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Biomarker Profiles in Asthma With High vs Low Airway Reversibility and Poor Disease Control

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BACKGROUND: High bronchodilator reversibility in adult asthma is associated with distinct clinical characteristics. This analysis compares lung function, biomarker profiles, and disease control in patients with high reversibility (HR) and low reversibility (LR) asthma.

METHODS: A retrospective analysis was performed with data from two completed clinical trials of similar design. Patients were divided into HR and LR subgroups based on their response to bronchodilators (HR = ΔFEV_1 postbronchodilator $\geq$ 20%). Blood eosinophil count, serum IgE level, and fraction of exhaled nitric oxide concentration, biomarkers commonly used to stratify patients into T-helper (Th)-2-high vs Th2-low phenotypes, were measured in patients with not well controlled (1.5 $\leq$ Asthma Control Questionnaire [ACQ] $\leq$ 2.143) and very poorly controlled (ACQ $>$ 2.143) disease.

RESULTS: The majority of patients in the HR and LR subgroups displayed Th2-low biomarker profiles and very poor disease control. HR was more frequently associated with Th2-high biomarker profiles (40.1% vs 29.4%, $P = .006$), lower lung function (FEV_1, 63.5 $\pm$ 7.7% predicted vs 67.9 $\pm$ 8.4% predicted; $P < .001$), and atopy (93.7% vs 86.5%, $P = .005$).

CONCLUSIONS: HR is a physiologic indicator of reduced lung function and is more often associated with elevations in Th2 biomarkers than LR in moderate to severe asthma. However, the majority of patients with HR and LR asthma in this analysis had a Th2-low biomarker profile. Moreover, a Th2-high biomarker profile was not associated with worse disease control.

CHEST 2015; 148(6):1489-1496

Manuscript received October 3, 2014; revision accepted July 1, 2015; originally published Online First July 30, 2015.

ABBREVIATIONS: ACQ = Asthma Control Questionnaire; CART = classification and regression tree; FENO = fraction of exhaled nitric oxide; GINA = Global Initiative for Asthma; HR = high reversibility; IPI = Immune Profile Index; LR = low reversibility; NWC = not well controlled; PPV = positive predictive value; SARP = Severe Asthma Research Program; Th = T-helper; VPC = very poorly controlled

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FUNDING/SUPPORT: This study was supported by Amgen Inc, Thousand Oaks, CA.

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DOI: 10.1378/chest.14-2457

Data from large patient registries have identified clusters of patients with asthma and severe airflow limitation and disease that is difficult to control with conventional therapy.[1-4] Characteristics differentiating these patients from the general asthma population include longer duration of disease, high reversibility (HR) of the airway following bronchodilator administration, sputum neutrophilia, increased oral corticosteroid use, and increased health-care resource utilization.[1,4] Indeed, HR is a distinctive physiologic characteristic of cluster 4 and 5 patients in the Severe Asthma Research Program (SARP), the two cohorts displaying the worst disease control and highest frequency of clinic and ED visits and hospitalizations. Serum and BAL fluid biomarkers have been analyzed to characterize the immune processes modulating disease severity.[2,5] However, limited information exists regarding how serum biomarkers define endotypes across the asthma population, correlate with disease control, and relate to distinctive physiologic features such as HR. The present study uses combined baseline datasets from two randomized controlled trials involving patients with moderate to severe asthma to examine the relationship between immune pathway biomarkers and disease control in those with HR and low reversibility (LR).

Materials and Methods

A retrospective exploratory analysis was performed using data from two phase 2 clinical trials involving patients with partly or poorly controlled moderate to severe asthma (GINA [Global Initiative for Asthma] steps 3 and 4). The first trial was initiated on December 21, 2009, to assess the efficacy of a dual inhibitor of the prostaglandin D2 and chemoattractant receptor homologous molecule expressed on T-helper (Th) 2 cells.[6] Full baseline datasets were available for 358 of 396 enrolled patients. The second trial was initiated October 4, 2010, to assess the efficacy of a monoclonal antibody against the IL-17 receptor A subunit in a similar cohort.[7] Full baseline datasets were available for 272 of 302 enrolled patients. Although both trials were interventional, treatment responses were not considered in this analysis. Data collected beyond the baseline time point were used solely to assess stability of phenotypic characteristics in patients who did not receive experimental therapy.

