Association between Depression, Lung Function and Inflammatory Markers in Patients with Asthma and Occupational Asthma

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Abstract

Objective: Depression is associated with autonomic and immune dysregulation, yet this remains poorly explored in asthma. We assessed associations between depressive disorder, lung function, and inflammatory markers in patients under investigation for occupational asthma (OA).

Methods: 112 patients under investigation for OA (60% men) underwent a psychiatric interview to assess depressive disorder, and spirometry, a methacholine test, sputum induction and specific inhalation challenge (SIC) to assess OA. Blood and sputum inflammatory markers were assessed.

Results: There was a statistically significant association between depressive disorder (p=.0195) and FEV_1 responses, with the drop in FEV_1 post-SIC smaller in patients with OA and depression, versus OA with no depression, ($p < .001$).

Conclusions: The presence of depressive disorder may influence FEV_1 in patients with OA, which may be via autonomic pathways. However, further studies are warranted in order to determine the mechanisms which underlie these effects.

Keywords: inflammation, depression, occupational asthma

Running title: Depression and Inflammation in Occupational Asthma

Abbreviations and Acronyms

ANS – Autonomic Nervous System

BMI – Body Mass Index

CRP – C-reactive Protein

DSM-IV – Diagnostic and Statistical Manual of Mental Disorders

FEV_1 – Forced Expiratory Volume in 1 second

ICS – Inhaled Corticosteroids

IL-6 – Interleukin-6

MDD – Major Depressive Disorder

NA – No Asthma

Non-OA – non-Occupational Asthma

OA – Occupational Asthma

PC_{20} - a significant fall in Forced Expiratory Volume in 1 second (FEV_1) which was greater than 20%

PRIME-MD - Primary Care Evaluation of Mental Disorders

SCID - Structured Clinical Interview for DSM

SIC – Specific Inhalation Challenge

Introduction

Asthma is a chronic respiratory disease which is characterized by airway inflammation and reversible airflow limitation [1], and is one of the most common diseases in the world [2]. Between 10 to 25% of new adult onset asthma is attributed to work exposure [3]. Asthma related to work can be classified as either work-aggravated asthma or occupational asthma, the latter being caused by either immunologically-mediated sensitization or by exposure to high concentrations of an irritant. Classically, occupational asthma (OA) refers to sensitization and is defined as a respiratory disease with variable airflow limitation and/or airway hyper-responsiveness in response to exposure to stimuli present in an individual's work environment, and not to stimuli encountered outside the workplace [4]. The most common causal agents in developed countries are isocyanates in paints and industrial processes, flour, chemical agents (e.g., fluxes, enzymes), wooddusts, drugs, proteins (e.g., plant and animal-based), metals, resins and glues, latex, cereals and grains [4,5].

Psychological stress, which refers to the emotional and physiological reactions experienced when an individual confronts a situation in which the demands go beyond their coping resources [6], is also believed to contribute to the burden of disease in both asthma and OA [7]. Indeed, there is a disproportionately high rate of depression (which reflects excess feelings of sadness and/or loss of interest in performing the activities of daily life)[8] among patients with asthma and OA. In an early study, we found depressive symptoms causing clinically significant levels of psychological distress in almost half (47.5%) of 40 confirmed subjects with OA [9]. More recently, we have reported rates of depressive disorders (including major depression, minor depression and dysthymia, which reflect clinically significant levels of depression [8]) among asthma and OA patients of 20% and 29% respectively [10,11]. Given that depression has been associated with increased asthma severity, increased use of emergency services, increased symptoms reporting, poorer pulmonary function, lengthier hospital stays, increased use of reliever medication, worse asthma control, and worse asthma-related quality of

life among asthmatics in general [12-16], it has been suggested that depression may also play a role in OA.

Depression has been shown to be associated with dysregulated autonomic and immune responses [17-22], and there is some evidence linking stress-related depression with altered autonomic function (e.g., decreased lung function) in asthmatic children [23] and increased inflammatory responses in atopic asthmatics [24]. However to our knowledge, no studies have assessed how depression affects these responses in patients with OA. The objective of the current study was to explore the associations between depression (depressive disorder), lung function, and inflammatory markers in patients under investigation for occupational asthma (OA) with eventual diagnoses of OA, non-Occupational asthma (Non-OA) and no asthma (NA).

Methods

Participants

This is a sub-study of a prospective cohort study where aspects of the methods have been reported elsewhere [11,25]. Briefly, 219 consecutive participants who had been refered for possible work-related asthma were recruited from the outpatient asthma clinic of the CIUSSS-NIM (formerly Hôpital du Sacré-Coeur de Montréal) from December 2004 to December 2008. Participants were eligible if they were between 18 and 75 years of age and fluent in English or French. Participants were excluded if they had a previously diagnosed comorbid medical condition conferring greater risk for morbidity than asthma (e.g., lung cancer), severe psychopathology (e.g., schizophrenia) or substance abuse. Of the 247 participants who presented to the clinic for investigation of OA during the study period, 241 were approached to participate, and 219 agreed to be in the main study. For this sub-study, only participants who were diagnosed as having OA, non-Occupational asthma (Non-OA) or no asthma (NA) were included, which yielded a final sample of 112 participants (see Figure 1). Final diagnosis of OA, Non-OA and NA was determined based on FEV_1, clinical characteristics including blood cell

counts and variability in the responses to methacholine assessment. This study was approved by the research ethics committee of CIUSSS-NIM (formerly Hôpital du Sacré-Coeur de Montréal), and written informed consent was obtained from all participants.

