Ventilation defects on hyperpolarized helium-3 MRI in asthma are predictive of 2-year exacerbation frequency

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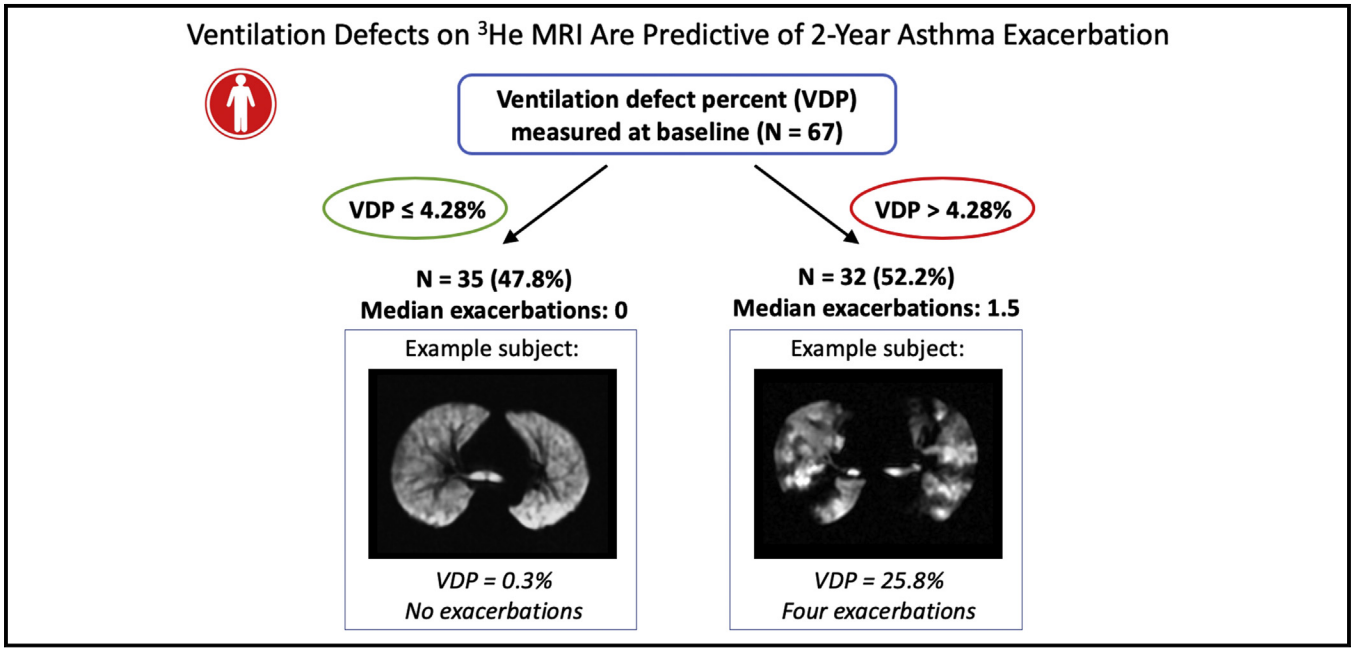
GRAPHICAL ABSTRACT



Background: There is an unmet need for an objective biomarker to predict asthma exacerbations.
Objective: Our aim was to assess the ventilation defect percent (VDP) on hyperpolarized helium-3 magnetic resonance imaging as a predictor of exacerbation frequency following imaging.
Methods: Subjects underwent hyperpolarized helium-3 and conventional clinical measurements, including pulmonary function tests, during a period of disease stability, and exacerbations were recorded prospectively over the following 2 years. We used a Poisson regression tree model to estimate an optimal VDP threshold for classifying subjects into high- versus low-exacerbation groups and then used statistical regression to compare this VDP threshold against conventional clinical measures as predictors of exacerbations.
Results: A total of 67 individuals with asthma (27 males and 40 females, 28 with mild-to-moderate asthma and 39 with severe asthma) had a median VDP of 3.75% (1.2% [first quartile]-7.9% [third quartile]). An optimal VDP threshold of 4.28% was

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selected on the basis of the maximum likelihood estimation of the regression tree model. Subjects with a VDP greater than 4.28% (n = 32) had a median of 1.5 exacerbations versus 0.0 for subjects with a VDP less than 4.28% (n = 35). In a stepwise multivariate regression model, a VDP greater than 4.28% was associated with an exacerbation incidence rate ratio of 2.5 (95% CI = 1.3-4.7) versus a VDP less than or equal to 4.28%. However, once individual medical history was included in the model, VDP was no longer significant. Nonetheless, VDP may provide an objective and complementary quantitative marker of individual exacerbation risk that is useful for monitoring individual change in disease status, selecting patients for therapy, and assessing treatment response.

Conclusion: VDP measured with magnetic resonance imaging shows promise as a biomarker of prospective asthma exacerbations. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: *Asthma, magnetic resonance imaging, functional, airway obstruction, patient outcomes assessment*

Abbreviations used

ACQ:	Asthma control questionnaire
ACT:	Asthma Control Test
BMI:	Body mass index
CT:	Computed tomography
ED:	Emergency department
FENO:	Fractional exhaled nitric oxide
FVC:	Forced vital capacity
GERD:	Gastroesophageal reflux disease
^{3}He MRI:	Helium-3 magnetic resonance imaging
ICS:	Inhaled corticosteroid
IRR:	Incidence rate ratio
MRI:	Magnetic resonance imaging
OCS:	Oral corticosteroid
Q1:	First quartile
Q3:	Third quartile
SARP III:	National Heart, Lung, and Blood Institute Severe Asthma Research Program III
UW-Madison:	University of Wisconsin-Madison
VDP:	Ventilation defect percent

Prevention and management of exacerbations is of paramount concern in the care of patients with asthma. In addition to their impact on patient quality of life, exacerbations are associated with increased health care costs.[1] Multiple risk factors for asthma exacerbation have been proposed, including FEV_1,[2] bronchodilator reversibility,[3] and sputum and blood eosinophils.[3,4] However, there remains a need for a strong individualized predictor of asthma exacerbation in moderate and severe asthma to support decision making regarding personalized therapy and to enable clinical trials in smaller populations of individuals with asthma over shorter time scales. Here, we examine the use of hyperpolarized helium-3 magnetic resonance imaging (^{3}He MRI) as a biomarker of exacerbation in a subpopulation of individuals with asthma drawn from the National Heart, Lung, and Blood Institute Severe Asthma Research Program III (SARP III) study.