Inclusion and exclusion criteria for the two trials were identical: (1) age 18 to 65 years, (2) disease requiring inhaled corticosteroids with and without a long-acting β-agonist, (3) baseline Asthma Control Questionnaire (ACQ) score $\geq$ 1.5, (4) FEV_1 % predicted between 50% and 80%, (5) reversibility in $FEV_1 \geq 12\%$ (and at least 200 mL) following administration of a short-acting β-agonist, (6) no evidence of active infection, and (7) no medical conditions associated with immune suppression. Patients were excluded from study participation if they had COPD or asthma-COPD overlap syndrome,[8] were receiving maintenance oral corticosteroids or IgE antibody therapy, were current or recent smokers, had a history of intubation within 3 years of enrollment, or had OSA.

Subjects were classified into (1) an HR group of those with high airway reversibility defined as a $\geq$ 20% increase in FEV_1 following administration of a short-acting bronchodilator during screening and baseline pulmonary function testing and (2) an LR group of those with reversibility below this level.[1,4] Subgroups were further divided into subjects with not well controlled (NWC) (ACQ score $\geq$ 1.5 and $\leq$ 2.143) or very poorly controlled (VPC) (ACQ $>$ 2.143) disease. The ACQ cut point defining VPC disease was derived from a linear extrapolation of ACQ scores of well controlled (five total ACQ points corresponding to an ACQ cut point of 0.75) and NWC (10 total ACQ points corresponding to an ACQ cut point of 1.5) disease.[9] VPC disease was defined as corresponding to an additional five-point increment in total ACQ score beyond the NWC cut point (ie, ACQ $>$ 2.143). Disease control was summarized in terms of both ACQ6 and ACQ7 values. Biomarkers reflecting Th2 immune activation were assessed in each patient and included serum IgE level, circulating eosinophil count, and fraction of exhaled nitric oxide (Feno) concentration. For each biomarker, cut points used to define a high level were as follows: IgE $\geq$ 100 IU/mL, eosinophil count $\geq$ 300/μL, and Feno $\geq$ 30 parts per billion.[10,11] Patients were classified as having either a positive or a negative Th2 Immune Profile Index (IPI) (positive IPI indicates elevation in two or more Th2 biomarkers; negative IPI indicates elevation in one or no Th2 biomarkers).

Results are reported using descriptive statistics as mean $\pm$ SD, median, or quartiles as appropriate. Comparisons of baseline data between the two trials[6,7] and between the HR and LR subgroups were performed by either Student *t* or Wilcoxon rank sum test. Correlations between baseline and week 12 biomarker assessments were performed using the Pearson product-moment correlation method. Assessments of the significance of relationships between Th2 biomarker elevations and disease control were performed by χ^2 test (applying Yates correction as appropriate). A classification and regression tree (CART) analysis was included in the sensitivity analysis of cut points defining Th2 biomarker status. Statistically significant differences between groups were defined by $P < .05$.

Results

Demographics, biometrics, medical history, pulmonary function values, and medication use for all patients (N = 698) in both studies are summarized in Table 1. Patients studied in the first clinical trial[6] were more likely to be atopic (93% vs 83%, $P < .001$) and had a higher FEV_1 % predicted (66.9% $\pm$ 8.24% vs 65.2% $\pm$ 8.53%, $P = .0081$) than those in the second trial.[7] However, these differences were small and not clinically significant or substantial enough to affect interpretation of results when considered as a single combined cohort.

Two hundred thirty-seven patients (38%) in the combined study cohort met the HR criterion. Relative to those with LR (n = 385), patients with HR asthma tended to be younger; were more often atopic; and had a significantly lower BMI, lower baseline pulmonary function, higher mean Feno and IgE values, and higher ACQ7 scores (Table 2). One hundred thirty-six patients (63.6%) with HR asthma met the criterion for VPC

TABLE 1] Comparison of Patients in the Clinical Trials Used to Define HR and LR Subgroups for the Present Analysis

