Neutrophilic asthma is characterised by increased rhinosinusitis with sleep disturbance and GERD

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Summary

Background: **Asthma is a heterogeneous inflammatory disease and eosinophilic, non-eosinophilic and neutrophilic forms are recognised. While clinically similar to eosinophilic asthma, patients with non-eosinophilic asthma have different responses to treatment and little is known about the triggers of symptoms and inflammation.**

Objective: **This study sought to characterise asthma control, exacerbation frequency and potential triggers of non-eosinophilic and specifically neutrophilic asthma such as infection, gastroesophageal reflux disease, and rhinosinusitis.**

Methods: **Adults with asthma (n=65; doctor's diagnosis plus demonstrated response to bronchodilator and/or airways hyperresponsiveness to hypertonic saline) were recruited from the Respiratory and Sleep Medicine Ambulatory Care Service at John Hunter Hospital, NSW, Australia. Questionnaires were administered to assess gastroesophageal reflux disease, rhinosinusitis and asthma control. A sputum induction was performed and sputum was processed for assessment of inflammatory cells, infection, and lipid laden macrophages (Oil Red O).**

Results: **Participants with neutrophilic asthma (n=11, 23%) had a higher frequency of primary care doctor visits for asthma exacerbations and a high prevalence (>70%) of chest infections in the previous 12 months. There was also an increased prevalence of rhinosinusitis (64%) and increased symptoms of gastroesophageal reflux disease compared to those with eosinophilic asthma.**

Conclusions: **The clinical pattern of neutrophilic asthma is different from paucigranulocytic and eosinophilic asthma with evidence of abnormal upper airways responses. Specific and targeted treatment of these airway problems may assist in the control and management of neutrophilic asthma.** ***(Asian Pac J Allergy Immunol 2014;32:66-74)***

Key words: *asthma, rhinosinusitis, reflux, neutrophils*

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Introduction

Asthma is a heterogeneous inflammatory disease and non-eosinophilic forms of asthma are increasingly recognised as important inflammatory subtypes of asthma.[1-3] We have recently reported the presence of four distinct inflammatory subtypes of asthma where eosinophilic, neutrophilic, paucigranulocytic and mixed granulocytic inflammatory patterns have been identified.[4] There are few reported differences between clinical characteristics of the phenotypes, and little is known regarding the triggers of neutrophilic asthma.

Potential triggers of neutrophilic inflammation include infection, which can trigger an acute neutrophilic bronchitis, gastroesophageal reflux disease (GERD) with aspiration and rhinosinusitis. The role of acute infection in exacerbations of asthma is well known and viral infections result in the majority of acute exacerbations and many hospitalisations. The presence of GERD and rhinosinusitis in the airways of patients of different asthma inflammatory subtypes is unknown but could result in chronic inflammation with influx of neutrophils into the airways.

The aims of this study were to investigate differences in asthma control and exacerbation frequency between asthma inflammatory subtypes, to examine potential triggers of neutrophilic asthma including the frequency of viral and bacterial

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pathogens, and the presence of GERD and rhinosinusitis across asthma inflammatory phenotypes. We hypothesised that neutrophilic asthma would be associated with poor asthma control, an increased exacerbation frequency, presence of pathogens, and higher rates of GERD and rhinosinusitis

Methods

Participants

In this observational cross-sectional study we recruited adults with asthma (n=65) in a tertiary care setting, from the Respiratory and Sleep Medicine Ambulatory Care Service at John Hunter Hospital, NSW, Australia between 2008-2011. Patients were eligible to be included as a participant if they had a doctor's diagnosis plus demonstrated response to bronchodilator and/or airways hyperresponsiveness to hypertonic saline. Patients were not eligible to participate if they were current smokers, had a current or recent chest infection (past 4 weeks), had altered their asthma therapy (past 4 weeks), and/or had a primary diagnosis of an airway disease other than asthma. Participants gave written informed consent. The Hunter New England Area Health Service and University of Newcastle Research Human Ethics Committees approved this study (approval number 07/10/24/5.03).

Design

Participants attended two study visits, at the first visit an assessment of symptoms including cough, atopy (skin prick test), medication use, smoking status, spirometry and sputum induction was undertaken. Questionnaires were administered to examine gastroesophageal reflux disease,[5] rhinosinusitis (SNOT 20)[6] and asthma control (ACQ).[7] Sputum was processed for assessment of inflammatory cells, infection, and lipid laden macrophages (Oil Red O).

