, Ventolin prn	No	ICS, LTRA	
023R	Advair 500/50 bid, Ventolin 2 puffs every 4 h, DuoNeb prn	No		
024R	Theophylline 300 mg 3×/d; albuterol neb prn; Ventolin HFA prn; Symbicort 160-4.5 μg 2 puffs bid; Spiriva HandiHaler 18 μg qd; Alvesco 160 μg 2 puffs bid; Accolate 20 mg bid; prednisone 2 mg/d	Yes	LAMA, LTRA, Theophylline	
025R	Advair 500/50 bid, ProAir HFA prn	No		
026R	Singulair 10 mg, Daliresp 500 mg, Advair 500/50 bid; Alvesco 160 bid; prednisone taper prn on monthly basis, Proventil MDI prn; albuterol neb prn; Xolair	Yes	LTRA, roflumilast, Xolair	
028R	Prednisone 20 mg bid; Symbicort 160/4.5 two puffs bid and QVAR 80 μg 2-4 puffs BID; albuterol HFA or neb PRN	Yes		

(Continued)

TABLE E5. *(Continued)*

Patient ID	Asthma medications (ICS, LABA, oral steroid and SABA)	Oral steroid	Medication by pharmacologic class	Others
029R	20 mg prednisone; theophylline 300 mg 3×/d; Spiriva neb prn every 2-4 h; albuterol every 2-4 h; Zyflo 600 mg bid; Advair 230/21 two puffs bid; Xolair 150 mg every 2 mo	Yes	LAMA, LTRA, theophylline, Xolair, zileuton	
030R	ProAir HFA; prednisone taper ongoing; Dulera 200-5 2 puffs bid	Yes		
031R	30 mg Prednisone; albuterol/ipratropium neb bid; Dulera 200/5 two puffs bid; Albuterol prn; Xolair	Yes	LAMA, Xolair	
032R	Advair 230/21 two puffs BID	No		
033R	Symbicort 160/4.5 two puffs BID; DuoNeb	No		
034R	Advair 500/50 BID; ProAir 2 puffs prn; QVAR 80 three puffs bid	No	ICS	
035R	Spiriva 18 μg/d; Flovent HFA 220 μg 2 puffs bid; Ventolin and Xopenex prn	No	LAMA	
036R	Advair 500/50 with QVAR 80 two puffs bid	No		
037R	10 mg Prednisone/d; Advair 500/50 bid; Pro-Air prn	Yes		
038R	Albuterol HFA prn; Proventil HFA 108 prn; Pulmicort 180 2 puffs bid; QVAR 2 puffs bid	No	ICS	
039R	Symbicort 160/4.5 two puffs bid; QVAR 2 puffs bid; ProAir and albuterol neb prn; Zyrtec 10 mg	No	ICS	
041R	Albuterol neb prn; Albuterol HFA prn; Dulera AERO 2 puffs bid; Prednisone 10 mg every other day	Yes		
042R	5 mg/d Prednisone, Advair 500/50 bid; Spiriva 18 μg/d; albuterol neb daily prn	Yes	LAMA	
043R	Symbicort 160 two puffs bid; Flovent 110 two puffs once/day; Xopenex neb BID; Spiriva qd	No	ICS, LAMA	
046R	Symbicort 160/4.5 two puffs bid; prednisone 20 mg every other day	Yes		
047R	Ipratropium-albuterol for neb prn; Advair HFA AERO	No	LAMA	
048R	Albuterol prn; Dulera 200-5 two puffs bid	No		
049R	Advair 500/50 bid; Prednisone 10 mg 3×/wk; albuterol prn; Xolair; ProAir HFA 2 puffs every 4-6 h	Yes	Xolair	
050R	Dulera 100/5 two puffs bid; QVAR 80 two puffs bid; Zyflo 1200 mg bid; prednisone 20 mg qd; nasal saline washes	Yes		
051R	Advair 500/50 BID	No		
052R	Dulera 200/5 2 puffs bid; Ventolin prn; Zyrtec 10-20 mg; Nasonex prn; Albuterol neb prn	No		
053R	Advair HFA 230 two puffs bid; fluticasone nasal spray, theophylline 300 bid, albuterol prn	No		
054R	Symbicort 160/4.5 two puffs bid; QVAR 80 μg 3 puffs bid; montelukast 10 mg; Allegra 180 mg; Fluticasone 50 μg; Ventolin prn; NSW	No		
055R	Albuterol nebs/MDI prn; Brovana 15 μg neb bid; budesonide 1000 mg neb bid; Pulmicort; Omeprazole 40 mg bid; ranitidine	No		
056R	Albuterol neb tid; Symbicort 160/4.5 two puffs bid, Singulair 10 mg; saline nasal rinse with budesonide 0.5 mg twice a day	No		
057R	Albuterol nebulizer treatment every 4 h; Dulera 200/5 two puffs bid; Asmanex to 2 puffs bid, ProAir prn; Spiriva daily; Flonase; Singulair	No		
058R	Dulera 200-5MCG/ACT 2 puffs bis; ProAir inhaler prn; Singulair 10 mg; Zyrtec; Zetonna; QVAR 2 puffs bid, Nexium 40 mg	No	Xolair, Zetonna	
059R	Prednisone 10 mg/d, ProAir HFA prn; Dulera 200/5 2p bid; DuoNeb prn	Yes		
060R	Xopenex and Atrovent nebulizers prn; Symbicort 160 two puffs bid	No		
061R	Singulair; albuterol nebulizer prn; Advair 500/50 bid, Cetirizine prn, Flonase	No		
062R	Advair 500 μg bid, albuterol MDI/neb	No		
063R	Alvesco, methylprednisolone 4 mg qd, Prilosec, Qnasl, Spiriva, Symbicort 160, Theophylline 300 mg bid, Ventolin prn, Zyrtec, Nasacort, Patanase nasal solution	Yes	Theophylline	
066R	Pulmicort 180 μg 2 puffs bid, Symbicort 160/4.5 two puffs bid, theophylline, DuoNeb	No	Theophylline	
067R	QVAR 80 μg 2 puffs twice daily; Symbicort 160/4.5 two puffs twice daily; albuterol nebs prn; Singulair 10 mg/d	No	Flonase prn; NSW prn	Lipitor, Lisinopril
068R	Albuterol inhaler/neb prn, Symbicort 160 two puffs bid, QVAR 80 two puffs bid, Zyrtec	Yes		