Variable	Study NCT01018550[6] (N = 396)	Study NCT01199289[7] (N = 302)	*P* Value[a]
Baseline ACQ7 score	2.5 ± 0.60	2.5 ± 0.65	.4552
Baseline ACQ6 score	2.3 ± 0.67	2.3 ± 0.74	.8223
Age, y	44.8 ± 11.37	45.7 ± 11.40	.3088
BMI, kg/m^2	31.2 ± 7.08	30.2 ± 7.12	.0917
Baseline eosinophil count, cells/μL	199.5 (122, 292)	191.5 (130.5, 294.5)	.8815
Baseline FENO, parts/billion	23.5 (15.0, 36.3)	23.5 (14.8, 35.8)	.6111
Baseline FEV_1, L	2.22 ± 0.54	2.16 ± 0.57	.1945
Baseline FEV_1 % predicted	66.9 ± 8.24	65.2 ± 8.53	.0081
Baseline IgE, IU/mL	176.2 (58.6, 423.3)	132.2 (51.7, 319.7)	.0572
Airway reversibility, % change	17.9 ± 12.13	19.4 ± 13.29	.1168
Female sex	60	59	.8777
Positive atopy status	93	83	< .0001
Elevated eosinophil count	24	24	.9746
High-dose ICS	47	53	.1312
High-dose ICS + LABA	40	41	.6811

Data are presented as mean ± SD, median (quartile 1, quartile 2), or %. ACQ = Asthma Control Questionnaire; FENO = fraction of exhaled nitric oxide; HR = high reversibility; ICS = inhaled corticosteroid; LABA = long-acting β-agonist; LR = low reversibility.
[a]*P* value from two-sample *t* test for means and from Wilcoxon rank sum test for medians.

disease defined as ACQ6 > 2.143. Compared with patients with HR and NWC asthma (ACQ, 1.5-2.143), the subgroup with VPC asthma tended to be older (44.1 ± 11.0 years vs 42.0 ± 12.3 years, $P = .184$), had lower baseline FEV_1 values (2.06 ± 0.51 L vs 2.19 ± 0.59 L, $P = .1038$), and had a lower FEV_1 % predicted (62.8% ± 7.50% vs 64.9% ± 8.04%, $P = .062$) (Table 3). Patients with HR and NWC disease did not differ from those reporting VPC disease with respect to sex distribution, BMI, medication use, or degree of airway reversibility. A parallel analysis using ACQ7 as the measure of disease control applying the same cut point showed similar findings. Patients with LR and NWC disease did not differ significantly from those with VPC disease with respect to demographics, baseline biomarker profiles, lung function, bronchodilator reversibility, or medication use (Table 4).

TABLE 2] Comparison of HR (n = 237; $\Delta FEV_1 \geq 20\%$) vs LR (n = 385; $\Delta FEV_1 < 20\%$) Subgroups From the Clinical Trials[6,7] Used in the Present Analysis

Variable	HR (n = 237)	LR (n = 385)	*P* Value[a]
Baseline ACQ7 score	2.6 ± 0.62	2.4 ± 0.63	.0247
Baseline ACQ6 score	2.3 ± 0.70	2.2 ± 0.71	.2799
Age, y	43.1 ± 11.63	46.4 ± 11.14	.0004
BMI, kg/m^2	29.9 ± 6.71	31.3 ± 7.03	.0154
Baseline eosinophil count, cells/μL	214.0 (136.0, 303.0)	191.0 (126.0, 288.0)	.173
Baseline FENO, parts/billion	25.7 (16.0, 40.0)	22.0 (14.3, 31.7)	.004
Baseline FEV_1, L	2.1 ± 0.54	2.2 ± 0.56	.006
Baseline FEV_1 % predicted	63.5 ± 7.72	67.9 ± 8.35	< .0001
Baseline IgE, IU/mL	192.9 (71.7, 400.3)	127.5 (50.3, 357.6)	.0111
Airway reversibility, %Δ	31.2 ± 11.39	11.2 ± 5.76	< .0001
Female sex	149 (62.9)	223 (57.9)	.2217
Positive atopy status	222 (93.7)	333 (86.5)	.0050

Data are presented as mean ± SD, median (quartile 1, quartile 2), or No. (%). See Table 1 legend for expansion of abbreviations.
[a]*P* value from two-sample *t* test for means and from Wilcoxon rank sum test for medians.