Smoking

A smoking history was elicited and smoking pack-years determined. Past smoking history was not used as an exclusion criterion. Participants were asked to provide an exhaled carbon monoxide (eCO) measurement, determined by electrochemical detection using a Smokerlyzer (Bedfont Kent UK; detection limit of 1ppm). All included participants had an eCO of less than 10ppm confirming their non-smoking status.

Pulmonary Function Tests

Participants withheld bronchodilators for their duration of action before testing. Three reproducible measurements of FEV_1 and FVC were obtained (KoKo PD Instrumentation USA) before and after inhalation of 200µg salbutamol via a metered dose inhaler with valved holding chamber (Volumatic, Allen and Hanbury's, Australia) using predicted values according to Knudson et al.[8] Hypertonic (4.5%) saline bronchoprovocation was performed as described previously.[9]

Sputum induction and analysis

Sputum induction with hypertonic saline (4.5%) was performed as previously described.[10] The selected sputum portions were dispersed using dithiothreitol (DTT).[10] A total cell count of leucocytes and viability was performed on the filtered suspension. Following centrifugation, supernatant was stored at -80°C. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained from 400 non-squamous cells. Inflammatory subtype was assigned as eosinophilic asthma where sputum eosinophils ≥3%, neutrophilic asthma where sputum neutrophils ≥61%, paucigranulocytic asthma where sputum eosinophils <3% AND sputum neutrophils <61%, mixed granulocytic asthma where sputum eosinophils ≥3% AND sputum neutrophils ≥61%.[11]

Lipid laden macrophage (LLM) index

Induced sputum cytospins were stained with Oil Red O (Sigma-Aldrich Sydney, Australia) to detect cytoplasmic lipid droplets. One hundred consecutive macrophages were evaluated using a five-point scale,[12] (0: the macrophage has no inclusions at all; 1: the macrophage has a couple of inclusions; 2: the macrophage has approximately 1/2 of cell covered in inclusions; 3: the macrophage has approximately 2/3 of cell covered in inclusions; 4: the entire macrophage is covered in inclusions). The LLM index was calculated by multiplying the number of macrophages by the grade given then adding up all the scores. All assessments were carried out by the same laboratory scientist, who was blinded to the clinical details of the participants.

Microbiology/Virology

Presence of viral infection was determined using real-time PCR as previously described.[13] Sputum RNA was extracted, reverse transcribed to cDNA and combined with master mix (5'Prime, Hamburg, Germany) and virus primers for either rhinovirus , enterovirus , respiratory syncytial virus types A and B and metapneumovirus, and influenza types A and B, or coxsackie virus in a real-time PCR reaction (ABI Real Time PCR System Applied Biosystems,

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Table 1. Participant demographics and sputum cell counts from participants with neutrophilic, eosinophilic and paucigranulocytic asthma