The widely accepted metric for measuring extent of ventilation defect by using hyperpolarized gas magnetic resonance imaging (MRI) is the ventilation defect percent (VDP), which is defined as the ratio of ventilation defect volume to total lung volume.[5-7] VDP has been shown to predict exacerbations in chronic obstructive pulmonary disease.[8] In asthma, VDP has recently been shown to be associated with history of severe clinical outcomes, including asthma-related emergency department (ED) visits and hospitalizations.[9] Although prior studies have investigated multiple aspects of VDP in the context of asthma, including response to bronchial thermoplasty,[6] sputum eosinophilia,[10] and mucus plugs on computed tomography (CT),[11] no study to date has examined VDP as a predictor of prospective asthma exacerbation. The purpose of this study was to evaluate VDP as a predictor of asthma exacerbation frequency in the 2-year period following imaging in a mixed population of individuals with mild-to-moderate or severe asthma. Establishing VDP as a biomarker of asthma exacerbation is of interest in evaluating drug efficacy in clinical trials, selecting patients for targeted therapies, and monitoring treatment response.

METHODS

Study population

Nonsmoking subjects with asthma aged 6 and older were recruited as part of the SARP III multisite study[12,13] (National Institutes of Health ClinicalTrials.gov identifier NCT01606826), with a particular emphasis on subjects who had used high-dose inhaled corticosteroids (ICSs) and a second controller therapy for the last 3 months at recruitment. Asthma diagnosis was confirmed by demonstration of relative change in postalbuterol FEV_1 of at least 12% or a methacholine provocative concentration less than or equal to 16 mg/mL associated with a 20% decline in FEV_1. The imaging-based study population was drawn from the larger SARP III population recruited at the University of Wisconsin-Madison (UW-Madison), and the study was approved by the UW-Madison Health Sciences IRB (HS-2012-0571). The use of hyperpolarized ^{3}He gas as an inhaled contrast agent was regulated by the US Food and Drug Administration (Investigational New Drug No. 064867). Informed consent was obtained for all subjects. The SARP III–wide inclusion and exclusion criteria, together with specific UW-Madison exclusion steps, are shown in the Online Repository for this article (available at www.jacionline.org). Note that 8 subjects were excluded from the analysis because of images that were not of diagnostic quality (ie, a coil and/or gas delivery issue, incomplete coverage of thoracic cavity, or a signal-to-noise ratio less than 5.0).

Exacerbations were determined from self-reported number of asthma-related oral corticosteroid (OCS) courses lasting 3 or more days within the previous year. This information was collected at the baseline imaging visit and at visits occurring 1 year and 2 years following baseline, with phone call interviews at 6 months between the longitudinal visits.

Imaging methods

Image data for this analysis were collected after withholding bronchodilators for 4 hours for short-acting agonists and 6 to 24 hours for long-acting products. Specific MRI acquisition parameters and techniques are described in detail in the Online Repository (available at www.jacionline.org). Proton MRI was registered to hyperpolarized ^{3}He MRI by using a 3-dimensional rigid registration algorithm implemented with use of ANTs (http://picsl.upenn.edu/software/ants/). The lung boundary on hyperpolarized ^{3}He MRI was segmented with reference to the proton MRI, and regions of ventilation defect were classified on hyperpolarized ^{3}He MRI with a semiautomated adaptive k-means algorithm.[7]

Subject characterization

Characterization procedures were adapted from the SARP I and SARP II studies[13-17] and included spirometry, maximum reversibility following bronchodilator, measurements of blood serum and sputum eosinophil levels, fractional exhaled nitric oxide (FENO), GERD and sinusitis assessment, and

administration of the Asthma Control Test (ACT) and asthma control questionnaire (ACQ) surveys. Percent predicted values for FEV_1 and forced vital capacity (FVC) were generated by using the Global Lung Function Initiative reference values.[18] Blood, sputum, and FENO assessments were all administered on the same day as imaging. Spirometry was also performed on the same day as imaging unless the subject had already undergone spirometry within the previous 6 weeks as part of his or her consent visit, in which case those values were used.

ICS dosing level at baseline was assessed in our data set on an ordinal scale of 0 to 3, corresponding to none, low, medium, and high. High dose was determined by site coordinators with reference to treatment history codes. Medium dose and low dose were determined from dosing information provided from 2 ICS inhalers, with the threshold for medium dose defined as at least half of the high-dose definition and low dose being half of the medium dose. The reported dose was assumed to be that prescribed, and determination of category was made without regard for reports of less-than-daily use.

Statistical methods

Specific predictors under consideration included VDP, age, sex, body mass index (BMI), FEV_1 percent predicted, FVC percent predicted, FEV_1/FVC, ACT, ACQ, GERD and sinusitis status, serum and sputum eosinophil levels, FENO, and maximum reversibility following bronchodilator use.

We assessed associations between these predictors and 2-year exacerbation frequency in 2 stages. First, we used Poisson model–based recursive partitioning[19] to examine the association between VDP and cumulative exacerbations and selected an optimal VDP threshold for dividing the population into high and low exacerbators based on maximum likelihood estimation. We then used 3 types of negative binomial regression models of exacerbation frequency to assess the performance of the VDP threshold with respect to other demographic and clinical metrics gathered at baseline as predictors of cumulative 2-year exacerbations. These models included (1) individual univariate models for each predictor, (2) a multivariate model with all predictors included, and (3) a parsimonious multivariate model with a subset of predictors chosen by using stepwise model selection based on the Akaike information criterion. The relationships between baseline predictor variables and subsequent exacerbation counts are summarized as exacerbation incidence rate ratios (IRRs) with 95% CIs obtained by exponentiating the regression coefficient estimates. Predictor variables were scaled or log-transformed as needed to facilitate easier interpretation of results as follows: IRRs were estimated with respect to 10-unit changes in age, BMI, FVC percent predicted, and FEV_1 reversibility, and 2-fold changes in FENO or eosinophil levels. Variables were termed significant if the 95% CI for the estimated exacerbation IRR did not include 1.

Differences in measurements between subjects with and without exacerbations, between the groups above and below the VDP threshold, and in other subgroup comparisons were assessed by using the Wilcoxon rank sum test or the chi-square test for categoric variables when appropriate. Correlations between VDP and other predictors of exacerbation were assessed by using the Spearman correlation. Analyses were conducted by using R software (version 3.6.0).[20]

RESULTS

Study population

The study population consisted of 67 subjects, with 28 classified as individuals with mild-to-moderate asthma and 39 classified as having severe asthma per the SARP III criteria.[15] These subjects in aggregate experienced a total of 78 exacerbations in the 2 years following imaging, with 46 exacerbations (59.0%) occurring in the first year and 32 (41.0%) occurring in the second year. A total of 36 subjects (53.7%) experienced 1 or more exacerbations, with the remaining 31 subjects (46.3%) experiencing 0 exacerbations. Subjects with 1 or more exacerbations experienced a median (quartile 1 [Q1]-quartile 3 [Q3]) of 2.0 (1.0-3.0) exacerbations. In all, 4 subjects (6.0%) received biologic therapy within 3 months before baseline. Over the course of the study, 31 subjects (46.3%) were taking the same ICS dose at baseline and 2-year follow-up, 6 subjects (9.0%) had an increased ICS dose at follow-up, 19 subjects (28.4%) had a decreased ICS dose at follow-up, and 11 subjects (16.4%) were not taking an ICS at either time point.