TABLE 3] Comparison of Patients With HR Based on Disease Control Assessed Through ACQ6

Variable	Very Poorly Controlled (n = 136)	Not Well Controlled (n = 78)	*P* Value[a]
Baseline ACQ7 score	3.0 ± 0.45	2.1 ± 0.18	< .0001
Baseline ACQ6 score	2.8 ± 0.51	1.8 ± 0.17	< .0001
Age, y	44.1 ± 11.04	42.0 ± 12.28	.1841
BMI, kg/m^2	30.0 ± 6.44	29.6 ± 6.73	.6767
Disease duration at study enrollment, y	25.3 ± 13.44	23.4 ± 12.66	.3081
Baseline eosinophil count, cells/μL	219.5 (146.5, 311.0)	188.0 (129.0, 298.0)	.2486
Baseline FENO, parts/billion	25.3 (15.5, 40.1)	26.0 (15.0, 42.0)	.7655
Baseline FEV_1, L	2.06 ± 0.51	2.19 ± 0.59	.1038
Baseline FEV_1 % predicted	62.8 ± 7.50	64.9 ± 8.04	.0620
Baseline IgE, IU/mL	197.7 (61.1, 396.2)	192.8 (91.6, 430.7)	.4567
Age at disease onset, y	18.9 ± 16.79	18.6 ± 16.35	.9000
Airway reversibility, %Δ	31.8 ± 12.39	30.3 ± 10.06	.3663
Female sex	67	60	.2443
Positive atopy status	91	99	.0262
High-dose ICS	48	49	.8271
ICS + LABA	39	39	.9989

Data are presented as mean ± SD, median (quartile 1, quartile 2), or %. See Table 1 legend for expansion of abbreviations.
[a]*P* value from two-sample *t* test for means and from Wilcoxon rank sum test for medians.

To assess the consistency of Th2 biomarker values as indicators of inflammatory status in this cohort, we compared blood eosinophil counts, serum IgE levels, and FENO concentrations at baseline with those at week 12 in subjects assigned to placebo treatment. The results are summarized in Figures 1A-C and show significant correlations between baseline and week 12 for all three Th2 biomarkers. We also examined within-patient temporal consistency of bronchodilator reversibility measurements and ACQ assessments using a similar

TABLE 4] Comparison of Patients With LR Based on Disease Control Assessed Through ACQ6

Variable	Very Poorly Controlled (n = 202)	Not Well Controlled (n = 149)	*P* Value[a]
Baseline ACQ7 score	2.9 ± 0.52	2.0 ± 0.19	< .0001
Baseline ACQ6 score	2.8 ± 0.57	1.8 ± 0.17	< .0001
Age, y	47.3 ± 11.19	45.8 ± 10.97	.2110
BMI, kg/m^2	32.0 ± 7.48	30.7 ± 6.46	.1092
Disease duration at study enrollment	26.6 ± 14.30	27.9 ± 14.72	.4023
Baseline eosinophil count, cells/μL	188.0 (123.0, 288.0)	205.0 (140.0, 290.0)	.2499
Baseline FENO, parts/billion	21.3 (14.0, 30.5)	24.5 (14.7, 34.3)	.1074
Baseline FEV_1, L	2.17 ± 0.58	2.33 ± 0.54	.0087
Baseline FEV_1 % predicted	67.4 ± 8.58	69.0 ± 8.05	.0805
Baseline IgE, International Units/mL	112.9 (40.7, 311.0)	143.2 (64.7, 356.3)	.0808
Age at disease onset, y	20.7 ± 16.25	18.0 ± 15.01	.1021
Airway reversibility, %Δ	11.2 ± 5.66	11.0 ± 6.09	.7280
Female sex	62	54	.1574
Positive atopy status	87	88	.7217
High-dose ICS	53	48	.4141
ICS + LABA	43	40	.5720

Data are presented as mean ± SD, median (quartile 1, quartile 2), or %. See Table 1 legend for expansion of abbreviations.
[a]*P* value from two-sample *t* test for means and from Wilcoxon rank sum test for medians.

