# Severe Asthma

## Lessons Learned from the National Heart, Lung, and Blood Institute Severe Asthma Research Program

**Nizar N. Jarjour[1], Serpil C. Erzurum[2], Eugene R. Bleecker[3], William J. Calhoun[4], Mario Castro[5], Suzy A. A. Comhair[2], Kian Fan Chung[6], Douglas Curran-Everett[7], Raed A. Dweik[2], Sean B. Fain[1], Anne M. Fitzpatrick[8], Benjamin M. Gaston[9], Elliot Israel[10], Annette Hastie[3], Eric A. Hoffman[11], Fernando Holguin[12], Bruce D. Levy[10], Deborah A. Meyers[3], Wendy C. Moore[3], Stephen P. Peters[3], Ronald L. Sorkness[1], W. Gerald Teague[9], Sally E. Wenzel[12], and William W. Busse[1]; for the NHLBI Severe Asthma Research Program (SARP)***

[1]University of Wisconsin, Madison, Wisconsin; [2]Cleveland Clinic, Cleveland, Ohio; [3]Wake Forest School of Medicine, Winston-Salem, North Carolina; [4]University of Texas Medical Branch, Galveston, Texas; [5]Washington University, St. Louis, Missouri; [6]Imperial College School of Medicine, London, United Kingdom; [7]University of Colorado-Denver, Colorado; [8]Emory University, Atlanta, Georgia; [9]University of Virginia, Charlottesville, Virigina; [10]Brigham & Women's Hospital, Boston, Massachusetts; [11]University of Iowa, Iowa City, Iowa; and [12]University of Pittsburgh, Pittsburgh, Pennsylvania

**The National Heart, Lung, and Blood Institute Severe Asthma Research Program (SARP) has characterized over the past 10 years 1,644 patients with asthma, including 583 individuals with severe asthma. SARP collaboration has led to a rapid recruitment of subjects and efficient sharing of samples among participating sites to conduct independent mechanistic investigations of severe asthma. Enrolled SARP subjects underwent detailed clinical, physiologic, genomic, and radiological evaluations. In addition, SARP investigators developed safe procedures for bronchoscopy in participants with asthma, including those with severe disease. SARP studies revealed that severe asthma is a heterogeneous disease with varying molecular, biochemical, and cellular inflammatory features and unique structure–function abnormalities. Priorities for future studies include recruitment of a larger number of subjects with severe asthma, including children, to allow further characterization of anatomic, physiologic, biochemical, and genetic factors related to severe disease in a longitudinal assessment to identify factors that modulate the natural history of severe asthma and provide mechanistic rationale for management strategies.**

**Keywords:** asthma; remodeling; inflammation; bronchoscopy; imaging

In asthma, patients with severe disease represent the greatest unmet need in terms of understanding mechanisms, morbidity, healthcare costs, and effective treatment. To meet these needs, two events occurred in 2000 to change direction in the study of severe asthma: (*1*) the National Heart, Lung, and Blood Institute (NHLBI) convened a workshop to review severe asthma pathophysiology and provide recommendations for future directions, which led to the funding of a Severe Asthma Research Program (SARP) (1); and (*2*) the American Thoracic Society (ATS) published the proceedings of a workshop on refractory asthma, which included a working definition of severe asthma (2). The initial efforts of SARP benefited greatly from a preceding effort by the European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA), which conducted a cross-sectional study comparing 163 subjects with severe asthma to 158 subjects with well-controlled asthma. In that study, patients with severe asthma were more likely to be female, and they had neutrophil-predominant inflammation based on sputum analysis (3). However, the study had limited support, thus restricting its sample size and scope of evaluations performed in each subject. To gain much-needed insight into severe asthma and eventually improve its treatment, a collaborative network approach was believed to be essential, because one site would not have sufficient subjects required to address these questions. A major innovative aspect of SARP was a requirement that its investigators, with independently funded mechanistic studies, collaborate within the program to recruit subjects and obtain biological samples that were made available to all investigators in the network for site-specific and network-wide mechanistic studies. The results of SARP investigations led to the largest and most comprehensively characterized cohort of patients with severe asthma ever assembled, significantly advanced our understanding of asthma in general and severe asthma in particular, and have positioned SARP and other investigators on a trajectory to advance the treatment of severe asthma.

### DEFINITION OF SEVERE ASTHMA

Asthma guidelines classify disease severity as mild, moderate, and severe, largely based on symptoms and lung functions, but do not fully account for the medications required to maintain control (4). The ATS workshop on refractory asthma proposed two major criteria to define severe asthma based on medications (daily use of high-dose inhaled corticosteroids and/or use of systemic corticosteroids) and seven minor criteria (symptoms; frequent, severe, or life-threatening exacerbations; lung function; controller use; and loss of control when corticosteroids were tapered). The workshop recommendations for refractory asthma required at least one major and two minor criteria, which became the working definition for severe asthma in SARP (2, 5). Implicit in this definition is the establishment of

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Correspondence and requests for reprints should be addressed to Nizar N. Jarjour, M.D., Professor and Head, Allergy, Pulmonary, and Critical Care Division, Department of Medicine, School of Medicine and Public Health, K4/914 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792-9988. E-mail: nnj@medicine.wisc.edu