A summary of population characteristics stratified by presence or absence of 1 or more prospective exacerbations is presented in Table I. All measurements except for FENO (n = 64 [95.5%]) and sputum eosinophil levels (n = 50 [74.6%]) were acquired for all subjects. Of all the predictors under consideration, only sex, FVC percent predicted, and VDP were significantly different between the 2 groups at a P value of .05, although GERD status was on the cusp of significance. VDP was greater in those subjects with 1 or more 2-year exacerbations, with a median (Q1-Q3) value of 5.5 (3.0-9.8) versus 2.2 (0.9-4.9) in subjects with no exacerbations (P = .010). Subjects with 1 or more exacerbations were more likely to be female (P = .024) and have a decreased FVC percent predicted (P < .01). The number of exacerbations in the year before imaging was also significantly increased in the exacerbation group (P < .001). Subjects who underwent an exacerbation in the second year only (n = 10) had a median (Q1-Q3) VDP of 4.4% (1.3%-6.4%) versus 6.1% (3.6%-13.5%) for subjects with an exacerbation in the first year (n = 26) but the difference was not significant (P = .16). Example images from 3 individual subjects demonstrating different patterns and the incidence of 2-year exacerbations are shown in Fig 1.

Correlations between VDP and clinical measures

Fig 2 shows a box and whisker plot comparing VDP in groups of subjects without and with 1 or more exacerbations. The median (Q1-Q3) VDP was 5.5% (3.0%-9.8%) in subjects with 1 or more exacerbations versus 2.2% (0.9%-4.9%) in subjects with no exacerbations (P = .010).

Correlations between VDP and selected clinical measurements are shown in Table II. VDP was negatively and moderately correlated with FVC percent predicted, maximum reversibility following bronchodilator use, sputum eosinophil level, and ACQ and ACT scores. VDP was not correlated with FENO or serum eosinophil level. For categoric variables, specifically GERD and sinusitis status, VDP was not significantly different across the 2 categories (P = .12 and P = .73 respectively). VDP was also correlated with FEV_1 percent predicted (P < .001; ρ = –0.47) and FEV_1/FVC (P <.001; ρ = –0.60), but both of these measurements were also correlated with FVC percent predicted (P < .001; ρ = 0.90 and P < .001; ρ = 0.56, respectively) and have been omitted from the table for clarity.

Determining a maximum likelihood VDP threshold

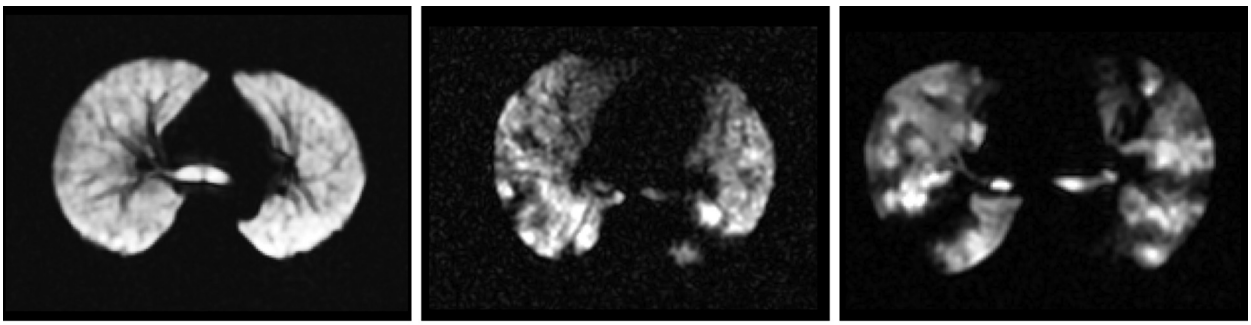
Through use of maximum likelihood estimation on the Poisson regression tree model fit, a VDP of 4.28% was selected as the threshold that most accurately classified subjects on the basis of cumulative exacerbation history (Fig 3).

Subjects with a VDP exceeding the 4.28% threshold (n = 32) had a median (Q1-Q3) of 1.5 (0.75-3.0) exacerbations versus 0.0 (0.0-1.0) for subjects at or below the threshold (n = 35), as shown in Fig 4. Study population descriptive statistics stratified by VDP

TABLE I. Summary of population characteristics stratified by number of prospective exacerbations

Baseline characteristic	0 exacerbations	≥ 1 exacerbations	Difference
No. subjects	31 (46.3%)	36 (53.7%)	—
Severe asthma	13 (41.9%)	26 (72.2%)	$P = .012$
Sex (F)	14 (45.2%)	26 (72.2%)	$P = .024$
Age (y)	30.7 (19.9-49.0)	46.5 (19.9-58.8)	$P = .18$
BMI	25.9 (23.6-29.1)	27.6 (22.8-32.3)	$P = .35$
Pre-BD FEV_1 %P	86.2 (80.0-99.0)	78.7 (57.9-96.0)	$P = .11$
Pre-BD FVC %P	95.0 (88.2-104.5)	89.8 (71.6-102.7)	$P < .01$
Pre-BD FEV_1/FVC	0.76 (0.72-0.78)	0.71 (0.63-0.80)	$P = .32$
Pre-BD FEV_1/FVC z score	(1.22) (–1.73 to –0.87)	–1.59 (–2.47 to –0.55)	$P = .41$
ACQ	0.71 (0.57-1.22)	1.15 (0.71-1.86)	$P = .07$
ACT	20.0 (19.0-21.5)	20.0 (15.0-22.3)	$P = .40$
FENO*	19.5 (11.3-36.0)	22.5 (12.3-32.3)	$P = .97$
Blood eosinophil level (eosinophils/μL)	201.0 (126.5-278.0)	327.0 (147.8-510.2)	$P = .09$
Sputum eosinophil level (%)†	0.70 (0.20-1.88)	0.75 (0.00-3.05)	$P = .86$
GERD	10 (32.3%)	20 (55.6%)	$P = .056$
Sinusitis	7 (22.6%)	14 (38.9%)	$P = .15$
Max reversibility following BD administration (%)	10.6 (6.1-15.2)	10.8 (4.3-17.4)	$P = .99$
VDP (%)	2.15 (0.91-4.89)	5.51 (2.95-9.82)	$P = .010$
Taking a LABA	16 (51.6%)	31 (86.1%)	$P < .01$
Taking a high-dose ICS	15 (48.4%)	28 (77.8%)	$P = .012$
Taking a LABA + a high-dose ICS	13 (41.9%)	27 (75.0%)	$P < .01$
No. exacerbations in prior year	0 (0-0)	2 (0-3)	$P < .0001$

BD, Bronchodilator; *LABA*, long-acting β-agonist; *%P*, percent predicted.
Results given as median (Q1-Q3) or percent of total. All measurements except maximum reversibility were acquired before BD administration.
*n = 64.
†n = 50.