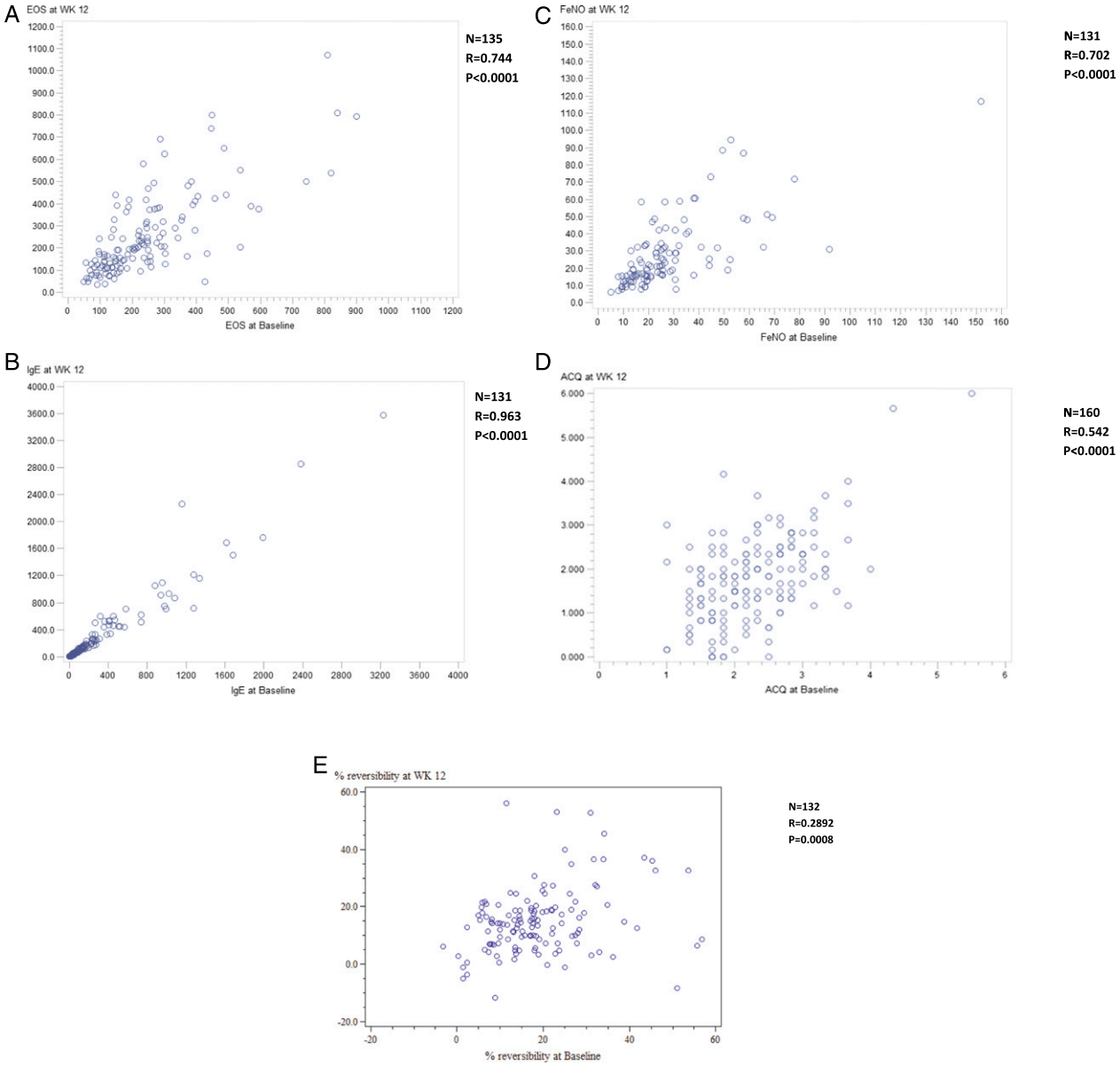

Figure 1 – *A-E, Correlations between baseline and wk 12 blood eosinophil counts (cells/μL³) (A), serum IgE levels (IU/mL) (B), FeNO concentrations (parts per billion) (C), disease control assessed in terms of ACQ6 (U) (D), and bronchodilator reversibility (E) are summarized for patients who did not receive active therapy in the clinical trials studied.*[6,7] *The results confirm the stability of these measures out to 12 wk. ACQ = Asthma Control Questionnaire; EOS = eosinophil count; FeNO = fraction of expired nitric oxide.*

approach. The results summarized in Figures 1D and 1E confirm that these were also quite stable over the 12-week assessment interval.