Procedure and Measures

Sociodemographic and Medical Information

Sociodemographic information and medical history (including medications) was obtained using a brief structured interview. Participants self-reported smoking history and height and weight were used to calculate body mass index (BMI). Adherence to daily ICS medication (if applicable) was also self-reported. All clinical data were verified by medical chart review.

Depression

Participants underwent a brief (approximately 15 minute) structured psychiatric interview, the Primary Care Evaluation of Mental Disorders (PRIME-MD) [26] to assess the presence of a depressive disorder including major depressive disorder (MDD) and other depressive disorders (minor depression, dysthymia). The PRIME-MD generates clinical diagnoses based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [27] criteria, and has demonstrated reliability (Kappa = 0.71), sensitivity (83%) and specificity (88%) for the diagnosis of MDD comparable to longer interviews such as the Structured Clinical Interview for DSM, (SCID) [26,28]. The interview was administered by a single, trained clinical research assistant who was supervised by a licensed clinical psychologist (KLL).

Physiological Measures: Spirometry, Blood and Sputum

Spirometry and OA Evaluation

The full procedure has been described fully elsewhere [29], and is described briefly below. Firstly, spirometry was assessed per the American Thoracic Society guidelines [30]. Methacholine

inhalation challenge was performed with a Wright nebulizer (output 0.14 mL/min) at tidal volume breathing for 2 minutes as described elsewhere [31]. Sputum was induced using inhalations of increasing concentrations of hypertonic saline solution (3-5%) and then processed [32]. Blood was drawn in every participant before the SIC challenge to obtain a blood cell count [29]. Blood measures included: total white blood cell count, and the cell counts for neutrophils, eosinophils and lymphocytes. Sputum measures included: total cell count by weight (10^6 c/ml), and the respective total cell counts and percentage counts of neutrophils, eosinophils, and lymphocytes.

Specific inhalation challenges (SIC) were performed in the laboratory or in the workplace [33]. On the first testing day, participants were exposed to a sham/inert substance (e.g., lactose or thinner) for 30 minutes to ensure that stability of their spirometry. Subsequently, participants were exposed to the occupational agent suspected of causing their symptoms. If the SIC to this suspected substance was negative or if exposure in the workplace could not be replicated in the laboratory, the participants returned to their workplace with a respiratory technologist where their respiratory function was assessed hourly over a 7-hour period for 2 consecutive days. A positive SIC test and diagnosis of occupational asthma (OA) was defined by a significant fall in Forced Expiratory Volume in 1 second (FEV_1) which was greater than 20% after exposure (PC_{20}), inconjunction with other characteristics including variation of repeated methacholine tests, increased cellularity of blood and eosinophil profile (e.g., heightened immune profile in blood). At the end of the control day, and at the end of the exposure day a methacholine challenge and sputum induction were completed. Long-acting β2-agonists and short-acting β2-agonist were stopped for 72 hours and 8 hours respectively before the challenge; inhaled corticosteroids (ICS) use was maintained throughout the SIC test, however the total daily dose was administered 12 hours prior to the SIC or in the evenings of the SIC days.

At the end of the OA evaluation process, participants were classified as having occupational asthma (OA); pre-existing or non-occupational asthma (Non-OA); or no asthma (NA) (further

information as to diagnosis procedure has been described fully elsewhere [34]). Those meeting diagnostic criteria for another non-asthma-related diagnosis (e.g., rhinitis, hyperventilation syndrome, eosinophilic bronchitis, n= 54) were excluded from the present sub-study, plus participants who did not complete an adequate SIC (n=30) (see Figure 1).

Data Analysis

All analyses were completed using SAS version 9.4 (Cary, NC). Prior to statistical analysis, the distributions of variables were examined. Variables demonstrating notable skewness were log transformed to normalize the distribution. Means (SD) and proportions (percentages and numbers) were presented to describe continuous and categorical variables, respectively. A series of Chi-square statistics and General Linear Models were used to determine demographic and sample characteristics for categorical and continuous variables respectively. Using Rubin's rules [35] our missing data analysis procedures used multiple imputation [36] with missing at random (MAR) assumptions. Using the PROC MI method of multiple multivariate imputation in SAS, we independently analyzed 20 copies of the data. PROC MIANALYZE was used according to Harrell's guidelines [37]. Significance was set at $p < .05$ for all analyses. The primary analyses were general linear models (GLMs) examining the association between depressive disorder, final asthma diagnosis (OA; non-OA; NA) and our physiological outcomes of interest. These were 1) Pulmonary function (measured as FEV_1 reactivity, and PC20 responses to methacholine); 2) Mean percentage of sputum cell differentials percentages of neutrophils, lymphocytes and eosinophils in sputum); and 3) Total mean blood cell differentials (total blood cell counts for neutrophils, lymphocytes and eosinophils). These analyses were adjusted for age, sex, smoking status, symptoms post SIC test, and prescribed dose of ICS. Each physiological parameter was the dependent variable; the independent variables were depressive disorder and final asthma diagnosis. The potential interacting effects between depressive disorder and final asthma diagnosis were also assessed.

Results

Socio-demographic and medical characteristics

Demographic characteristics are summarized in Table 1 as a function of asthma group; there were group differences in the proportion of participants taking ICS and long acting asthma medications and in ICS dose, with more participants in the OA and Non-OA groups being prescribed asthma medications and having higher ICS doses than those in the NA group. As expected, there were also differences in the fall in FEV_1 during the SIC challenge and the total cell counts for eosinophils, with those in the OA group having greater drops in FEV_1 and higher eosinophil cell counts relative to those in the NA group. Further details on sputum immune concentrations can be found in supplementary table 1.