bid, Twice daily; *HFA*, hydrofluoroalkane; *LABA*, long-acting β-agonist; *LAMA*, long-acting muscarinic antagonist; *LTRA*, leukotriene receptor antagonist; *MDI*, metered-dose inhaler; *prn*, as necessary; *qd*, one daily; *tid*, 3 times daily.

TABLE E6. Correlations among clinical and laboratory variables

Variables	Correlation FEV_1 (% *[r]*)	*P* value	Correlation with FVC (% *[r]*)	*P* value	Correlation with reversibility *(r)*	*P* value
Inflammatory cells						
PMN, BAL fluid	−0.16	NS	−0.32	.01	0.15	NS
Eosinophils, BAL fluid	−0.23	NS	−0.20	NS	0.15	NS
Lymphocytes, BAL fluid	0.03	NS	0.05	NS	−0.15	NS
Eosinophils, blood	0.02	NS	0.04	NS	0.06	NS
BAL biomolecules						
Myoglobin	−0.24	.05	−0.27	.03	0.12	NS
Prostasin	0.27	.03	0.15	NS	−0.27	.04

TABLE E7. GO (biological process) pathway analysis

Annotation (pathway/process)	XD score	Fisher *q* value	Gene set size	Pathway size	Overlap size
GO:0090023~positive regulation of neutrophil chemotaxis	0.5976409	0.58840	1	15	1
GO:0044344~cellular response to fibroblast growth factor stimulus	0.5976409	0.58840	1	15	1
GO:0050930~induction of positive chemotaxis	0.5270527	0.58840	1	17	1
GO:0048566~embryonic digestive tract development	0.4476409	0.58840	1	20	1
GO:0031623~receptor internalization	0.3190695	0.58840	1	28	1
GO:0071347~cellular response to IL-1	0.2976409	0.58840	1	30	1