We next examined whether within-patient changes from baseline to week 12 in individual biomarkers correlated among one another or with changes in either bronchodilator reversibility or ACQ over this same interval. No correlations of significance were found between baseline to week 12 changes among Th2 biomarkers or between biomarker changes and changes in bronchodilator reversibility or ACQ.

The relationships between biomarkers and disease control in the HR and LR subgroups are summarized in Figure 2. Among patients with HR asthma, an elevated blood eosinophil count was somewhat predictive (positive predictive value [PPV], 62%; $P = .368$ by χ^2 test) of VPC disease. However, circulating eosinophil count was not a sensitive indicator (0.279) of disease control status because 98 of the 136 patients with VPC disease and HR had low eosinophil counts. FeNO and serum IgE levels were also not associated with disease control in patients with HR. An elevated serum IgE level had moderate PPV (0.548) for identifying patients with VPC

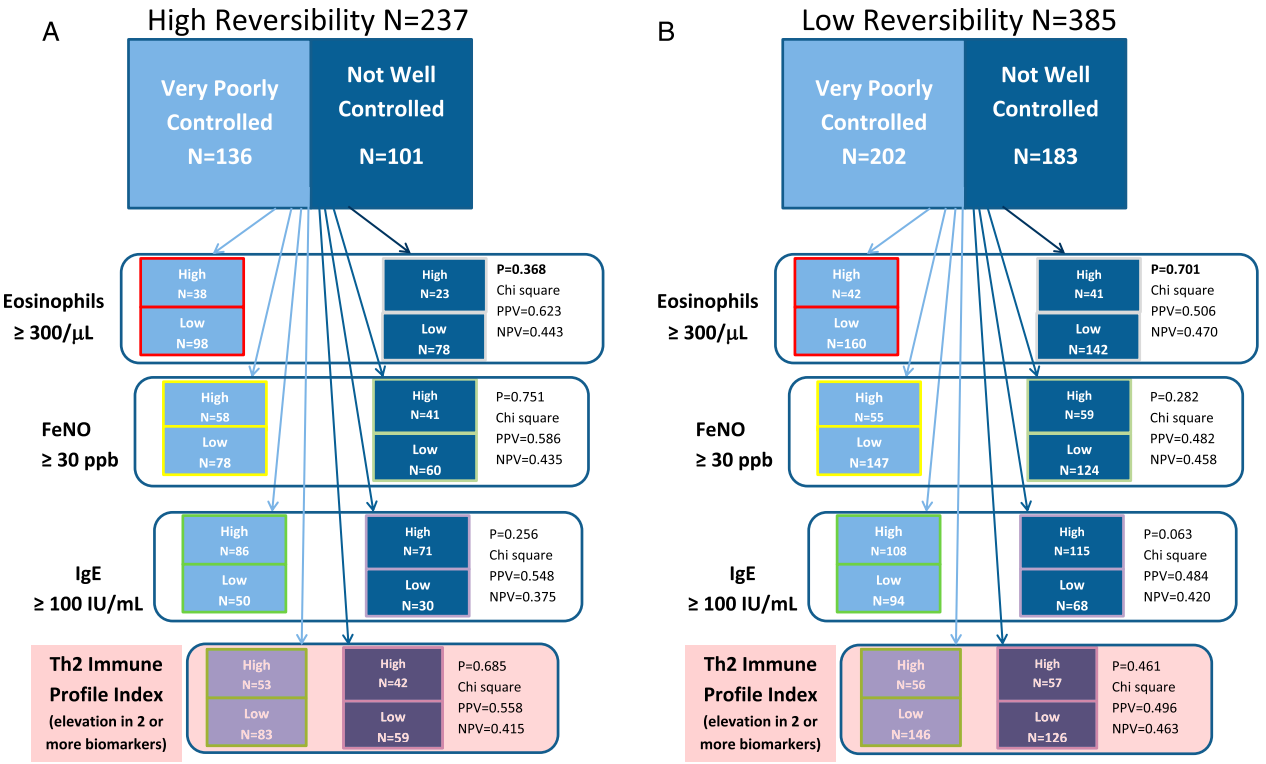

Figure 2 – *A, Patients with very poorly controlled and not well controlled disease with high reversibility assessed via ACQ6. B, Patients with very poorly controlled and not well controlled disease with low reversibility assessed via ACQ6. Patients with high reversibility (A) and low reversibility (B) phenotypes are partitioned into those with poor and adequate disease control and then further subdivided based on individual Th2 biomarkers and the aggregate Th2 Immune Profile Index. The results of χ^2 analysis assessing the statistical significance of the association of each biomarker with disease control status and PPV and NPV of each biomarker for predicting disease control status are shown. NPV = negative predictive value; PPV = positive predictive value; Th2 = T-helper 2. See Figure 1 legend for expansion of other abbreviations.*