Pulmonary Function

Analyses were undertaken to determine the relationships between depressive disorder, asthma diagnosis, and pulmonary function (measured as FEV_1 reactivity, and PC20 responses to methacholine). As expected (see supplementary table 2), there was a main effect of diagnostic group (β=7.56 ± 0.95; 95%CI = 5.68-9.45; $p < .001$) on FEV_1 responses with the greatest decreases in FEV_1 observed in the OA group. There were also main effects of depressive disorder (β=3.59 ± 1.53; 95%CI = 0.57-6.60; $p = .0195$) on FEV_1 responses. Participants with a depressive disorder exhibited significantly smaller FEV_1 decreases FEV_1 compared to those without a depressive disorder (-7.03% ± 6.56% vs -11.69% ± 10.24%), irrespective of asthma diagnosis group. Analysis of the impact of depression in each group revealed that in participants with OA, we observed a specific effect of depressive disorder on FEV_1 responses (OA without depression vs OA with depression; -21.5% ± 8.50% vs -12.1% ± 9.70; $p < .001$). This suggests that among OA participants, having a depressive disorder

attenuates (decreases) FEV_1 drops in response to SIC challenge. There was no specific effect of depressive disorder on FEV_1 in either the Non-OA or NA participants.

In participants with a depressive disorder, we observed specific differences in FEV_1 responses between participants with OA and NA (-12.05% ± 9.70% vs -4.89% ± 1.88%; p = .025), with unsurprisingly larger decreases in FEV_1 observed in OA participants. Likewise, there were differences in FEV_1 between participants with OA and Non-OA (-12.05% ± 9.70% vs -4.55% ± 2.75%; p = .003), where again OA participants had greater decreases in FEV_1. There were no differences seen between the FEV_1 responses of participants with depression and Non-OA or NA (Non-OA =-4.55% ± 2.75% vs -4.98% ± 1.88% (OA); p = .43). As seen in Figure 2, in participants without a depressive disorder, there was a similar pattern of response. There was no depressive disorder by diagnostic group interaction effects (p > .05).

There were no main effects of depressive disorder on PC_{20} responses (β=0.01 ± 0.11; 95%CI = -0.20-0.22; p = .92), however there was a main effect of diagnostic group (β=0.27 ± 0.07; 95%CI = 0.12-0.42; p = .0003) revealing, unsurprisingly, higher (better) $LogPC_{20}$ responses in participants with NA in contrast to participants with OA (p < .001) and Non-OA (p = .003). There were no differences in PC_{20} responses from pre-SIC to post-SIC testing between participants with OA vs Non-OA (p = .43) and there was no interaction between depressive disorder and diagnostic group (p > .05).

Immune Parameters

Sputum cell differentials

Mean sputum cell differentials can be found in Table 2, with total sputum cell counts in Supplementary table 3. Depressive disorder was not associated with pre-SIC sputum total cell counts (β=-0.28 ± 1.35; 95%CI = -2.94 – 2.37; p = .83). Depressive disorder was also not associated with % counts for neutrophils (β=1.48 ± 4.88; 95%CI = -8.08 – 11.05; p = .76),

eosinophils (β=0.09 ± 1.87; 95%CI = -3.57 – 3.75; *p* = .96) or lymphocytes (β=0.49 ± 0.40; 95%CI = -0.29 – 1.27; *p* = .22). There were no main effects of diagnostic group for sputum total cells (β=-1.05 ±0.81; 95%CI = -2.66-0.55; *p* = .20). For the percentage of immune markers that were measured in sputum, there were no main effects of diagnostic group for % sputum neutrophils (β=-3.33 ± 3.12; 95%CI = -9.45-2.79; *p* = .29), or % lymphocytes: (β=-0.12 ± 0.25; 95%CI = -0.61-0.37; *p* = .62), but there was a main effect for % eosinophils, (β=-4.96 ± 1.17; 95%CI = -5.55 - -3.88; *p* < .001). Post-hoc analyses showed lower % eosinophils in participants with Non-OA and NA compared to OA (*p* < .001). However, there were no significant differences in % eosinophils in participants with Non-OA vs NA (*p* = .86).

Diagnosis group was not associated with pre-SIC sputum total cell counts (β=-1.05 ± 1.35; 95%CI = -2.66 – 0.55; *p* = .20). There were no interactions between depressive disorder and diagnostic group for any of the sputum cell counts or percentages (*p's* > .05) (Table 2).

Blood cell differentials

Mean blood cell differentials can be found in Table 3. There was a main effect of depressive disorder for pre-SIC neutrophils and lymphocytes. Depressive disorder was associated with higher blood neutrophils (β=0.05 ± 0.02; 95%CI = 0.01 – 0.10; *p* = .013) and lower lymphocyte cell counts (β=-0.05 ± 0.02; 95%CI = -0.09 – -0.02; *p* = .004). Post-hoc analyses showed that there was also a marginal effect of depressive disorder for neutrophils in participants with NA, with higher neutrophils in those with a depressive disorder compared to those with OA or Non-OA (*p* = .058). There was a similar effect of depressive disorder on lymphocyte counts in participants with NA but not OA or Non-OA (*p* = .019), with lower lymphocytes seen in those with a depressive disorder. There was no relationship between depressive disorder and eosinophil counts (β=0.002 ± 0.006; 95%CI = -0.01-0.01; *p* = .70).