	Neutrophilic	Eosinophilic	Paucigranulocytic	*P*
n (%)	11 (20)	19 (34)	26 (46)	
Age, median (q1, q3)	59.2 (36, 75)	47 (24, 73)	55 (22, 86)	0.108
Gender male/female	2/9	7/12	13/12	0.165
Ex-smoker, n (%)	4 (36)	6 (32)	9 (35)	0.960
Smoking Pack years median (q1, q3)	14.3 (2.9, 41.55)	5.1 (0.45, 17.0)	10.4 (1.80, 29.80)	0.648
Years since smoking ceased, mean (SD)	17.4 (19.0)	27.6 (16.9)	25.7 (16.0)	0.628
Atopy, n (%)	6 (55)	14 (74)	22 (85)	0.152
Aspergillus	4 (50)*	0 (0)	6 (24)	0.007
Altenaria	2 (25)	2 (12)	8 (32)	0.326
Dust mite	7 (88)	13 (76)	20 (80)	0.813
Cockroach	3 (38)	6 (35)	10 (40)	0.953
Grass	6 (75)	6 (35)	15 (60)	0.152
CCI median (q1,q3)	3 (2, 4)	1 (1, 4)	2 (1, 3)	0.253
ICS dose, median (q1,q3)	1500 (700, 2000)	500 (500, 1000)	1000 (400, 2000)	0.389
FEV_1% predicted (pre bronchodilator), mean (SD) Trend *p* value	71 (20)	73 (23)	84 (19)	0.082 0.033
FEV_1% predicted (post bronchodilator), mean (SD)	79 (24.5)	83 (15.4)	92 (20.2)	0.146
FEV_1/FVC %, mean (SD)	68.1 (12.1)	69.4 (13.8)	73.1 (5.9)	0.380
FIF50% actual, mean (SD)	2.83 (0.73)	3.81 (1.51)	4.02 (1.26)	0.051
FIF50% predicted, mean (SD)	76.5 (19.7)	90.8 (32.6)	96.3 (31.2)	0.220
BDR, n (%)	2 (18)	12 (63)	9 (36)	0.045
PD15, median (q1,q3)	4.5 (3.4-5.7)	4.2 (1.9-6.6)	10.5 (7.1-14.5)	0.107
DRS, median (q1,q3)	2.40 (0.73-3.99)	1.88 (0.64-4.45)	0.62 (0.22-1.26)*	0.036
Jqol Total, mean (SD)	6.5 (0.5)	6.5 (0.7)	6.7 (0.4)	0.730
Sputum Cell Counts				
Total cells x 106/mL, median (q1,q3)	4.32 (2.70,7.47)#	2.61 (1.62,4.68)	2.25 (1.08,3.06)	0.002
Viability, %, median (q1,q3)	92 (86,94)*#	69 (58,86)	70 (64,79)	<0.001
Neutrophils, %, median (q1,q3)	63.75 (62.25,75.25)*#	20.5 (9.75,27.00)	23.88 (11.00,39.00)	<0.001
Eosinophils, %, median (q1,q3)	0.25 (0.00,2.00)*	4.5 (2.5,15.0)	0.50 (0.00,0.75)*	<0.001
Macrophages, %, median (q1,q3)	30.75 (20.50,35.00)*#	67.5 (50.5,76.75)	70.38 (57.50,80.25)	<0.001
Lymphocytes, %, median (q1,q3)	0.50 (0.25,1.25)	0.75 (0.25,1.75)	1.00 (0.25,2.00)	0.469
Squamous, %, median (q1,q3)	1.96 (1.23,3.38) *#	6.75 (3.38,9.5)	6.92 (2.90,10.11)	0.048
Columnar Epithelials, %, median (q1,q3)	0.5 (0.00,1.75)	1.0 (0.5,2.75)	2.25 (0.49,5.25)	0.117
C2R positive eosinophils, %, median (q1,q3)	0.25 (0.00,2.50)*	6.5 (3.75,16.25)	1.00 (0.50,1.25)*	<0.001

* *P* <0.017 v eosinophilic asthma, # *P* <0.017 v paucigranulocytic asthma

CCI: Charlson Co-morbidity Index

ICS dose is calculated as beclomethasone equivalents where 1µg of beclomethasone = 1µg budesonide = 0.5µg fluticasone

PD15: provocation dose of saline resulting in a 15% drop in FEV_1 from baseline

DRS: Dose response slope: %fall FEV_1/mL 4.5% saline

Jqol: Juniper Asthma Quality of Life Questionnaire

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Foster City, CA, USA). An aliquot of sputum was selected using a positive displacement pipette and used for bacteriological culture.[14] Sputum samples were homogenized using dithiothreitol (DTT) diluted with PBS to 10^{-3}, 10^{-4} and 10^{-5} and used to inoculate chocolate bacitracin and blood agar plates. Plates were transported for Gram staining and identification to the Microbiology Department of the Hunter Area Pathology Service for culture and reporting. All plates were incubated at 37°C 5% CO2 and examined for bacterial growth after 24 and 48 h by trained microbiologists. Biochemical testing was performed on pathogenic bacteria and confirmatory tests were performed prior to reporting.

Analysis

Data were analysed using Stata 11 (Stata Corporation, College Station Texas USA). Results are reported as mean (SD) or median (q1,q3) unless otherwise indicated. Analysis was performed using the two-sample Wilcoxon rank sum test, and the Kruskal-Wallis test was used for more than two groups. Fishers' exact test was used for categorical data. Associations between data were determined using the Spearman rank correlation. All results were reported as significant when $p < 0.05$.