disease but lacked specificity (0.375). Likewise, an elevated FeNO had a moderate PPV of VPC (0.586) but was neither sensitive (0.481) nor specific (0.435) as an indicator of level of disease control. The aggregate Th2 IPI also failed to reflect disease control status in the HR subgroup. A positive profile was indeed associated with poor disease control, but the majority of patients with VPC disease did not display a Th2-high profile.

Similar poor associations between biomarker status (high vs low) and extent of disease control were observed in the LR subgroup (Fig 2B, Table 4). Th2 biomarkers alone and in aggregate when expressed as Th2 IPI failed to accurately reflect the level of disease control. This finding indicates that Th2 biomarkers in both the HR and the LR subgroups are not reliable indicators of disease control.

To assess whether application of various biomarker cut points might have rendered different findings, we performed a CART analysis. The CART analysis aimed to maximize differences between subgroups by exploring the continuous range of values for each Th2 biomarker to identify optimal cut point values to differentiate patients with NWC from those with VPC disease. CART results were unrevealing, however, because no cut point strategy, including > 25% of either the HR or the LR population, clearly separated NWC from VPC patients.

Discussion

To better understand endotype diversity in patients with asthma and identify profiles to guide therapy, patients have been grouped, or clustered, based on clinical data and biomarkers. Using this approach, SARP investigators identified subgroups differing with respect to age of disease onset, sex distribution, biometrics, health-care resource utilization, airway hyperresponsiveness, and bronchodilator reversibility.[1,2] High airway bronchodilator reversibility has emerged as a reliable physiologic biomarker associated with severe airflow obstruction, increased health-care resource utilization, and poor disease control. By contrast, classic serum and blood biomarkers have not consistently correlated with disease activity.[2,5] Indeed, several studies paradoxically suggest that higher levels of some cytokines may be associated with milder phenotypes.[5]

The present study attempts to better characterize this HR phenotype by comparing biomarker profiles and disease control status in patients with HR and LR asthma. The present analysis focused on three biomarkers (blood eosinophil counts, serum IgE levels, and FENO concentrations) and used ACQ to assess disease control status. Results presented in Figure 1 show that these three biomarkers are stable indicators of immune status. Patients not receiving active therapy in either clinical trial[6,7] had 12-week biomarker values that highly correlated with baseline values. Although some variability was observed, 49% to 93% of the week 12 signal, as assessed in terms of r^2 value, was accounted for by the baseline value, with FENO concentration showing the poorest correlation over time. A similar analysis involving ACQ and bronchodilator reversibility indicated that within-subject measures of disease control and airway reversibility also remained relatively stable over this time frame.

Although baseline Th2 biomarker levels appear to be stable indicators of inflammation in a given patient, they were not useful indicators of HR vs LR or VPC vs NWC phenotypes. As shown in Figures 2A and 2B, the fraction of patients with a Th2-high biomarker profile in the HR subgroup was higher than in the LR subgroup (40.1% vs 29.3%, $P = .006$). However, the majority of patients in both subgroups displayed a Th2-low biomarker profile. Serum IgE level was elevated in a similar fraction of the HR (66.2%) and LR (57.9%) subgroups, consistent with the high incidence of atopy in both populations (93.7% and 86.5%, respectively). However, blood eosinophil counts and FENO concentrations were elevated in a minority of patients in both subgroups. This could reflect the effects of corticosteroid use on biomarker expression because this class of medication is known to affect circulating eosinophil counts and FENO levels and may influence cytokine expression.[12,13] Thus, although the HR relative to the LR subgroup is enriched for patients with a Th2-high biomarker profile, the majority of patients with moderate to severe asthma in both studies[6,7] had a Th2-low profile.