Similarly, there was a main effect of asthma diagnosis for neutrophils and lymphocytes. These effects are summarized in Table 3. There was a relationship between asthma diagnosis

group and lymphocytes (β=0.03 ± 0.01; 95%CI = 0.01 – 0.05; p = .010), where lymphocytes were lower for OA compared to NA (p = .011) but not for OA vs Non-OA (p = .11) or Non-OA vs NA (p = .79). There was also a relationship between asthma diagnosis and neutrophils, (β=-0.028 ± 0.01; 95%CI = -0.06 - -0.001; p = .044), with a similar pattern observed whereby neutrophils were higher for OA compared to NA (p = .054) but not for OA vs Non-OA (p = .09) or Non-OA vs NA (p = .72). There was no effect of group on eosinophils cell count (β=-0.00 ± 0.00; 95%CIs = -0.01 – 0.01; p = .99). There was no depressive disorder by asthma diagnosis group interaction effects (p > .05).

Discussion

The aim of the study was to examine the association between depressive disorder and lung function and inflammatory responses in participants with OA, Non-OA, and NA. In summary, participants with (vs without) a depressive disorder exhibited smaller decreases in FEV_1 responses (i.e., the reduction in FEV_1 was greater in patients without depressive disorder). Further, we found that in participants with OA, having a depressive disorder was associated with lower decreases in FEV_1 post-SIC, which was not seen in the other two diagnostic groups. Having a depressive disorder was also associated with increased blood neutrophils and decreased blood lymphocytes, but only among those without asthma; a pattern that was not observed in sputum. Overall, these results suggest that depression is associated with attenuated Autonomic Nervous System (ANS) responses, particularly in participants with OA, and with dysregulated immune responses that are more pronounced in participants without asthma (but who present with asthma-like symptoms by virtue of them being under investigation for OA). This suggests that among those without asthma, depression may be accounting for at least some aspects of asthma-like symptom presentation via depression-related immune pathways [38].

Relationships between physiological measures and depression

Our results show that in participants with a depressive disorder and OA, that there was a smaller drop in FEV_1 that would seem to reflect less severe or attenuated airway responses. This contradicts previous studies showing that depression tends to increase or enhance ANS-driven responses in asthmatics [23]. This suggests that our findings point to a dysregulation of the ANS, rather than a upregulation of the ANS, which is a key characteristic of asthma [39] and consistent with some manifestations of depression [40-43]. For example, depression is associated with both increases *and* decreases in sleep, appetite and weight; it is also associated with both psychomotor agitation *and* retardation [8]. It is possible that among asthmatics whose ANS is already upregulated, that depression may interfere with this, resulting in an attenuation of ANS-driven responses [44] including FEV_1. Depression may also attenuate FEV_1 drops through behavioral pathways. A cardinal feature of depression is decreased motivation and fatigue [8], which may have influenced performance of spirometry which is in part behaviorally mediated [30]. Depression has been previously shown to interfere with the measurement of lung volumes [45], so it is possible that depressed participants may have been less performant during spirometry testing, resulting in lower drops in FEV_1. Further studies directly assessing spirometric performance in depressed vs. non-depressed participants and its impact on diagnosis and outcomes are needed.

There was no association between depressive disorder and PC_{20} responses post-SIC, suggesting similar levels of airway obstruction and hyper-reactivity across participants with and without depression regardless of asthma status. It should be noted that airway obstruction in asthma is variable such that mild asthmatics often display normal spirometry between periods of acute exacerbations. Indeed, most participants under investigation for OA present with normal lung function when FEV_1 and FVC are initially measured in the clinic [46]. Furthermore, even confirmed cases of OA may present with normal bronchial reactivity as captured by PC_{20} in the clinic, especially if tested after several days away from the causal workplace environment [46,47].

Therefore it is possible that in a non-clinic or 'real life' setting that there is an effect of depression but that this is not evident in our results. Other studies have also shown no effect of depression on PC_{20} responses in asthma patients [48,49], so it is perhaps not surprising that we did not see this in our participants who were undergoing assessment for OA.

Interestingly, immune analyses demonstrated that having a depressive disorder was associated with an increased number (and proportion) of neutrophils and a decreased number (and proportion) of lymphocytes in blood pre-SIC challenge. However, this was only observed in participants without asthma and not seen in participants with OA or Non-OA. These findings are consistent with previous depression research in the general population [50,51] and with the general finding that depression is linked to increases in immune markers, however this was not the case for lymphocytes in our sample which is counter to previous research [50,52,53]. Our results add to the growing evidence that there is a relationship between depression and airway immune markers in asthma populations [54,55]. However, these immune markers were derived from pre-SIC assessments of blood and sputum, and as such our results need to be interpreted cautiously relative to the rest of the literature.