	Subject A	Subject B	Subject C
Severity:	Mild/Moderate	Severe	Severe
Age:	38	56	61
Sex:	F	F	F
BMI:	32	43	26
FEV1 %P:	88%	47%	58%
FVC %P	95%	60%	76%
FEV1/FVC:	0.77	0.63	0.60
VDP:	0.3%	6.3%	25.8%
# Exacerbations:	0	2	4

FIG 1. Example ^{3}He MRI images from subjects with a range of clinical patterns, VDPs, and exacerbations in the 2 years following baseline. *F*, Female.

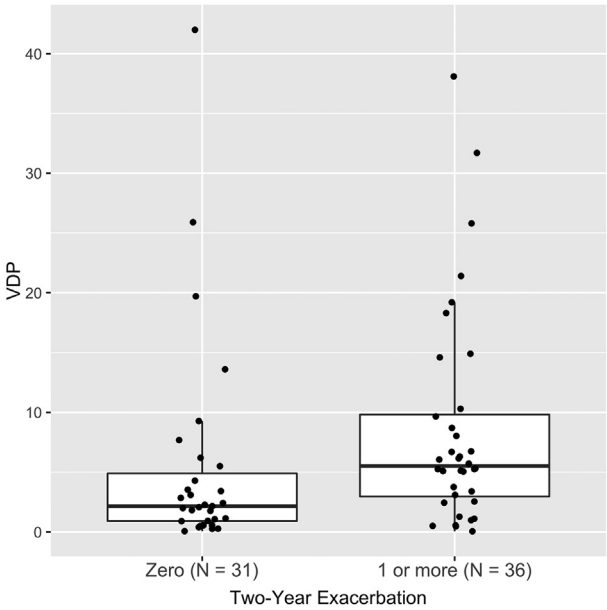


FIG 2. VDP in subjects with 0 (*left*) and 1 or more (*right*) 2-year exacerbations. The median and quartile range (Q1-Q3) values of VDP were 5.5% (3.0%-9.8%) in subjects with 1 or more exacerbations versus 2.2% (0.9%-4.9%) in subjects with 0 exacerbations ($P = .010$).

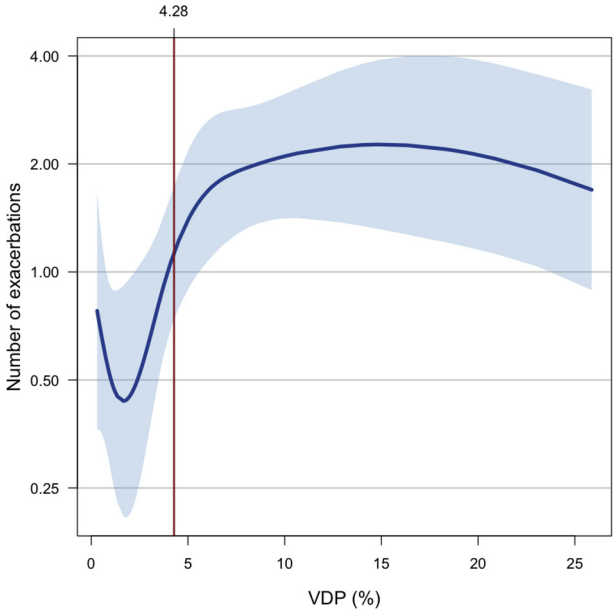


FIG 3. Number of exacerbations predicted by regression tree model of VDP versus exacerbations (*solid line*) and 95% CI (*shaded area*). The maximum likelihood estimate for a VDP threshold (4.28%) stratifying low (n = 35) versus high (n = 32) exacerbators is indicated by the solid vertical line.

TABLE II. Spearman correlation between VDP and clinical measures of lung function, inflammatory response, and asthma control status

Clinical measure	Correlation with VDP
FVC %P	$\rho = -0.45$; $P < .001$
ACQ	$\rho = 0.38$; $P < .01$
Sputum Eos level	$\rho = 0.31$; $P = .029$
Max reversibility	$\rho = 0.29$; $P = .018$
ACT	$\rho = -0.25$; $P = .038$
Blood Eos	$\rho = 0.16$; $P = .20$
FENO	$\rho = -0.01$; $P = .95$

Eos, Eosinophil; *Max*, maximum; *%P*, percent predicted.

threshold are shown in Table E1 (in this article's Online Repository at www.jacionline.org).

Regression models

Table III shows exacerbation IRR values with 95% CIs from negative binomial regression models of cumulative exacerbations gathered for each study participant. These include estimates from (1) individual univariate models for each predictor, (2) a multivariate model with all predictors included, and (3) a parsimonious multivariate model with a subset of predictors chosen by using stepwise model selection based on the Aikake information criterion. Note that because FEV_1 percent predicted and FEV_1/FVC were both correlated with FVC percent predicted as noted earlier, we chose to include only FVC percent predicted in the model-based analyses, because initial analysis (see Table I) showed that it was significantly reduced in the exacerbation versus no-exacerbation groups, whereas neither FEV_1 percent predicted nor FEV_1/FVC was significantly different across the 2 groups.