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the diagnosis of asthma and addressing known comorbidities. Assessment of adherence was based on patient history, which resulted in limitations in the interpretation of some SARP studies. An initial characterization of patients with asthma recruited into SARP found that a major factor differentiating severe from mild/moderate asthma was a significantly greater frequency and severity of high-risk outcomes, such as emergency department visits, hospitalizations, intensive care admissions, and intubations. Subjects with severe asthma not only had a greater need for more medications, more persistent symptoms, and lower lung function but also had serious outcomes despite what would be considered effective treatment. Similar characteristics were identified in children (6). Based on improved understanding of the distinguishing features of severe asthma gained from SARP and other global efforts in severe asthma, an ATS and European Respiratory Society Workshop was recently organized to further refine the definition of severe asthma and distinguish it from difficult to manage asthma, thus extending current knowledge to a worldwide level (7).

## RISK FACTORS FOR SEVERE ASTHMA

SARP studies identified several key risk factors associated with severe asthma, including sex, race, obesity, and environmental tobacco exposure. Severe asthma was shown to be more prevalent in women after puberty (8, 9). In addition, obesity appeared to be associated with increased asthma severity in adult-onset disease (10), which in obese women may be related to sex hormones (8) or obesity-related inflammation (11, 12). Asthma in blacks was diagnosed at an earlier age, with IgE level and a family history of asthma being strong independent risk factors for severe disease (13). Secondhand smoke exposure, as measured by urinary cotinine levels, was associated with greater airflow obstruction and hyperresponsiveness as well as lower levels of serum superoxide dismutase (SOD) activity (14). Finally, a history of gastroesophageal reflux, sinopulmonary infections, and pneumonia was more commonly seen in patients with severe asthma (5, 10).

## LUNG STRUCTURE–FUNCTION RELATIONSHIPS

In addition to incomplete reversal with bronchodilation, severe asthma was associated with a marked increase in air trapping across the range of airflow limitation, suggesting a disproportionate involvement of small airways (Figure 1) (5, 10, 15). After inhaled bronchodilator, the improvement in $FEV_1$ appears due, in large part, to reversal of the air trapping (15). In children aged 6 to 17 years, the airway closure/air trapping component of airway obstruction was more prominent in boys (6, 16). Severe asthma, male sex, and age were independent predictors of incomplete reversal post-bronchodilator (16, 17).

To further investigate airway structure–function abnormalities, SARP incorporated imaging modalities, including multidetector computed tomography (CT) and magnetic resonance imaging with hyperpolarized helium ($He^3$). The network performed 424 CT studies using a common protocol tailored for measurement of the airways and parenchyma. Airway wall thickness and area were increased in patients with severe asthma and correlated with epithelial thickness on biopsies from a subset of participants (Figure 2). In addition to airway wall thickness, the degree of air trapping was also associated with severity of disease (18). Risk factors for the air trapping phenotype included atopy, neutrophilic inflammation, duration of disease, and history of pneumonia. Magnetic resonance imaging with $He^3$ demonstrated focal ventilation defects, which are areas of reduced signal distal to airway obstruction. In asthma, ventilation defects had a heterogeneous pattern, occurred more frequently in severe disease, persisted over time, and often coincided with regions of air trapping on multidetector CT (19). Molecular imaging techniques, such as positron emission tomography, that image areas of inflammation, when combined with CT allow colocalization of pulmonary inflammatory signals to specific structures, further advancing the characterization of patients with asthma. These findings could provide useful correlates for lung physiology and lung inflammation parameters measured directly by bronchoscopic techniques (19).

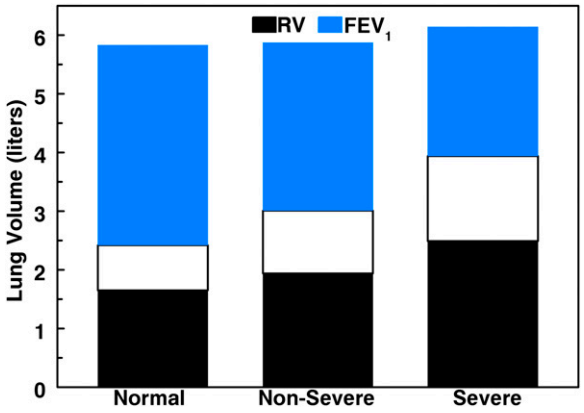


*Figure 1.* Comparison of physiological characteristics among adults with normal airways (n = 51), nonsevere asthma (n = 109), and severe asthma (n = 61), showing total lung capacity (box height), $FEV_1$, and residual lung volume (RV). Note prominent air trapping (elevated RV) and airflow limitation (reduced $FEV_1$) in severe asthma. Lung volumes are adjusted for differences due to height, age, sex, and race.