Surprisingly, we did not observe any significant relationships between depressive disorder and sputum measures of inflammation post-SIC challenge. To our knowledge, this is the first study to examine relationships between depressive disorder and sputum lymphocyte populations in both OA and Non-OA populations. While blood lymphocytes may provide information about global immune alterations, sputum lymphocytes are of interest in asthma research because they provide more direct observation of immune function in the airways. Our results are somewhat contrary to those of Liu et al, who assessed the inflammatory responses during antigen challenge among medical students with asthma during a "high stress" period (exam stress) [56]. Liu and colleagues found that both sputum eosinophils and eosinophil derived neurotoxin levels increased in response to antigen challenge during the stress period, which were observed in the context of significantly increased levels of anxiety and depression [56]. Thus,

it was surprising to see no relationship to eosinophil counts in relation to depression in this study. One possible explanation is that we were investigating responses to SIC tests rather than antigen challenge, where patterns of physiological responses may differ. Additionally, given that both asthma and depression are inflammatory diseases, it is possible that in participants with both depression and asthma, there is no direct relationship with early cascade immune markers as the immune system is already flooded with more late-phase cascade inflammatory markers. However, as it was beyond the scope of the study to examine inflammatory markers such as Interleukin-6 (IL-6) and C-reactive protein (CRP), which are more typically related to depression [57], we cannot ascertain if this theory is accurate, and further investigation in participants with different asthma diagnoses is needed to help determine the relationships to sputum inflammation and depression in asthma.

Our study contains some potential limitations that should be noted. Firstly, as the study is cross-sectional, future longitudinal research will be required to examine the potential causal effects of depression over time and the possible mediating role of immune parameters on measures of respiratory function. To explore this, one direction for future studies could be to objectively measure physiological responses in depressed versus non-depressed participants with OA, Non-OA or NA, capturing respiratory and immune function before and after laboratory exposure to a psychological stressor or a physiological challenge (e.g., antigen exposure). Our inability to examine additional markers of inflammation (e.g., IL-6, CRP) which are more typically assessed in the context of depression should also be considered a limitation.

Despite these limitations, the present study also has several important strengths. It is the first to examine depressive disorder in relation to clinical and physiological characteristics in participants with OA and Non-OA compared to NA. Though our sample size is modest at 112, it is considered very large for this highly specialized patient population (participants under investigation for OA). Relative to the broader asthma literature, this sample was well defined and included verified medication prescriptions and objectively assessed asthma and depression

diagnoses. All our measures (blood, sputum, SIC testing) were completed in a specialized clinic, which enhances the validity of our diagnoses. Finally, analyses adjusted for many important covariates (e.g., age, sex, smoking status, symptoms post SIC test, and prescribed dose of inhaled corticosteroid).

Conclusion

In summary, our study demonstrated interesting and novel links between depression, ANS-influenced measures of lung function (FEV_1) and immune markers in an understudied population of participants undergoing testing for OA. Overall, our results suggests that depression is potentially associated with attenuated ANS responses, particularly in participants with OA, and with dysregulated immune responses that are more pronounced in participants without asthma. These results add to the extant literature linking depression to altered physiological response patterns. Findings suggest that depression may influence respiratory and immune responses to pulmonary function and SIC tests among participants undergoing evaluation for OA, indicating that depression screening may be warranted to improve interpretation of diagnostic findings.

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Table 1. ***Participant characteristics as a function of diagnostic group***

	Occupational Asthma (N=40)	**Non-Occupational Asthma (N=37)**	**No Asthma (N=35)**	**F value or X^2 value**	**p**	**Missing N**
Sociodemographics						
Age (years)	41.16 ± 12.48	42.51 ± 11.57	40.01 ± 10.02	0.43	.65	0
Years of Education	11.2 ± 3.25	11.57 ± 2.93	11.97 ± 2.27	0.68	.51	0
BMI (kg/m^2)	27.55 ± 6.19	27.78 ± 5.81	27.16 ± 6.33	0.09	.91	1
Sex (% Men, [N])	62.5 [25]	59.5 [22]	57.1 [20]	0.23	.89	0
Cohabitation (%[N])	52.5 [21]	64.9 [24]	65.7 [23]	1.77	.41	0
Presence of any psychiatric disorder (%[N])	32.5 [13]	23.4 [12]	48.6 [17]	2.66	.26	0
Depressive disorder (%[N])	27.5 [11]	29.7 [11]	37.1 [13]	0.87	.65	
Smoking status (%[N])				2.81	.59	0
Current smoker	20 [8]	32.4 [12]	28.6 [10]			
Past smoker	35 [14]	37.8 [14]	40 [14]			
Never smoker	45 [18]	29.7 [11]	31.4 [11]			

	Occupational Asthma (N=40)	Non-Occupational Asthma (N=37)	No Asthma (N=35)	F value or X^2 value	p	Missing N
Clinical Characteristics						
Max % drop in FEV_1 for control day	-3.75 ± 4.00	-5.05 ± 4.30	-3.01 ± 2.82	2.68	.0735	4
Max % drop in FEV_1 for SIC day	-18.93 ± 9.72	-5.86 ± 5.69	-4.79 ± 2.74	49.55	<.001	2
Geometric Mean PC_{20} (mg/ml) - control day	19.57 ± 29.61	7.42 ± 13.29	81.34 ± 48.15	44.68	< .0001	13
Geometric Mean PC_{20} (mg/ml) - SIC day	15.39 ± 32.28	9.04 ± 25.20	92.39 ± 44.09	51.97	<.001	21
Total dose of inhaled corticosteroids (µg/day)	682.69 ± 531.78	921.88 ± 771.04	173.91 ± 372.60	10.36	<.0001	31
Taking short-acting asthma medication (%[N])	80 [32]	89.2 [33]	68.6 [24]	4.7	.095	0
Taking inhaled corticosteroids (%[N])	90 [36]	86.5 [32]	51.4 [18]	18.5	< .001	0
Taking long-acting asthma medication (%[N])	40 [16]	62.2 [23]	22.9 [8]	11.5	.0032	0