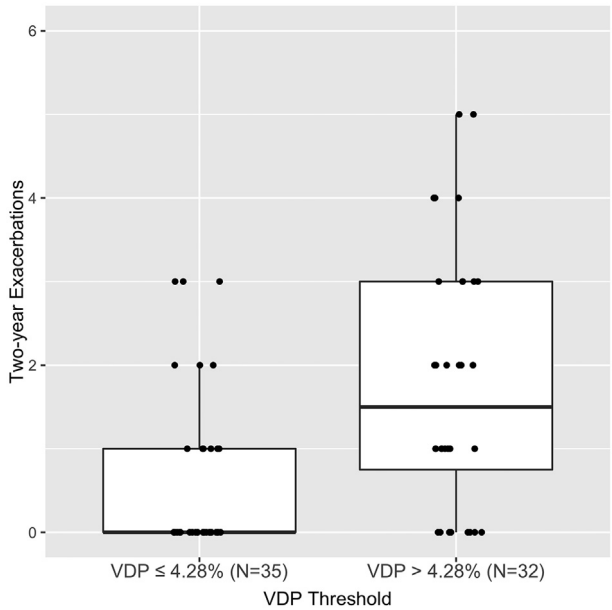


FIG 4. Box and whisker plot of 2-year exacerbation frequency for subjects above and below the VDP threshold. Subjects above the threshold experienced a median of 1.5 exacerbations versus 0 exacerbations for subjects below the threshold ($P < .001$).

In all 3 sets of models, VDP was a strong predictor of subsequent exacerbations, with subjects over the threshold having more than twice the exacerbation IRR as subjects at or below the threshold, as illustrated in Fig 5. In the univariate models, age, GERD, and FVC percent predicted were significant predictors in addition to VDP. In the full multivariate model, only VDP

TABLE III. Exacerbation IRRs with upper and lower limits of the 95% CIs from negative binomial regression models of exacerbation counts for each participant (n = 67)

	Univariate model			Multivariate model			Stepwise multivariate model		
Predictor	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**
VDP >4.28	2.97	1.68	5.40	2.29	1.08	4.97	2.46	1.32	4.70
Age (per 10-y period)	1.20	1.04	1.39	1.04	0.86	1.26	-	-	-
Female sex	1.35	0.72	2.56	1.44	0.77	2.72	-	-	-
BMI (per 10 pt)	1.12	0.70	1.80	0.64	0.38	1.05	0.69	0.44	1.06
ACT	0.98	0.90	1.05	1.05	0.95	1.16	-	-	-
ACQ	1.41	0.98	2.04	1.29	0.71	2.32	-	-	-
GERD	1.87	1.04	3.41	1.39	0.77	2.53	1.76	1.05	2.98
Sinusitis	1.52	0.81	2.87	1.52	0.85	2.68	-	-	-
Serum Eos level (log_2)	1.17	0.89	1.55	1.13	0.87	1.47	-	-	-
FENO level (log_2)	0.97	0.72	1.31	0.94	0.69	1.30	-	-	-
FVC (per 10%)	0.78	0.66	0.91	0.87	0.68	1.11	0.83	0.7	0.98
Max reversibility (per 10%)	1.11	0.91	1.38	1.05	0.81	1.37	-	-	-

- *(dash),* Predictor not chosen in by stepwise model; *Eos*, eosinophil; *Max*, maximum; *pts*, patients.
Transformations or multipliers to each variable are listed inline where applicable.

was a significant predictor. In the stepwise multivariate model, BMI, GERD, and FVC percent predicted were selected in addition to VDP, with GERD and FVC percent predicted being significant and BMI not being significant.

Incorporation of baseline ICS dose and exacerbation history

To test the predictive power of VDP when medical history was known, we ran 2 additional sets of models, each with 1 added predictor: in the first set of models, we added categoric ICS dose at baseline, and in the second set of models, we added the number of exacerbations in the year before baseline.

When categoric ICS dose at baseline was included in the multivariate model, VDP was no longer significant (IRR = 1.57 [95% CI = 0.74-3.39]), although it was selected in the multivariate stepwise model. Subjects taking a medium- or high-dose ICS were significantly more likely to experience exacerbation (IRR = 10.7 [95% CI = 1.7-208.6] and IRR = 9.5 [95% CI = 1.7-180.0], respectively) than were subjects with no ICS use. Complete results from these models are shown in Table E2 (in this article's Online Repository at www.jacionline.org).

When exacerbation history was incorporated into the multivariate model (IRR = 1.36 [95% CI = 1.20-1.6] per prior exacerbation), the VDP threshold no longer provided significant additional predictive power (IRR = 1.15 [95% CI = 0.53-2.53]). Complete results for these models are shown in Table E3 (in this article's Online Repository at www.jacionline.org).

DISCUSSION

We found that the VDP on hyperpolarized gas MRI is predictive of asthma exacerbation rate in the 2 years following imaging. VDP was a significant predictor of exacerbation rate in both univariate and multivariate regression models incorporating a suite of conventional demographic and clinical measures assessed at baseline. In each of these models, a VDP measurement over the threshold of 4.28% was associated with more than double the exacerbation rate for subjects with a VDP at or below the threshold. This finding adds to previously published results showing that VDP was associated with a history of 1 or more severe exacerbations before imaging.[9] However, the work presented here differs from and extends that result in several key aspects. First and foremost, the prospective rather than retrospective study design enables an assessment of predictive value rather than a retrospective association. Second, the definition of exacerbation in this work was broadened: rather than tracking severe clinical outcomes (ED visits and hospitalizations), exacerbations were defined as any event requiring 3 consecutive days of OCS use, thus allowing assessment of the ability of VDP to predict less severe outcomes. Finally, we tracked *cumulative* exacerbations (ie, exacerbation frequency) over a set time period of 2 years prospective to imaging instead of a binary indicator of exacerbation at any time before imaging.

We also found that FVC percent predicted was significantly lower in subjects who went on to have 1 or more exacerbations (Table I), a risk factor related to air trapping identified in previous studies,[21] whereas FEV_1 percent predicted, FEV_1/FVC, and FEV_1 reversibility following bronchodilator showed no significant difference. FVC percent predicted was also a significant predictor in our stepwise multivariate model of 2-year exacerbations (Table III), even when that model was expanded to include exacerbation history (see Table E1), highlighting the role of air trapping as a driver of instability in this population. Given that VDP is individually correlated with FVC percent predicted across the population as a whole, it is plausible that the increase in VDP in the exacerbation group is driven to a large extent by peripheral air trapping.

Consistent with this hypothesis is the fact that bronchodilator reversibility was not associated with exacerbation risk in this study. Although some previous literature has shown that increased bronchodilator response is associated with asthma exacerbation[3,22] this effect was not observed by Koga et al, who found that subjects with asthma with multiple exacerbations had persistent airflow limitation and reduced reversibility.[23] Notably, three-fourths of subjects in our study who experienced at least 1 exacerbation were already taking a high-dose ICS at the time of imaging as part of their clinical care. This treatment may be partially suppressing central airway hyperresponsiveness and thereby accentuating the relative roles of persistent airway limitation and peripheral air trapping (as reflected in decreased FVC percent predicted) in driving exacerbation.