## AIRWAY INFLAMMATION AND REMODELING

Although several studies reported on the safety of investigative bronchoscopy in asthma, few included significant numbers of patients with severe disease (20). National Institutes of Health workshops on investigative bronchoscopy concluded that more experience was needed to establish the safety of bronchoscopy in severe asthma (21–23). To meet this need, SARP investigators developed guidelines for bronchoscopy in participants with increasingly severe airflow obstruction and disease. The experience of SARP with 505 bronchoscopies, of which 151 were performed on individuals with severe asthma, confirmed that bronchoscopy is safe and well tolerated (5, 10, 24), thus enabling SARP investigators to obtain airway samples to define the accompanying histopathology and immunobiology of severe asthma (25–33). Lung inflammation specific to severe asthma has been poorly defined. Studies from SARP revealed that mast cells differed in severe asthma by numbers, phenotypes, location, and Th2 pathway genotype with the chymase-positive mast cell subtype present in higher numbers in severe asthma, particularly in the epithelium (26, 34). Although severe asthma was not characterized by mucosal eosinophilia on biopsy, a combined increase in both eosinophils and neutrophils in sputum identified individuals with the lowest lung function, worst asthma control, increased symptom severity, and higher healthcare use (35). The airway epithelium and lamina reticularis were thicker in airway biopsies compared with patients with mild asthma, healthy individuals, or patients with chronic bronchitis (29), and strongly correlated with $FEV_1$, suggesting that remodeling contributes to airway obstruction in severe asthma. The role and contribution of other cell types is under study (36, 37).

## BIOMARKERS OF SEVERITY

SARP studies have identified novel biomarkers associated with asthma severity and expanded on previously identified biomarkers.

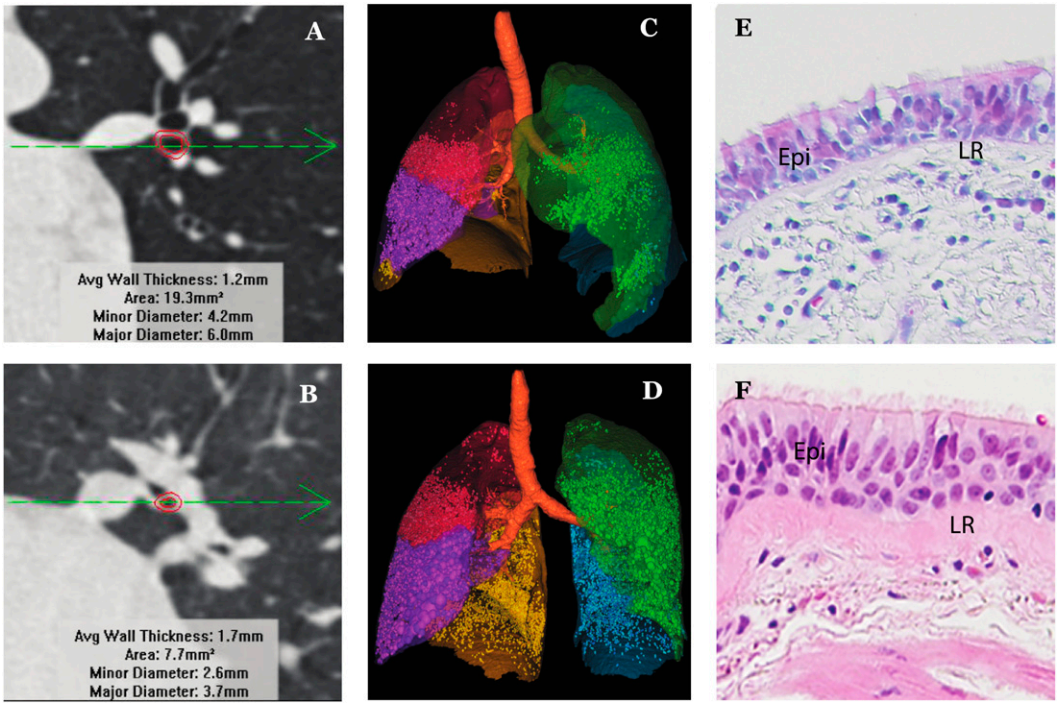


***Figure 2.*** Chest multidetector computed tomography (MDCT) images and bronchial biopsy from subjects with mild and severe asthma. Chest MDCT scans were performed in (*A*) mild asthma and (*B*) severe asthma. A screen capture of the cross-sectional MDCT image demonstrates an outline of a central airway and reported average wall thickness. Note that the average wall thickness is significantly greater in the subject with severe asthma. Quantitative CT using the Pulmonary Workstation software (VIDA) allows accurate assessment of air trapping (defined as voxels within the lung field falling below −856 Hounsfield units) as demonstrated by sphericals proportional to area of air trapping (volume rendered view). Each lobe is color-coded. Note the increase in air trapping in all lobes of (*D*) the subject with severe asthma compared with (*C*) the subject with mild asthma. Representative images from hematoxylin-eosin sections from an endobronchial biopsy from (*E*) a subject with mild asthma and (*F*) a subject with severe asthma are demonstrated. The epithelial layer (Epi) and lamina reticularis (LR) are indicated. This histopathology demonstrates key features of airway remodeling that can now be correlated with quantitative imaging of the airways (airway wall thickness) and lungs (air trapping).