Results

The demographics/clinical characteristics of participants are summarised in Table 1. Sputum was collected from 60 (92%) participants and an adequate cell count was obtained in 56 (93%) of those samples, which were used for analysis. There were 19 (36%) participants with eosinophilic asthma, 26 (46%) with paucigranulocytic asthma and 11 (20%) with neutrophilic asthma. The two participants who had mixed granulocytic asthma were included in the neutrophilic asthma group as previously described.[4] Sputum inflammatory cell counts are shown Table 1.

The participants in each subtype were of similar age, gender, past smoking history, lung function, ICS dose comorbidity profile and quality of life (Table 1). Those with neutrophilic asthma had the lowest rate of atopy (55%) and the highest rate of skin test allergy to *Aspergillus fumigatus* (Table 1 and 4). Pre and post bronchodilator FEV_1% predicted was highest in those with paucigranulocytic asthma and lowest in those with neutrophilic and eosinophilic asthma. Pariticpants with neutrophilic asthma had significantly lower odds of having a response to bronchodilator compared to those with eosinophilic asthma (Table 4). Participants with paucigranulocytic asthma tended to have less responsive airways (dose response slope to hypertonic saline, Table 1).

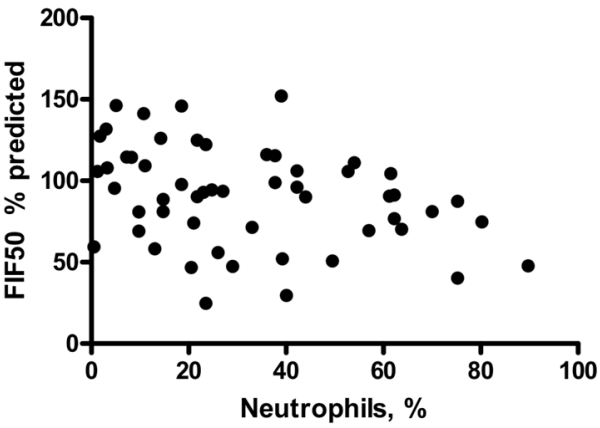

Figure 1. Scatter plot of FIF50 % predicted and sputum neutrophil % showing a significant inverse correlation. Spearman rho -0.354 P =0.009.

FIF50 tended to be lowest in those with neutrophilic asthma (Table 1). There was a significant inverse correlation between sputum neutrophils and FIF50% predicted with spearman's rho -0.326, $p < 0.015$ (Figure 1).

Asthma control

Participants with eosinophilic asthma had poorest asthma control with a higher ACQ score (P =0.029, Table 2) and a lower prevalence of controlled asthma (32%, P =0.011). Participants with neutrophilic asthma were more 1.8 times more likely to have controlled asthma compared to eosinophilic asthma (Table 4).

Asthma exacerbations

The frequencies of severe exacerbations (defined by requirement of a course of oral corticosteroids in the previous 12 months) were similar across inflammatory subtypes (Table 2). However participants with paucigranulocytic asthma and neutrophilic asthma had more than double the amount of primary care visits for uncontrolled asthma in the past year (Table 2, P =0.015) and in particular those with neutrophilic asthma had 4.5 times the odds of requiring a visit to their general practice physician for their asthma compared to those with eosinophilic asthma (Table 4). No participant had a hospital admission in the past year.

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Table 2. Asthma control, exacerbations, infection and chronic bronchitis in asthma inflammatory subtype.

	Neutrophilic	Eosinophilic	Paucigranulocytic	*P*
Asthma Control				
ACQ7, median (q1,q3)	0.86 (0.71,1.14)	1.14 (0.71,1.57)	0.57 (0.29,0.71)*	0.029
Asthma Controlled (ACQ7<0.75), n(%)	5 (45)	6 (32)	19 (76)*	0.011
Exacerbations				
Prescribed OCS in past year for asthma, n(%)	2 (18)	2 (11)	9 (36)	0.141
Visit GP for asthma in past year, n(%)	6 (55)	4 (21)	16 (64)*	0.015
Patient report of chest infection, n(%)	8 (73)	7 (37)	16 (64)	0.111
Pathogen detected, n (%)	2 (25)	4 (22)	0 (0)	0.020
Virus detected, n (%)	0 (0)	2 (10)	0 (0)	0.667
Bacteria detected, n (%)	2 (18)	3 (16)	0 (0)	0.073
Chronic Bronchitis, n (%)	2 (18)	4 (19)	8 (32)	0.665

*P<0.017 vs. eosinophilic asthma.
OCS: oral corticosteroids

Infection

Chest infections were more common in those with neutrophilic asthma. More than 70% reported a chest infection in the previous 12 months, and a higher prevalence of bacterial pathogens was detected when asthma was stable (Table 2). Overall there was a low rate of positive culture with a bacterial pathogen identified in five participants (10%), one each with *Moraxella catarrhalis, Haemophilus influenzae, Pseudomonas aeruginosa* and 2 *with Staphylococcus aureus.*

Chronic bronchitis was present in 27% of participants, and not different between inflammatory phenotypes (P =0.665, Table 2).