	Occupational Asthma (N=40)	Non-Occupational Asthma (N=37)	No Asthma (N=35)	F value or X^2 value	p	Missing N
Immune markers						
SIC day – sputum only						
Total sputum cell count for SIC day (10^6 c/g)	5.90 ± 6.36	4.66 ± 6.95	2.39 ± 4.57	2.17	.12	28
Total sputum neutrophil count for SIC day (10^6 c/g)	3.28 ± 5.21	3.01 ± 6.16	1.30 ± 3.53	1.06	.35	28
Total sputum eosinophils count for SIC day (10^6 c/g)	0.71 ± 0.94	0.11 ± 0.31	0.02 ± 0.03	10.53	<.001	28
Total sputum lymphocytes count for SIC day (10^6 c/g)	0.13 ± 0.20	0.10 ± 0.16	0.02 ± 0.04	2.98	.0565	28
Percentage sputum neutrophil count for SIC day (10^6 c/g)	45.76 ± 24.87	46.47 ± 26.85	39.01 ± 23.12	0.65	.52	28
Percentage sputum eosinophils count for SIC day (10^6 c/g)	12.21 ± 14.48	2.10 ± 5.13	10.7 ± 1.87	11.94	< .0001	28
Percentage sputum lymphocytes count for SIC day (10^6 c/g)	2.20 ± 2.44	1.88 ± 2.13	1.22 ± 1.39	1.45	.24	28

SIC day – blood only						
Total blood neutrophil count for SIC day (10^6 c/g)	0.64 ± 0.13	0.60 ± 0.08	0.60 ± 0.11	1.83	.17	11
Total blood eosinophils count for SIC day (10^6 c/g)	0.03 ± 0.03	0.02 ± 0.02	0.03 ± 0.04	0.31	.73	12
Total blood lymphocytes count for SIC day (10^6 c/g)	0.25 ± 0.10	0.30 ± 0.07	0.31 ± 0.10	2.95	.060	12

Presented as Mean ± SD or Percent; Note: OA = occupational asthma; BMI = Body Mass Index; Max % drop in FEV_1 = Maximum % drop in forced expiratory volume in 1 second (FEV_1), expressed as a percentage of the patient's predicted value to the test; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1; ICS = inhaled corticosteroid steroid; SIC = Specific Inhalation Challenge

Table 2. Mean immune cell counts in pre-SIC sputum, presented by diagnostic group and depressive disorder.

	Occupational Asthma (OA)		Non-Occupational Asthma (Non-OA)		No Asthma (NA)			
	No depression	**Depression**	**No depression**	**Depression**	**No depression**	**Depression**	**Main effect of depression**	**Main effect of asthma diagnosis**
Total cell count for SIC day (10^6 c/g)	6.64 ± 7.17	2.47 ± 1.94	6.11 ± 8.69	3.48 ± 3.32	1.32 ± 0.74	1.84 ± 1.18	β=-0.28 ± 1.35; 95%CI = -2.94 – 2.37;	β=-1.05 ± 1.35; 95%CI = -2.66 – 0.55;
% neutrophil count for SIC day sputum	48.35 ± 25.8	41.24 ± 19.1	42.71 ± 27.94	45.44 ± 18.43	34.10 ± 19.8	32.88 ± 24.20	β=1.48 ± 4.88; 95%CI = -8.08 – 11.05;	β=-3.33 ± 3.12; 95%CI = -9.45-2.79;
% eosinophils count for SIC day sputum	12.02 ± 14.90	10.63 ± 9.53	2.76 ± 4.58[ab]	5.25 ± 8.43[b]	2.86 ± 5.27[ab]	6.06 ± 7.38[b]	β=0.09 ± 1.87; 95%CI = -3.57 – 3.75;	β=-4.96 ± 1.17; 95%CI = -5.55 - -3.88;
% lymphocytes count for SIC day sputum	2.23 ± 2.55	1.69 ± 1.42	1.87 ± 1.74	2.39 ± 2.62	1.39 ± 1.75	2.06 ± 1.47	β=0.49 ± 0.40; 95%CI = -0.29 – 1.27;	β=-0.12 ± 0.25; 95%CI = -0.61-0.37;

Note: [a] indicates significantly different from OA, No depression ($p < .05$); [b] = indicates significantly different from OA, Depression ($p < .05$);

Table 3. Mean immune cell counts in blood pre-SIC test, presented by diagnostic group and depressive disorder.

	Occupational Asthma (OA)		Non-Occupational Asthma (non-OA)		No Asthma (NA)			
	No depression	**Depression**	**No depression**	**Depression**	**No depression**	**Depression**	**Main effect of depression**	**Main effect of asthma diagnosis**
Total neutrophil count for SIC day (10^6 c/g)	0.621 ± 0.125	0.690 ± 0.119	0.594 ± 0.080 [a]	0.610 ± 0.080	0.574 ± 0.099 [a]	0.651 ± 0.116 [b]	β=0.05 ± 0.02; 95%CI = 0.01 – 0.10; *p* = .013	β=-0.028 ± 0.01; 95%CI = -0.06 - -0.001; *p* = .044
Total eosinophils count for SIC day (10^6 c/g)	0.027 ± 0.026	0.028 ± 0.030	0.023 ± 0.019	0.023 ± 0.020	0.027 ± 0.045	0.030 ± 0.033	β=0.002 ± 0.006; 95%CI = -0.01-0.01; *p* = .70	β=-0.00 ± 0.00; 95%CIs = -0.01 – 0.01; *p* = .99
Total lymphocytes count for SIC day (10^6 c/g)	0.271 ± 0.100	0.219 ± 0.102	0.305 ± 0.072 [a]	0.270 ± 0.055	0.337 ± 0.091 [a]	0.254 ± 0.097 [b]	β=-0.05 ± 0.02; 95%CI = -0.09 – -0.02; *p* = .004	β=0.03 ± 0.01; 95%CI = 0.01 – 0.05; *p* = .010

Note: [a] = indicates significantly different from OA, Depression ($p < .05$); [b] = indicates significantly different from NA, No depression ($p < .05$);

Online supplement
S1. Total sputum immune markers for the SIC day, presented as a function of asthma diagnosis.