For instance, the fraction of exhaled nitric oxide ($F_{ENO}$) exhibited broad heterogeneity in SARP and was partly determined by differences in atopy (30) and pH-dependent nitrite conversion to nitric oxide (38) and less dependent on corticosteroid therapy. High $F_{ENO}$ identified patients with severe asthma who were characterized by the greatest degree of airflow obstruction and hyperinflation as well as most frequent use of emergency care. Adult patients with low $F_{ENO}$ had increased arginase and decreased SOD activity, with low serum SOD activity being an independent biomarker for low $FEV_1$ in severe asthma (31, 39). Urinary bromotyrosine, a marker for eosinophil peroxidase activity, predicted risks for exacerbations, particularly in children with severe disease (40, 41). Consistent with our findings in adults, there were striking airway redox disturbances in children with severe asthma (6, 42–44) as well as increased catabolism of endogenous S-nitrosothiols (45), impaired airway macrophage function (46), and sustained expression of proinflammatory cytokines and chemokines resulting in a molecular phenotype that was neither Th1- nor Th2-predominant (42, 43, 47). Importantly, individual patients with severe asthma have overlapping features, highlighting the complexity and heterogeneity of this disease subclass. In addition to proinflammatory processes, persistent inflammation in severe asthma was related to a dysregulation of lipid proresolving systems with reduced lipoxin $A_4$ biosynthesis and receptor expression (48), which related to a greater corticosteroid use (49). Circulating chitinase-like protein (YKL-40) levels were also higher among patients with severe asthma in several cohorts, including SARP (50). Supervised and unsupervised cluster analysis of cytokines in bronchoalveolar lavage fluid samples from SARP identified distinct and novel molecular phenotypes, including one that is Th2-rich and another with evidence for innate immune activation (27, 28). These two molecular patterns corresponded to nonsevere and severe asthma, respectively. Importantly, it was a panel, not a single cytokine, that was required to define phenotypes (27, 28). Consequently, the biologic heterogeneity found among patients with severe asthma will likely serve as a reference for individualized therapeutic approaches that target specific cells or immune, biochemical, or endocrine responses (51, 52), such as antioxidant-mimetics and buffers, mast cell and Th2 pathway inhibitors, lipoxins, and/or prostaglandin D2 receptor antagonists.

## CORTICOSTEROID INSENSITIVITY

Because patients with severe asthma require large doses of corticosteroids in attempts to maintain disease control, relative corticosteroid insensitivity is implicated in this phenotype. SARP mechanistic studies identified that corticosteroid insensitivity in blood mononuclear cells and lung macrophages was linked to excessive activation of mitogen-activated protein kinases (53, 54) and decreased activity of nuclear histone deacetylase and histone acetyltransferase (55). Further SARP-wide studies have shown that tobacco smoke exposure and elevated oxidant burden are associated with corticosteroid insensitivity (14, 39, 42, 56, 57).

## GENETICS OF ASTHMA SUSCEPTIBILITY AND SEVERITY

The detailed subject phenotyping in SARP allowed for an examination of associations of genetic variations with asthma severity and its characteristics. Coding variants in IL-4 receptor α (IL4R-α), which regulates Th2 responses, were associated with more severe asthma (34). Mechanistically, IL4R-α polymorphisms were associated with increased numbers of tissue mast cells and higher levels of IgE bound to mast cells (34) and were more commonly found in African Americans. Polymorphisms in hedgehog interacting protein on chromosome 4q31, which is important in tissue development and cell proliferation, were associated with increased airflow limitation (58, 59). Furthermore, single-nucleotide polymorphisms in hedgehog interacting protein, combined with additional single-nucleotide polymorphisms in other

pulmonary function genes, predicted lung function abnormalities and asthma severity in whites (60). These studies suggest a genetic basis for asthma severity and associated airflow limitation, some of which may be shared among racial groups that are predisposed to more severe disease. SARP also contributed data on genome-wide association studies and actively participated in the NHLBI EVE consortium to gain better understanding of the variation in genetic risk patterns between European American, African American and African Caribbean, and Latino individuals (61). Association was found for several genes that were also observed in European genome-wide association metaanalyses (62), confirming their collective importance in asthma susceptibility, although each gene alone only conferred a minor risk. The results of these studies suggest that asthma heterogeneity, progression, and severity are caused by some of the same genes that are responsible for asthma susceptibility; however, there is increasing evidence that additional genes are important in asthma severity (63).

## CLINICAL PHENOTYPES IN ADULTS AND CHILDREN WITH ASTHMA

SARP studies have all identified clinical, physiologic, and biologic heterogeneity among patients with asthma. Studies linking clinical characteristics with biomarkers of inflammation, genetic analyses, and imaging should provide a framework for improved subject characterization in severe asthma and potentially allow for a stratified management approach. To identify potential unique clinical phenotypes in asthma, an unsupervised hierarchical cluster analysis was performed on adult SARP enrollees with asthma spanning the full spectrum of disease severity from mild to severe disease (10). This approach allowed for grouping of patients based on similarities free from *a priori* bias. Using 34 qualitative and quantitative variables that included age of asthma onset and duration, sex, race, lung function, atopy, and questionnaire data, five clusters emerged (Figure 3). Three clusters with divergent characteristics were more likely to include patients with severe disease (clusters 3, 4, and 5). A cluster analysis of nearly 300 children (aged 6–17 yr) who also participated in SARP (6, 47) confirmed that there is marked heterogeneity in childhood severe asthma similar to that seen in adults with severe asthma (47). However, in contrast to adults, children with severe asthma were highly atopic with peripheral blood eosinophilia, aeroallergen sensitivity, elevated serum IgE concentrations, and sustained increases in $Fe_{NO}$ (6, 30). The onset of puberty may be a critical phase of development wherein the phenotypic features of severe asthma progress toward the adult pattern (17). Characteristic features of severe asthma in comparison with nonsevere asthma in children and individuals after puberty are shown in Table 1. Longitudinal cohort studies will be required to define the outcomes of adults and children with severe asthma as well as to assess the effectiveness of cluster analysis to define heterogeneity in this population.