Rhinosinusitis

Rhinosinusitis was more common in neutrophilic asthma, with double the rate of that observed in eosinophilic asthma (64% compared to 32%, Table 3) and 3.8 and 4.5 times the odds compared to eosinophilic and paucigranulocytic asthma respectively (Table 4). The mean item score of the SNOT 20 was higher (but not statistically significant) in those with neutrophilic asthma and a more detailed examination of the rhinosinusitis questionnaire showed that those with neutrophilic asthma were more likely to report a problem with 'waking up at night' and 'fatigued' compared to those with eosinophilic and paucigranulocytic asthma (Table 3).

GERD

GERD was common in neutrophilic asthma (73%) and the odds were highest for the presence of GERD in patients with neutrophilic asthma compared to eosinophilic (4.6 times) and paucigranulcotic asthma (2.9 times, Table 4). While total symptoms were higher, the frequency of GERD symptoms was significantly higher in neutrophilic asthma, compared to both eosinophilic and paucigranulocytic asthma (P =0.025, Table 3). The ORO index was similar across phenotypes and there was not an increased rate of significant ORO score (p =0.917).

Discussion

This study identified differences between asthma inflammatory subtypes with increased exacerbations and more prevalent GERD and rhinosinusitis in neutrophilic asthma. Patients with neutrophilic asthma reported more primary care visits in the previous 12 months for asthma exacerbations and almost half had uncontrolled asthma. Chest infections were more common in those with neutrophilic asthma and pathogenic bacteria were identified in 18%. There was also an increased

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Table 3. Rhinosinusitis and GERD by asthma inflammatory subtype

	Neutrophilic	Eosinophilic	Paucigranulocytic	P
Rhinosinusitis				
Rhinosinusitis, n (%)	7 (64)	6 (32)	7 (28)	0.132
SNOT 20 mean item score, median (q1,q3)	1.45 (0.25,2.00)	0.65 (1.0,1.3)	0.55 (0.32,1.10)	0.178
SNOT20 question 12 wake up at night	3 (0,4)	0 (0,1)#	0 (0,1)	0.033
SNOT20 question 13 Lack of a good night's sleep	2 (0,4)	0 (0,1)#	1 (0,2)	0.031
SNOT20 question 15 fatigue	3 (0,4)	0 (0,12)	1 (0,2)	0.057
SNOT20 question 14 Wake up tired	3 (0,4)	0 (0,1)	1 (0,2)	0.106
SNOT20 question 17 Reduced concentration	2 (0,3)	0 (0,1)	0 (0,1)	0.064
GERD				
GERD, n (%)	8 (73)	7 (37)	12 (48)	0.180
GERD Frequency Score, median, (q1,q3)	8 (7,13)	6 (4,7)#	6 (4,7)#	0.025
GERD total score, median (q1,q3)	15 (11,23)	11 (7,17)	11 (8,13)	0.089
ORO Index, median (q1,q3)	23 (4,41)	10 (5,31)	19 (4,28)	0.917

# $P<0.017$ vs Neutrophilic Asthma.

prevalence of rhinosinusitis and increased symptoms of GERD observed in neutrophilic asthma.

Patients with paucigranulocytic asthma had the highest pre and post bronchodilator FEV1% and they also tended to have less responsive airways (lower dose response slope). These findings are similar to those reported in patients with severe asthma.[15] The frequencies of severe exacerbations were not different between the subtypes, but there was a higher rate of unplanned visits to primary care in those with neutrophilic and paucigranulocytic asthma. This combined with a lower asthma control score in neutrophilic and paucigranulocytic asthma suggests that asthma control, at least measured using the Juniper score may not be sensitive to the factors influencing control in patients without increased sputum eosinophilia.