	Occupational Asthma (N=40)	Non-Occupational Asthma (N=37)	No Asthma (N=35)	F value or X^2 value	p	Missing N
Immune markers						
SIC day – sputum only						
Total sputum cell count for SIC day (10^6 c/g)	5.90 ± 6.36	4.66 ± 6.95	2.39 ± 4.57	2.17	.12	28
Total sputum neutrophil count for SIC day (10^6 c/g)	3.28 ± 5.21	3.01 ± 6.16	1.30 ± 3.53	1.06	.35	28
Total sputum eosinophils count for SIC day (10^6 c/g)	0.71 ± 0.94	0.11 ± 0.31	0.02 ± 0.03	10.53	<.001	28
Total sputum lymphocytes count for SIC day (10^6 c/g)	0.13 ± 0.20	0.10 ± 0.16	0.02 ± 0.04	2.98	.0565	28

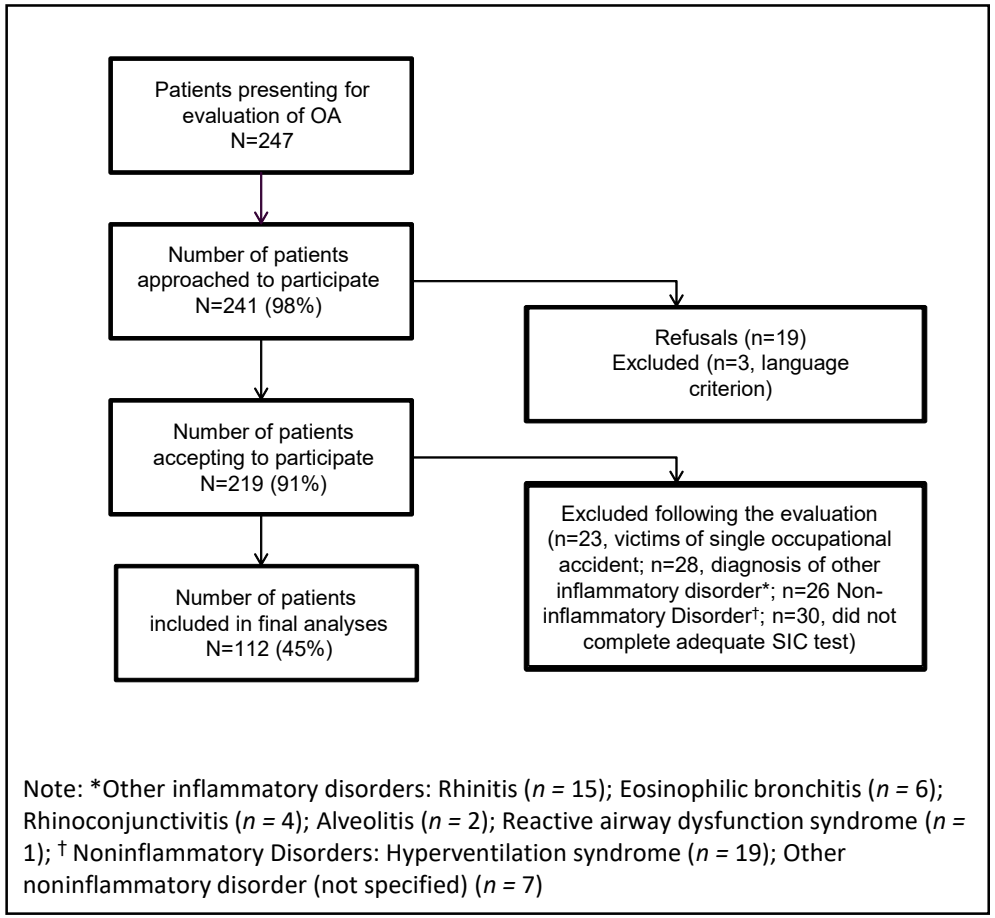
Table S2. FEV_1 responses, presented by asthma diagnosis and depressive disorder

	Occupational Asthma (OA)		Non-Occupational Asthma (Non-OA)		No Asthma (NA)			
	No depression	**Depression**	**No depression**	**Depression**	**No depression**	**Depression**	**Main effect of asthma group**	**Main effect of depressive disorder**
FEV_1 reactivity (%)	-21.54 ± 8.51	-12.05 ± 9.70	-6.42 ± 6.52	-4.55 ± 2.75	-4.96 ± 4.09	-4.89 ± 1.88	β=7.56 ± 0.95; 95%CI = 5.68-9.45; $p < .001$	β=3.59 ± 1.53; 95%CI = 0.57-6.60; $p = .0195$

Table S3. Total mean immune cell counts in sputum pre-SIC test, presented by diagnostic group and depressive disorder.