## ROLE OF SARP IN THE 2009 PANDEMIC H1N1 INFLUENZA

The contribution of influenza to asthma morbidity became evident during the 2009 H1N1 pandemic, when information on the efficacy and safety of the 2009 H1N1 pandemic vaccine in the high-risk asthma groups was lacking (64). The SARP cohort enabled rapid implementation of an open-label vaccination trial, with a two-month enrollment, to evaluate seroprotection and seroconversion in time to put vaccination strategies into practice for the public (65). Age, not asthma severity or corticosteroid use, was associated with diminished antibody response to the H1N1 vaccination. The study set a paradigm for use of established networks to rapidly implement clinical trials governed by federal mandates.

## LESSONS LEARNED THROUGH SARP

When the concept for SARP was initiated, selected investigators planned to use materials for study from many centers to accomplish their individual goals. It was essential to design and agree on uniform procedures for obtaining data, collecting samples, and establishing a database. This was a lengthy process, requiring nearly 2 years. The challenges of starting the unique program were rewarded by rigorous quality control, which has been

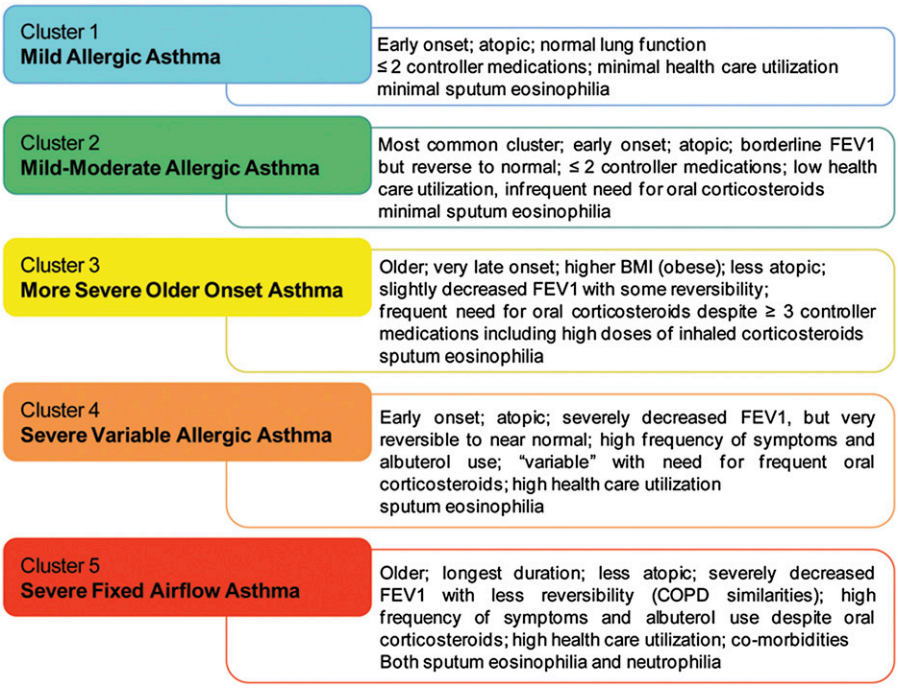


***Figure 3.*** Cluster analysis of all adults with asthma in the Severe Asthma Research Program. BMI = body mass index; COPD = chronic obstructive pulmonary disease.

**TABLE 1. FEATURES OF SEVERE ASTHMA IN ADULTS AND CHILDREN**

| | Adults (18 yr +) with Severe Asthma | Children (6–17 yr) with Severe Asthma |
|---|---|---|
| Feature | Observation (vs. Nonsevere Asthma) | Observation (vs. Nonsevere Asthma) |
| Symptoms | Daily or near-daily (5 of 7 d), with ~30% requiring daily oral corticosteroids | Daily or near-daily (5 of 7 d), with ~20% requiring daily oral corticosteroids |
| Healthcare use | Significant, with ~30% hospitalized in the preceding year and ~23% with lifetime history of intubation | Significant, with ~55% hospitalized in the preceding year and ~15% with lifetime history of intubation |
| Allergic sensitization | Varying degrees of atopy according to age of asthma onset and phenotype cluster | Highly atopic, with increased IgE, aeroallergen sensitization, and history of atopic dermatitis |
| Comorbid conditions | Increased prevalence of pneumonia (63%) requiring antibiotics; increased history of sinusitis (67%) | Increased prevalence of pneumonia (64%) requiring antibiotics; increased history of gastroesophageal reflux (35%); more frequent sinusitis (66%) in selected phenotypes |
| Exhaled nitric oxide | Not distinguishing feature of severe asthma, but associated with greater and more severe exacerbations | Sustained elevations |
| Airflow limitation | Moderate to severe airflow limitation often with incomplete reversal after bronchodilation | Some (mild) airflow limitation with near-complete reversal after bronchodilation; significant acceleration of airflow limitation in some adolescents after puberty |
| Air trapping | Increased air trapping (increased RV/TLC) at the same threshold of airflow limitation ($FEV_1$/FVC) | Increased air trapping (increased RV/TLC) at baseline, but reversible in girls and persistent in boys |