The present results further indicate that within the HR and LR subgroups, Th2 biomarker status does not align with disease control. Across both subgroups, the proportions of patients displaying Th2-high biomarker profiles with VPC and NWC asthma were similar (HR Th2-high, 39.0% vs 41.6%, respectively [$P = .685$]; LR Th2-high, 27.% vs 31.1%, respectively [$P = .461$]). To the extent that Th2 biomarkers levels accurately reflect inflammatory status, these findings do not support the hypothesis that disease activity in adult patients with poor asthma control is primarily driven by Th2 inflammation.

Several factors implicit in this analysis are relevant to interpreting these findings in the broader context of asthma research. The clinical trials[6,7] excluded patients on oral corticosteroids and IgE immunotherapy (ie, GINA step 5), and study eligibility required that patients have a baseline $FEV_1 > 50\%$ predicted and fewer than five exacerbations in the 12-month period before enrollment. Therefore, the findings apply specifically to a subset of patients in GINA steps 3 and 4. Extension of these findings to patients with different disease characteristics may not be valid. In addition, allocation of patients to Th2-high vs Th2-low biomarker profiles in this analysis is based on published IgE, eosinophil, and FENO cut points. Although the values used in this study are generally accepted and reasonable, results would vary if different cut points had been used. The CART analysis, however, failed to identify Th2 biomarker cut points that reliably separated patients with VPC and NWC asthma into either the HR or the LR subgroup. Finally, the ACQ-based cut points defining NWC and VPC are based on extrapolations from the literature, and although theoretically reasonable, these are specific to this analysis. A difference in cut point definition would affect comparisons of NWC vs VPC subgroups due to patient assignment. However, it would not likely change the overall conclusion that patients with HR asthma often display poor disease control along with Th2-low biomarker profiles.

In conclusion, the results indicate that conventional Th2 biomarker levels, including blood eosinophil counts, serum IgE levels, and FENO concentrations, are reasonably stable indicators of the inflammatory state of individuals with asthma receiving fixed medical therapy. Findings support prior results from SARP indicating that patients with HR asthma represent a distinct phenotype with worse pulmonary function and less-well-controlled disease. The data further suggest that the HR population, relative to the LR population, is enriched for patients displaying a Th2-high biomarker profile. However, the majority of patients in both the HR and the LR subgroups, although atopic, displayed Th2-low biomarker profiles. Finally, within the HR and LR subgroups with $ACQ6 \geq 1.5$, a poor relationship exists between disease control and Th2 biomarker levels, suggesting that factors other than Th2 inflammation drive disease activity in a majority of patients with moderate to severe asthma.

Acknowledgments

Author contributions: E. P. I. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. W. W. B. was a study investigator and contributed to the data analysis and writing and review of the manuscript; S. T. H. and S. S. W. were study investigators and contributed to the writing and review of the manuscript; P. K. was a sponsor medical director and contributed to the data analysis and review of the manuscript; Y. C. and J. F. contributed to the statistical analysis and table and figure preparation for the manuscript; E. P. I. was a sponsor medical director and contributed to the data analysis, table and figure preparation for the manuscript, and writing and review of the manuscript; and A. N. was the sponsor senior medical director and contributed to the data analysis and review of the manuscript.

Conflict of interest: W. W. B. has received personal fees from Novartis AG, Hoffmann-La Roche Ltd, AstraZeneca, Takeda Pharmaceutical Company Limited, Sanofi, Boston Scientific Corporation, and ICON plc. He also reports grants from the National Institute of Allergy and Infectious Diseases and National Heart, Lung, and Blood Institute, National Institutes of Health, outside the submitted work. S. T. H. has served as an occasional consultant to Amgen Inc. S. W. W. has been a principal investigator on multicenter industry-sponsored trials from Amgen Inc, AstraZeneca, sanofi aventis, Merck Sharp & Dohme Corp, and Pfizer Inc. She has received consulting fees from Amgen Inc, GlaxoSmithKline, AstraZeneca, Merck Sharp & Dohme Corp, Gilead, Novartis AG, and Boehringer Ingelheim GmbH. P. K., Y. C., J. F., and A. N. are employees of Amgen Inc. E. P. I. is a clinical medical director at Amgen Inc.

Role of sponsors: Amgen Inc funded the studies on which this manuscript is based. The data analysis, statistical assessments, and writing of the initial draft of the manuscript were performed by Amgen Inc employees.

Other contributions: The authors thank Maggie Wang, MS, for important contributions to the statistical analysis.

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