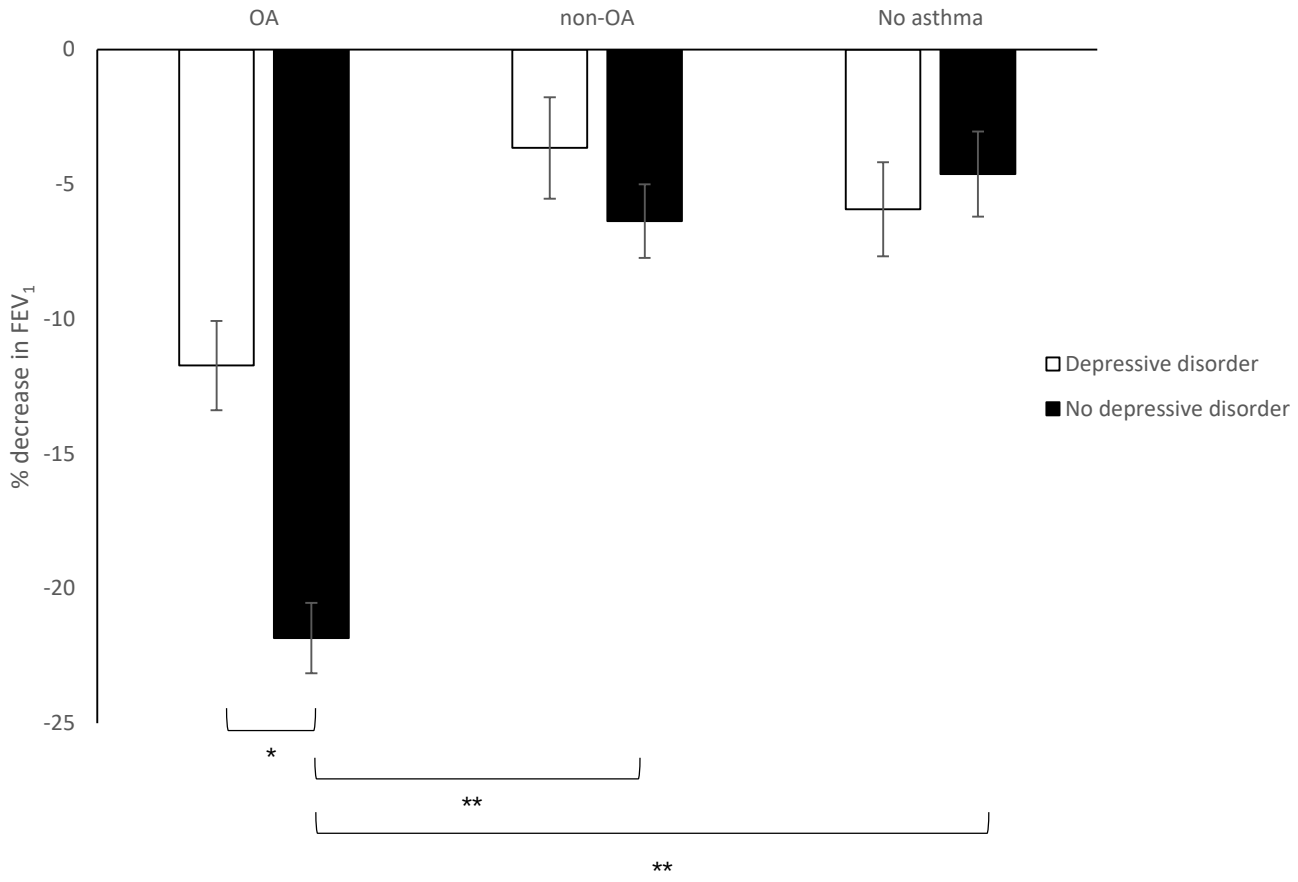
	Occupational Asthma (OA)		Non-Occupational Asthma (Non-OA)		No Asthma (NA)	
	No depression	**Depression**	**No depression**	**Depression**	**No depression**	**Depression**
Total cell count for SIC day (10^6 c/g)	6.639 ± 7.169	2.47 ± 1.936	6.114 ± 8.692	3.48 ± 3.321	1.318 ± 0.739	1.835 ± 1.176
Total neutrophil count for SIC day sputum (10^6 c/g)	3.852 ± 6.095	1.199 ± 0.957	4.237 ± 7.875	1.811 ± 2.182	0.538 ± 0.544	0.834 ± 0.597
Total eosinophils count for SIC day sputum (10^6 c/g)	0.859 ± 1.021	0.108 ± 0.198 [a]	0.076 ± 0.147[a]	0.214 ± 0.600	0.021 ± 0.029	0.022 ± 0.028
Total lymphocytes count for SIC day sputum (10^6 c/g)	0.149 ± 0.217	0.074 ± 0.095	0.129 ± 0.192	0.086 ± 0.095	0.013 ± 0.020	0.046 ± 0.056

Figure 1. Flow chart of participants



Note: *Other inflammatory disorders: Rhinitis (*n* = 15); Eosinophilic bronchitis (*n* = 6); Rhinoconjunctivitis (*n* = 4); Alveolitis (*n* = 2); Reactive airway dysfunction syndrome (*n* = 1); † Noninflammatory Disorders: Hyperventilation syndrome (*n* = 19); Other noninflammatory disorder (not specified) (*n* = 7)

Figure 2. FEV_1 responses (% decrease) by Asthma diagnosis and Depressive Disorder



Note: * = significantly different from no depressive disorder ($p < .001$); ** = significantly different from OA, no depressive disorder ($p < .05$)