RV = residual lung volume.

key to the success of the SARP in general and site-specific research programs. Perhaps the most important aspect of SARP was the development of a "team effort." Over the decade of SARP, the group evolved shared goals and studies, some of which were high risk and would not have been possible to develop in a traditional network structure or feasible in a single-site study. For example, SARP embarked on the application of imaging across several sites to investigate the structure–function relationship of the airway to clinical characteristics. The study of pathology of airway remodeling occurred through the bronchoscopic biopsies at all sites. The integration of individual strengths to achieve site-specific and common goals led to generous cooperation and innovative discoveries. Collectively, the science was enhanced and mechanistic studies were more comprehensively evaluated from multiple perspectives.

The recent renewal of SARP underscores a number of the lessons learned in the previous cycle that have been incorporated into the new program. First, the cross-sectional noninterventional nature of SARP limited assessment of adherence to asthma medications. To fully appreciate the presence and persistence of severe asthma, longitudinal studies and determinations of medication adherence are included in the upcoming SARP. Second, as asthma typically begins in childhood, a pediatric component, which was limited in the first 10 years, is now included in all participating sites. Third, although the use of imaging has been a major advance, its application in the past 10 years of SARP was in its infancy. This aspect has been changed with uniform and shared acquisition and analysis protocols across the network to enhance safety and usefulness of these studies. Finally, it is predetermined that investigations will have a common component. Participating sites continue to have individual hypotheses and aims, but they are now expected to also have a common SARP-wide longitudinal protocol with joint hypotheses and aims. Overall, the goal of longitudinal SARP is the comprehensive study of the evolution of severe asthma, so that mechanism-based diagnostic, prognostic, and treatment strategies can be applied to treat and in the future prevent severe asthma in children and adults.

## FUTURE DIRECTIONS AND IMPLICATIONS FOR SEVERE ASTHMA

The understanding of severe asthma has evolved substantially over the past decade. It is now recognized that severe asthma is a heterogeneous disease with varied phenotypes, each with potentially unique pathogenic mechanisms that may be linked to more effective therapy. Past efforts to characterize severe asthma were largely based on a single evaluation of history, physical examination, and physiology in limited numbers of patients, often without comparative groups or children. Investigations under SARP have advanced the study of severe asthma by allowing for a more comprehensive phenotyping including clinical characteristics, imaging, and biologic and genetic analyses. Future studies will be critical to define why certain phenotypes evolve, what leads to the disabling features of severe asthma, and what may be the most effective therapeutic approaches for these patients. These efforts in the United States will be complemented and enhanced by the recently funded European consortium, U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes). SARP and U-BIOPRED have initiated cooperative efforts at many levels. Extension of efforts on a worldwide basis holds the promise of accelerated discoveries, comparative and contrasting observations across populations and environments that deepen mechanistic understanding, and a robust variety of expertise to plan implementation of advances. As a consequence, the collective observations will inform a universal perspective on this phenotype and lower barriers to achieve optimal care in severe asthma.

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### References

1. Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. *J Allergy Clin Immunol* 2000;106:1033–1042.
2. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med* 2000;162:2341–2351.

3. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. *Eur Respir J* 2003;22:470–477.
4. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report. *J Allergy Clin Immunol* 1991;88:425–534.
5. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, Calhoun WJ, Castro M, Chung KF, Clark MP, *et al*. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;119:405–413.
6. Fitzpatrick AM, Gaston BM, Erzurum SC, Teague WG. Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. *J Allergy Clin Immunol* 2006;118:1218–1225.
7. Bel EH, Sousa A, Fleming L, Bush A, Chung KF, Versnel J, Wagener AH, Wagers SS, Sterk PJ, Compton CH. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax* 2011;66:910–917.
8. Farha S, Asosingh K, Laskowski D, Hammel J, Dweik RA, Wiedemann HP, Erzurum SC. Effects of the menstrual cycle on lung function variables in women with asthma. *Am J Respir Crit Care Med* 2009;180: 304–310.
9. Tantisira KG, Colvin R, Tonascia J, Strunk RC, Weiss ST, Fuhlbrigge AL. Airway responsiveness in mild to moderate childhood asthma: sex influences on the natural history. *Am J Respir Crit Care Med* 2008; 178:325–331.
10. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, Castro M, Curran-Everett D, Fitzpatrick AM, *et al.* Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315–323.
11. Holguin F, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, Fitzpatrick AM, Gaston B, Israel E, Jarjour NN, *et al*. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 2011;127:1486–1493.e2.
12. Holguin F, Fitzpatrick A. Obesity, asthma, and oxidative stress. *J Appl Physiol* 2010;108:754–759.
13. Gamble C, Talbott E, Youk A, Holguin F, Pitt B, Silveira L, Bleecker E, Busse W, Calhoun W, Castro M, *et al*. Racial differences in biologic predictors of severe asthma: data from the Severe Asthma Research Program. *J Allergy Clin Immunol* 2010;126:1149–1156.e1.
14. Comhair SA, Gaston BM, Ricci KS, Hammel J, Dweik RA, Teague WG, Meyers D, Ampleford EJ, Bleecker ER, Busse WW, *et al*. Detrimental effects of environmental tobacco smoke in relation to asthma severity. *PLoS ONE* 2011;6:e18574.
15. Sorkness RL, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Chung KF, Curran-Everett D, Erzurum SC, Gaston BM, Israel E, *et al.* Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation. *J Appl Physiol* 2008;104:394–403.
16. Sorkness RL, Teague WG, Penugonda M, Fitzpatrick AM. Sex dependence of airflow limitation and air trapping in children with severe asthma. *J Allergy Clin Immunol* 2011;127:1073–1074.
17. Fitzpatrick AM, Teague WG. Progressive airflow limitation is a feature of children with severe asthma. *J Allergy Clin Immunol* 2011;127:282–284.
18. Busacker A, Newell JD Jr, Keefe T, Hoffman EA, Granroth JC, Castro M, Fain S, Wenzel S. A multivariate analysis of risk factors for the air-trapping asthmatic phenotype as measured by quantitative CT analysis. *Chest* 2009;135:48–56.
19. Castro M, Fain SB, Hoffman EA, Gierada DS, Erzurum SC, Wenzel S. Lung imaging in asthmatic patients: the picture is clearer. *J Allergy Clin Immunol* 2011;128:467–478.
20. Jarjour NN, Peters SP, Djukanovic R, Calhoun WJ. Investigative use of bronchoscopy in asthma. *Am J Respir Crit Care Med* 1998;157:692–697.
21. Investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airways diseases. *Clin Exp Allergy* 1991;21:533–539.
22. Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, Kraft M, Levine RJ, Peters SP, Sullivan EJ. Investigative bronchoprovocation and bronchoscopy in airway diseases. *Am J Respir Crit Care Med* 2005;172:807–816.
23. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1985;132:180–182.
24. Moore WC, Evans MD, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Fan Chung K, Erzurum SC, Curran-Everett D, Dweik RA, *et al*. Safety of investigative bronchoscopy in the severe asthma research program. *J Allergy Clin Immunol* 2011;128:328–336.e3.
25. Aysola RS, Hoffman EA, Gierada D, Wenzel S, Cook-Granroth J, Tarsi J, Zheng J, Schechtman KB, Ramkumar TP, Cochran R, *et al*. Airway remodeling measured by multidetector CT is increased in severe asthma and correlates with pathology. *Chest* 2008;134:1183–1191.
26. Balzar S, Fajt ML, Comhair SA, Erzurum SC, Bleecker E, Busse WW, Castro M, Gaston B, Israel E, Schwartz LB, *et al.* Mast cell phenotype, location, and activation in severe asthma: data from the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2011;183:299–309.
27. Brasier AR, Victor S, Boetticher G, Ju H, Lee C, Bleecker ER, Castro M, Busse WW, Calhoun WJ. Molecular phenotyping of severe asthma using pattern recognition of bronchoalveolar lavage-derived cytokines. *J Allergy Clin Immunol* 2008;121:30–37.e6.
28. Brasier AR, Victor S, Ju H, Busse WW, Curran-Everett D, Bleecker E, Castro M, Chung KF, Gaston B, Israel E, *et al*. Predicting intermediate phenotypes in asthma using bronchoalveolar lavage-derived cytokines. *Clin Transl Sci* 2010;3:147–157.
29. Cohen L, E X, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, DeMartino S, Schechtman KB, Hussain I, Holtzman MJ, *et al*. Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007;176:138–145.
30. Dweik RA, Sorkness RL, Wenzel S, Hammel J, Curran-Everett D, Comhair SA, Bleecker E, Busse W, Calhoun WJ, Castro M, *et al*. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2011;181:1033–1041.
31. Lara A, Khatri SB, Wang Z, Comhair SA, Xu W, Dweik RA, Bodine M, Levison BS, Hammel J, Bleecker E, *et al.* Alterations of the arginine metabolome in asthma. *Am J Respir Crit Care Med* 2008;178:673–681.
32. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol* 2004;113:101–108.
33. Planaguma A, Kazani S, Marigowda G, Haworth O, Mariani TJ, Israel E, Bleecker ER, Curran-Everett D, Erzurum SC, Calhoun WJ, *et al*. Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. *Am J Respir Crit Care Med* 2008;178: 574–582.
34. Wenzel SE, Balzar S, Ampleford E, Hawkins GA, Busse WW, Calhoun WJ, Castro M, Chung KF, Erzurum S, Gaston B, *et al*. IL4R alpha mutations are associated with asthma exacerbations and mast cell / IgE expression. *Am J Respir Crit Care Med* 2007;175:570–576.
35. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, Bleecker ER. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010;125:1028–1036.e13.
36. Kotaru C, Schoonover KJ, Trudeau JB, Huynh ML, Zhou X, Hu H, Wenzel SE. Regional fibroblast heterogeneity in the lung: Implications for remodeling. *Am J Respir Crit Care Med* 2006;173:1208–1215.
37. Zhou X, Wu W, Hu H, Milosevic J, Konishi K, Kaminski N, Wenzel SE. Genomic differences distinguish the myofibroblast phenotype of distal lung from airway fibroblasts. *Am J Respir Cell Mol Biol* 2011;45: 1256–1262.
38. Shin HW, Shelley DA, Henderson EM, Fitzpatrick A, Gaston B, George SC. Airway nitric oxide release is reduced after PBS inhalation in asthma. *J Appl Physiol* 2007;102:1028–1033.
39. Comhair SA, Ricci KS, Arroliga M, Lara AR, Dweik RA, Song W, Hazen SL, Bleecker ER, Busse WW, Chung KF, *et al*. Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. *Am J Respir Crit Care Med* 2005;172:306–313.
40. Wedes SH, Khatri SB, Zhang R, Wu W, Comhair SA, Wenzel S, Teague WG, Israel E, Erzurum SC, Hazen SL. Noninvasive markers of airway inflammation in asthma. *Clin Transl Sci* 2009;2:112–117.
41. Wedes SH, Wu W, Comhair SA, McDowell KM, Didonato JA, Erzurum SC, Hazen SL. Urinary bromotyrosine measures asthma control and predicts asthma exacerbations in children. *J Pediatr* 2011;159:248–255.
42. Fitzpatrick AM, Teague WG, Burwell L, Brown MS, Brown LA. Glutathione oxidation is associated with airway macrophage functional impairment in children with severe asthma. *Pediatr Res* 2011; 69:154–159.