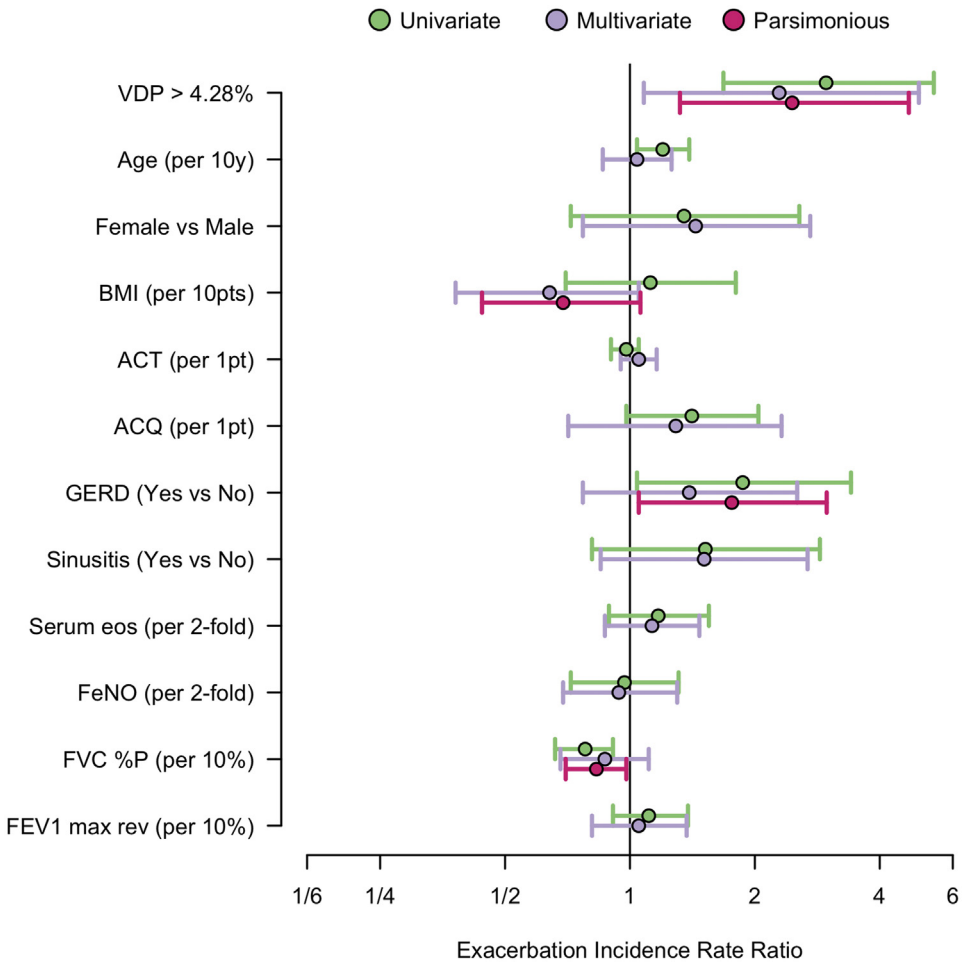


FIG 5. Exacerbation IRR and 95% CIs for model predictors. Within each predictor, the top line indicates the IRR in the univariate (individual) models, the second line indicates the inclusive multivariate model, and the third line (if present) indicates the parsimonious multivariate model. *Eos,* Eosinophil; *max rev,* maximum reversibility.

Interestingly, there were 8 subjects in our population who had a VDP greater than the threshold of 4.28% and yet did not go on to experience an exacerbation; 7 of these subjects were classified as having severe asthma under the SARP criteria. We did not see significant differences between the spirometry or inflammatory markers in these subjects and those in the 24 subjects who were also over the threshold and *did* go on to experience 1 or more exacerbations. However, only 2 of these 8 nonexacerbators (25%) had a record of an exacerbation in the year before imaging, versus 19 of 24 (79%) of the subjects who went on to experience exacerbation. This finding, together with the "severe" asthma status indicating a history of exacerbation and/or step-up in treatment, suggests that these subjects may have disease that has become more stabilized and controlled despite the presence of ventilation defects. In addition, mucus plugs scored on CT in asthma[24] were shown by Svenningsen et al to be associated with VDP,[11] and thus, we further speculate that some of these subjects may have persistent, long-term mucus plugging that is not associated with acute exacerbations. Research to test the associations of mucus plugs and air trapping on CT with regional defects is ongoing.

A previously published SARP III–wide analysis of 709 subjects[3] found that in a multivariate model (the basis for the models in this work), bronchodilator reversibility, blood eosinophil level, BMI, sinusitis, and GERD were associated with exacerbation frequency in the prior year. Here, we also found an association between prospective exacerbations and both GERD and sinusitis in univariate models, and with GERD in a stepwise multivariate model. Several of the predictors that we compared against VDP in this population have also been found to be associated with exacerbation risk in other asthma studies, including ACQ score,[25] blood eosinophil level,[26] and FENO.[27] However, we did not observe these associations in our population, possibly because of the relatively small study population compared with those of large-scale cohort studies. That a significant effect was observed for VDP despite the relatively modest size of our study population suggests that there is a potential for VDP to be a useful biomarker to support clinical research studies in smaller, more targeted populations, or even on an individual basis to guide therapy in difficult cases.

VDP was no longer a significant predictor of exacerbation in models that included either ICS dose category at baseline or

history of exacerbation. Specifically, subjects receiving a medium- or high-dose ICS were significantly more likely to experience exacerbation than were those not taking an ICS, and similarly, the number of exacerbations in the year before imaging was a stronger predictor of exacerbation than VDP when included in the comprehensive multivariate models. However, decision making regarding ICS dose is clearly based in a large part on medical history, and exacerbation history can be a subjective measure when applied on an individual basis. Thus, VDP may provide a useful quantitative means of assessing individual exacerbation tendency when disease status has changed over a short time period or when medical history is not currently reliable, such as when biologic therapy has recently been initiated or can no longer be administered. Because reducing exacerbations is an essential component of improving quality of life in asthma, longitudinal monitoring of VDP in a study population could provide a quantitative marker of individual exacerbation tendency and therapy response and also serve as a potential surrogate endpoint in clinical trials. VDP could also provide a means of screening to enrich for less stable disease and as a secondary endpoint for monitoring therapy response, thus enabling a potential reduction in both study size and duration. Thus, a VDP-based approach could provide a valuable means of assessing and monitoring response to therapy on a practical time scale for drug development or clinical decision-making.