The proportion of patients who had smoked cigarettes previously was not different between subtypes; however those with neutrophilic asthma tended to have the highest median pack year smoking history, three times that of the other subtypes. This is a similar observation to our larger study of 93 patients with asthma.[16] Similarly those with neutrophilic asthma had the highest median dose of inhaled corticosteroid and while this was not significantly different statistically it may reflect guideline based asthma therapy where inhaled corticosteroids are increased to manage patients' symptoms. Inhaled corticosteroids are also reported to increase neutrophil survival and therefore may have a direct influence on sputum neutrophil proportions.

Chronic rhinosinusitis affects up to 15% of the general population impacting on quality of life and daily activities. Similar to neutrophilic asthma, chronic rhinosinusitis without polyps is characterised by prominent neutrophilic inflammation, increased IL-8 and MMP-9, TIMP-1.[17] The presence of rhinosinusitis was twice as common in neutrophilic asthma as other phenotypes. The symptoms burden was generally similar; however there was a higher SNOT20 mean item score and significantly higher score for questions regarding night waking and lack of a good night's sleep. Chronic sinusitis induces extrathoracic airway narrowing and subsequently bronchial narrowing which improve following treatment with both nasal steroids and antibiotics.[18]

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Table 4. Odds ratio and confidence intervals for categorical outcomes

	Neutrophilic v Eosinophilic	Neutrophilic v Paucigranulocytic
Skin Test Aspergillus positive, n(%)	*Approaching ∞, Fishers exact P=0.002*	3.17 (0.43-22.50)
BDR n (%)	0.13 (0.01-0.95)	0.40 (0.04-2.64)
Asthma Controlled (ACQ7<0.75), n(%)	1.81 (0.30-10.70)	0.26 (0.05-1.51)
Visit GP for asthma in past year, n(%)	4.5 (0.69-30.80)	0.68 (0.13-3.71)
Rhinosinusitis, n (%)	3.79 (0.63-24.39)	4.5 (0.80-27.16)
GERD, n (%)	4.57 (0.73-34.14)	2.89 (0.51-20.03)

The increased sleep related symptoms were an unexpected finding and require further exploration.

GERD is a common problem and is often reported in people with respiratory diseases including asthma. A significant association has been shown between reflux symptoms and asthma symptoms independent of body weight or obstructive sleep apnoea which suggests that reflux may affect the respiratory tract directly rather than through other co-morbidities.[19] In this study, most patients with asthma reported mild reflux symptoms at least monthly. GERD was most common in those with neutrophilic asthma, and the frequency of symptoms was significantly higher compared to those with eosinophilic or paucigranulocytic asthma. The ORO index while highest in those with neutrophilic asthma was not different between the asthma inflammatory phenotypes, suggesting that GERD with aspiration was not different between groups. Increased neutrophils in tissue and BAL have been reported in children with GERD.[20] Similarly elevated levels of the neutrophil chemokines IL-8 have been reported in reflux esophagitis and improve with proton pump inhibitor treatment,[21] suggesting that identification and treatment of GERD in patients with neutrophilic asthma may lead to an improvement in inflammation.

Reduced FIF50% has been associated with snoring (both with and without obstructive sleep apnoea) suggesting increased upper airway resistance.[22] Children and adults with obstructive sleep apnoea (OSA) have airway inflammation characterised by a marked increase in neutrophils,[23] which is not reduced with CPAP, in adults the presence of OSA was also shown to result in delayed neutrophil apoptosis.[24] It is possible that neutrophilic asthma may have important upper airway abnormalities which require concurrent assessment and treatment with lower airway inflammation.

We have previously reported a higher prevalence of *Haemophilus influenzae* in the airways of patients with neutrophilic asthma compared to other asthma inflammatory subtypes[25] and have shown that patients with a potentially pathogenic bacteria isolated from their induced sputum sample have increased neutrophils compared to those who do not.[26] While we observed a low overall rate of detection of viruses or bacteria in this study, the rate of potentially pathogenic bacteria was highest in those with neutrophilic asthma.