43. Fitzpatrick AM, Teague WG, Holguin F, Yeh M, Brown LA. Airway glutathione homeostasis is altered in children with severe asthma: evidence for oxidant stress. *J Allergy Clin Immunol* 2009;123:146–152.e8.
44. Fitzpatrick AM, Brown LA, Holguin F, Teague WG. Levels of nitric oxide oxidation products are increased in the epithelial lining fluid of children with persistent asthma. *J Allergy Clin Immunol* 2009;124: 990–996.e1–9.
45. Greenwald R, Fitzpatrick AM, Gaston B, Marozkina NV, Erzurum S, Teague WG. Breath formate is a marker of airway s-nitrosothiol depletion in severe asthma. *PLoS ONE* 2010;5:e11919.
46. Fitzpatrick AM, Holguin F, Teague WG, Brown LA. Alveolar macrophage phagocytosis is impaired in children with poorly controlled asthma. *J Allergy Clin Immunol* 2008;121:1372–1378, 1378.e1-3.
47. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, Wenzel SE, Aujla S, Castro M, Bacharier LB, *et al.* Heterogeneity of severe asthma in childhood: Confirmation by cluster analysis of children in the national institutes of health/national heart, lung, and blood institute severe asthma research program. *J Allergy Clin Immunol* 2011; 127:382–389.e1–13.
48. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E. Diminished lipoxin biosynthesis in severe asthma. *Am J Respir Crit Care Med* 2005;172:824–830.
49. Bhavsar PK, Levy BD, Hew MJ, Pfeffer MA, Kazani S, Israel E, Chung KF. Corticosteroid suppression of lipoxin A4 and leukotriene B4 from alveolar macrophages in severe asthma. *Respir Res* 2010;11:71.
50. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, *et al.* A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;357: 2016–2027.
51. Gaston B. The biochemistry of asthma. *Biochim Biophys Acta* 2011; 1810:1017–1024.
52. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006;368:804–813.
53. Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM. P38 mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J Allergy Clin Immunol* 2002;109:649–657.
54. Bhavsar P, Hew M, Khorasani N, Torrego A, Barnes PJ, Adcock I, Chung KF. Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma. *Thorax* 2008;63:784–790.
55. Hew M, Bhavsar P, Torrego A, Meah S, Khorasani N, Barnes PJ, Adcock I, Chung KF. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. *Am J Respir Crit Care Med* 2006; 174:134–141.
56. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* 2003;168:1308–1311.
57. Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, Adcock IM. Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages. *FASEB J* 2001;15:1110–1112.
58. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, Franceschini N, van Durme YM, Chen TH, Barr RG, *et al.* Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet* 2010;42:45–52.
59. Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A, Zhai G, Vitart V, *et al.* Genome-wide association study identifies five loci associated with lung function. *Nat Genet* 2010; 42:36–44.
60. Li X, Howard TD, Moore WC, Ampleford EJ, Li H, Busse WW, Calhoun WJ, Castro M, Chung KF, Erzurum SC, *et al.* Importance of hedgehog interacting protein and other lung function genes in asthma. *J Allergy Clin Immunol* 2011;127:1457–1465.
61. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, *et al.* Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011;43:887–892.
62. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363:1211–1221.
63. Slager RELX, Meyers DA, Bleecker ER. Recent developments in the genetics of asthma susceptibility and severity. *Eur Respir Mon* 2011; 51:82–106.
64. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, Sugerman DE, Druckenmiller JK, Ritger KA, Chugh R, *et al.* Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. *N Engl J Med* 2009;361:1935–1944.
65. Busse WW, Peters SP, Fenton MJ, Mitchell H, Bleecker ER, Castro M, Wenzel S, Erzurum SC, Fitzpatrick AM, Teague WG, *et al.* Vaccination of patients with mild and severe asthma with a 2009 pandemic H1N1 influenza virus vaccine. *J Allergy Clin Immunol* 2011;127:130–137, 137.e1-3.