Further work is needed to evaluate temporal variability, bias, and precision to confirm VDP as a surrogate biomarker of exacerbations. Understanding the time course of VDP with respect to the build-up to an exacerbation event, measuring repeatability of VDP during stability, and evaluating potential bias when disseminated across a network of sites participating in a trial are all necessary goals for future research. Developing a surrogate biomarker of asthma exacerbations would be of clear benefit in view of the pressing need for a more reliable means for assessing suitability for costly targeted therapies (eg, biologics such as mepolizumab or benralizumab) in patients with severe asthma and for monitoring individual treatment response to guide dosing and new therapy regimens for tapering other controllers such as OCSs.

The 2 primary limitations in this work are (1) the relatively small sample size and (2) a study design that did not enable a more refined assessment of the timing of prospective exacerbation frequency and severity with respect to imaging beyond simply counting the number of self-reported exacerbations (regardless of degree and time since imaging) over a 2-year period. Although the self-reporting survey was designed to minimize misclassification, this method is still subject to potential recall bias. In addition, a more refined time scale for measuring exacerbations could permit a more accurate assessment of the relationship between VDP magnitude and exacerbation severity/frequency via a statistical approach such as a Cox proportional hazards model. Incorporating specific records of ED visits and/or hospitalizations (indicators of severe exacerbation and significant cost drivers) was not possible because the frequency of those events was too low in a 2-year period to allow for a meaningful model; a larger cohort and/or longitudinal monitoring over a longer time scale would enable an analysis of these relatively rare events. However, the definition of exacerbation used here is inclusive of both these severe events and less severe exacerbations that nonetheless required a treatment escalation (ie, OCSs). That the association between VDP and exacerbation nevertheless persists suggests that VDP is sensitive to ventilation deficits associated with milder outcomes as well as with those that require immediate emergency care, as examined previously.[9]

We found that the VDP on hyperpolarized gas MRI is predictive of exacerbation frequency in the 2 years following imaging, corroborating previously published results showing that VDP was associated with a history of severe exacerbation. VDP was no longer significant once individual medical history was added to the models. Nonetheless, these results suggest that VDP may provide a quantitative approach for patient selection and for monitoring of individual treatment response in clinical trials, especially in subjects with an uncertain or unknown medical history, or when disease has recently progressed, requiring consideration of a step-up in therapy.

Clinical implications: Lung ventilation measurements using hyperpolarized gas MRI are predictive of asthma exacerbation and may be useful for risk assessment, for monitoring therapy response, and as a secondary end point in clinical trials.

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METHODS

Exclusion criteria

- History of premature birth before 35 weeks gestation;
- Unwillingness to receive an intramuscular triamcinolone acetonide injection;
- Evidence of unreliability or poor adherence to asthma treatment or study procedures;
- Planning to relocate from the clinical center area before study completion;
- Any other criteria that placed the subject at unnecessary risk in the judgment of the principal investigator and/or attending physician(s) of record;
- Currently participating in an investigational drug trial;
- Pregnancy during the characterization phase;
- Current smoking, smoking history of more than 10 pack-years if at least 30 years of age or smoking history of more than 5 pack-years if younger than 30 years of age (note: if a subject has a smoking history, no smoking within the past year); and
- Pulmonary disorders associated with asthma-like symptoms, including (but not limited to)
    - Cystic fibrosis,
    - Chronic obstructive pulmonary disease,
    - Vocal cord dysfunction (that is the sole ca*use* of asthma symptoms and at the principal investigator's discretion),
    - Severe scoliosis or chest wall deformities that affect lung function, and
    - Congenital pulmonary disorders.

Specific exclusion criteria for the imaging substudy at UW-M are shown in Fig E1. The category nondiagnostic image quality comprises 4 subjects with coil and/or delivery issues, 2 subjects with coverage that did not include the entire thoracic cavity, and 2 subjects with a signal-to-noise ratio less than 5.0.

Specific MRI acquisition parameters and techniques

MRI studies were performed by using a 1.5T Signa HDx GE scanner (GE Healthcare, Waukesha, Wis) with a flexible wrap single-channel volume receiver coil (IGC Medical Advances, Milwaukee, Wis) or a rigid-body single-channel volume receiver coil (Rapid Biomedical, Columbus, Ohio) depending on patient size. Both coils were tuned to operate at the resonant frequency of ^{3}He and decoupled from the body radiofrequency coil so that hyperpolarized ^{3}He MRI and proton MRI could be performed without moving the subject. The ^{3}He studies in this work were conducted by using a Polarean IGI.9600 polarizer (Polarean Imaging plc, Durham, NC) with use of the SEOP method described previously.[E1] A 4.5-mM dose of hyperpolarized ^{3}He mixed with N_2 to 14% of the subject's total lung capacity was prepared in a Tedlar bag purged of oxygen to slow T1 relaxation. The bag was delivered directly to the subject positioned supine in the scanner, and the gas was inhaled from functional residual capacity through a small plastic tube attached to the bag. Subjects were then instructed to hold their breath through a 16- to 20-second acquisition. Subjects were monitored continuously for blood oxygen saturation by using a pulse oximeter to assess safety during the anoxic breath-hold and to ensure adequate recovery. Specific MRI acquisition parameters are summarized in Table E4.

Study population descriptive statistics stratified by VDP threshold

Study population descriptive statistics stratified by VDP threshold are shown in Table E1.

Model results including ICS dose

Complete results for models incorporating ICS dose category at baseline (none, low, medium, and high) are shown in Table E2.

Model results including prior exacerbations

Complete results for models incorporating history of exacerbation are shown in Table E3.

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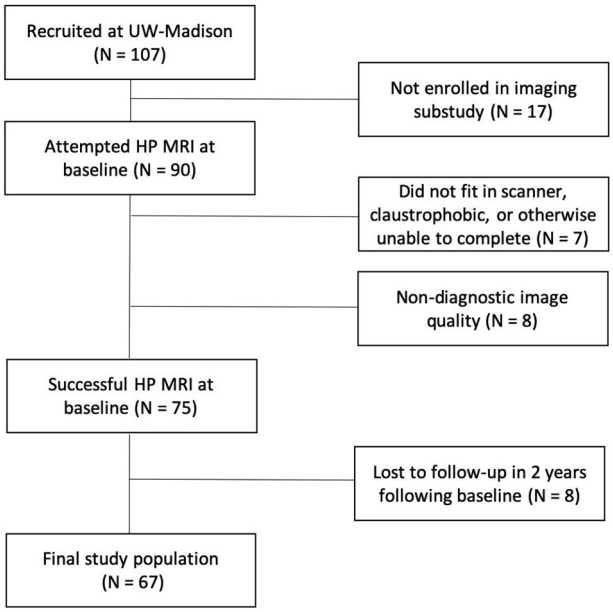

FIG E1. Specific exclusion criteria for the imaging substudy at UW-Madison.

TABLE E1. Summary of population characteristics stratified by VDP threshold derived from predictive model

Characteristic	VDP ≤4.28%	VDP >4.28%	Difference
No.	35	32	N/A
Severe asthma	12 (34.3%)	5 (78.1%)	$P = .08$
Sex (F)	19 (54.3%)	21 (65.6%)	$P = .34$
Age (y)	28.2 ± 16.5	48.2 ± 18.9	$P < .0001$
BMI	24.9 ± 5.7	29.9 ± 6.0	$P < .001$
FEV_1 %P	89.4 (82.0-98.4)	66.2 (53.2-94.2)	$P < .001$
FVC %P	97.7 (91.0-104.6)	79.3 (69.0-96.1)	$P < .001$
FEV_1/FVC	0.77 (0.74-0.80)	0.68 (0.62-0.75)	$P < .001$
ACQ	0.71 (0.6-1.1)	1.3 (0.7-2.0)	$P = .011$
ACT	21.0 (19.0-22.5)	19.0 (15.0-21.0)	$P = .029$
FENO level	20.0 (9.5-36.0)	20.0 (13.0-29.0)	$P = .98$
Blood eosinophil level (eosinophils/μL)	189.0 (118.5-375.5)	309.5 (156.2-519.2)	$P = .95$
Sputum eosinophil level (%)	0.35 (0.0-1.1)	0.85 (0.2-4.4)	$P = .15$
GERD	12 (34.3%)	18 (56.3%)	$P = .08$
Sinusitis	12 (34.3%)	9 (28.1%)	$P = .59$
Max FEV_1 reversibility following BD (%)	8.8 (4.3-13.1)	13.9 (7.8-19.6)	$P = .028$
Taking a LABA	17 (48.6%)	30 (93.8%)	$P < .0001$
Taking a high-dose ICS	17 (48.6%)	26 (81.3%)	$P < .01$
Taking a LABA + a high-dose ICS	14 (40.0%)	26 (81.3%)	$P < .001$
Cumulative 2-year exacerbations	0.0 (0.0-1.0)	1.5 (0.8-3)	$P < .001$

BD, Bronchodilator; *F*, female; *LABA*, long-acting β-agonist; *Max*, maximum; *N/A*, not applicable; *%P*, percent predicted.
Results given as means plus or minus SDs or medians (Q1-Q3). All measurements except for maximum FEV1 reversibility were acquired before bronchodilator administration.

TABLE E2. Exacerbation IRRs with upper and lower limits of the 95% CIs from negative binomial regression models of exacerbation counts for each participant (n = 67)

	Univariate models			Multivariate model			Stepwise multivariate model		
Predictor	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**
VDP >4.28%	2.82	1.59	5.12	1.57	0.74	3.39	1.86	0.97	3.68
Age (10 y)	1.20	1.03	1.39	1.12	0.94	1.34	1.12	0.96	1.31
Female sex	1.36	0.71	2.60	1.38	0.74	2.62	NA	NA	NA
BMI (per 10 patients)	1.13	0.70	1.86	0.60	0.35	1.02	0.57	0.34	0.94
ACT	0.98	0.90	1.05	1.08	0.97	1.20	1.08	0.99	1.20
ACQ	1.41	0.98	2.06	1.48	0.81	2.69	1.53	0.95	2.51
GERD	1.89	1.04	3.49	1.16	0.63	2.14	NA	NA	NA
Sinusitis	1.52	0.80	2.91	1.38	0.76	2.51	1.49	0.87	2.56
Serum Eos level (log)	1.18	0.89	1.56	1.06	0.81	1.4	NA	NA	NA
FENO level (log)	0.97	0.72	1.31	1.05	0.75	1.49	NA	NA	NA
FVC %P (per 10%)	0.84	0.72	0.96	0.96	0.79	1.17	NA	NA	NA
Max rev (per 10%)	1.11	0.90	1.39	1.04	0.78	1.37	NA	NA	NA
ICS low	7.20	0.84	153.37	3.63	0.41	77.57	3.9	0.45	82.71
ICS medium	20.57	3.61	390.71	10.7	1.73	208.61	10.82	1.8	208.94
ICS high	17.43	3.6	313.94	9.48	1.67	179.96	10.02	1.95	183.85

Eos, Eosinophil; *Max rev*, maximum reversibility; *NA*, variable was not chosen in stepwise multivariate model.

TABLE E3. Exacerbation IRRs with upper and lower limits of the 95% CIs from negative binomial regression models of exacerbation counts for each participant (n = 67)

	Univariate models			Multivariate model			Stepwise multivariate model		
Predictor	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**	**IRR**	**Lower**	**Upper**
VDP >4.28%	2.97	1.68	5.40	1.15	0.53	2.53	NA	NA	NA
Prior exac (per exac)	1.40	1.22	1.63	1.36	1.17	1.58	1.33	1.20	1.47
Age (10 y)	1.20	1.04	1.39	0.99	0.84	1.18	NA	NA	NA
Female sex	1.35	0.72	2.56	1.34	0.76	2.39	NA	NA	NA
BMI (per 10 pts)	1.12	0.70	1.80	0.75	0.48	1.16	NA	NA	NA
ACT	0.98	0.90	1.05	1.02	0.92	1.12	NA	NA	NA
ACQ	1.41	0.98	2.04	1.12	0.63	1.94	NA	NA	NA
GERD	1.87	1.04	3.41	1.86	1.08	3.24	1.82	1.15	2.90
Sinusitis	1.52	0.81	2.87	0.80	0.43	1.44	NA	NA	NA
Serum Eos level (log)	1.17	0.89	1.55	1.18	0.93	1.52	1.25	1.01	1.57
FENO level (log)	0.97	0.72	1.31	1.09	0.81	1.49	NA	NA	NA
FVC %P (per 10%)	0.78	0.66	0.91	0.79	0.63	0.99	0.81	0.70	0.93
Max rev (per 10%)	1.11	0.91	1.38	1.00	0.79	1.27	NA	NA	NA

Eos, Eosinophil; *Max rev*, maximum reversibility; *NA*, variable was not chosen in stepwise multivariate model; *pts*, patients.

TABLE E4. Hyperpolarized ^{3}He MRI acquisition parameters

MRI parameter	Value
Pulse sequence	Fast 2D gradient-recalled echo
TR/TE	7.7/4 ms
Flip angle	7°
Matrix	128 × 128 in plane
FOV	40 cm
Reconstructed voxel dimension	1.56 × 1.56 × 15 mm
Slices	Sufficient to cover lung volume (12-18 slices)

2D, Two-dimensional; *FOV*, field of view; *TE*, echo time; *TR*, repetition time.