The triggers and mechanisms of neutrophilic forms of asthma remain unknown; however growing evidence supports a role for the innate immune response with altered gene expression of toll like receptors and increased expression of genes from the IL-1β pathway observed in those with increased sputum neutrophils.[27,28] This suggests pathogen recognition and destruction processes may be altered and indeed macrophage phagocytosis is impaired in those with normal sputum eosinophil proportions[29] Similarly, air pollution and bacterial components may be prominent triggers of neutrophilic bronchitis as shown in a recent study were patients with the highest sputum neutrophils where from residences in close proximity to busy roads.[30]

We found no difference in smoking histories between patients of different inflammatory subtypes, and no difference in FEV_1/FVC. While the pre bronchodilator FEV_1 % predicted was lowest in those with neutrophilic asthma it was highest in the group with paucigranulocytic asthma who had similar pack years smoking histories. The presence of Asthma COPD overlap is common, especially in the older population and the presence of asthma is a risk factor for the development of COPD, the data in this study do not suggest that smoking history or the presence of mild or co-existing COPD are distinguishing features of neutrophilic asthma.

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The clinical pattern of neutrophilic asthma is different from those with paucigranulocytic and eosinophilic asthma with evidence of upper airways abnormalities and frequent chest infections. Specific and targeted treatment of these airway problems may assist in the control and management of neutrophilic asthma.

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References

1. Turner MO, Hussack P, Sears MR, Dolovich J, Hargreave FE. Exacerbations of asthma without sputum eosinophilia. Thorax. 1995;50:1057-61.
2. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma. Chest. 2001;119:1329-36.
3. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med. 2012;185:612-9.
4. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006;11:54-61.
5. Locke GR, Talley NJ, Weaver AL, Zinsmeister AR. A new questionnaire for gastroesophageal reflux disease. Mayo Clin Proc. 1994;69:539-47.
6. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg. 2002;126:41-7.
7. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Resp J. 1999;14:902-7.
8. Knudson RJ, Slatin RC, Lebowitz MD, Burrows B. The maximal expiratory flow-volume curve. Normal standards, variability, and effects of age. Am Rev Respir Dis. 1976;113:587-600.
9. Jones PD, Hankin R, Simpson J, Gibson PG, Henry RL. The tolerability, safety, and success of sputum induction and combined hypertonic saline challenge in children. Am J Respir Crit Care Med. 2001;164:1146-9.
10. Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AR, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. Am J Resp Crit Care Med. 1998;158:36-41.
11. Simpson JL, McElduff P, Gibson PG. Assessment and reproducibility of non-eosinophilic asthma using induced sputum. Respiration. 2010;79:147-51.
12. Parameswaran K, Anvari M, Efthimiadis A, Kamada D, Hargreave FE, Allen CJ. Lipid-laden macrophages in induced sputum are a marker of oropharyngeal reflux and possible gastric aspiration. Eur Resp J. 2000;16:1119-22.
13. Grissell TV, Powell H, Shaffren DR, Boyle MJ, Hensley MJ, Jones PD, et al. Interleukin-10 gene expression in acute virus-induced asthma. Am J Respir Crit Care Med. 2005;172:433-9.
14. Pye A, Stockley RA, Hill SL. Simple method for quantifying viable bacterial numbers in sputum. J Clin Pathol. 1995;48:719-24.
15. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol. 2010;125:1028-36 e13.
16. Simpson JL, Scott RJ, Boyle MJ, Gibson PG. Differential Proteolytic Enzyme Activity in Eosinophilic and Neutrophilic Asthma. Am J Resp Crit Care Med. 2005;172:559-65.
17. Eloy P, Poirrier AL, De Dorlodot C, Van Zele T, Watelet JB, Bertrand B. Actual concepts in rhinosinusitis: a review of clinical presentations, inflammatory pathways, cytokine profiles, remodeling, and management. Curr Allergy Asthma Rep. 2011;11:146-62.
18. Bucca C, Rolla G, Brussino L, De Rose V, Bugiani M. Are asthma-like symptoms due to bronchial or extrathoracic airway dysfunction? Lancet. 1995;346:791-5.
19. Mulrennan S, Knuiman M, Divitini M, Cullen D, Hunter M, Hui J, et al. Gastro-oesophageal reflux and respiratory symptoms in Bussleton adults: the affects of body weight and sleep apnoea. Intern Med J. 2011;doi: 10.1111/j.1445-5994.2011.02535.x. [epub ahead of print].
20. Chang AB, Cox NC, Faoagali J, Cleghorn GJ, Beem C, Ee LC, et al. Cough and reflux esophagitis in children: their co-existence and airway cellularity. BMC Pediatr. 2006;6:4.
21. Isomoto H, Wang A, Mizuta Y, Akazawa Y, Ohba K, Omagari K, et al. Elevated levels of chemokines in esophageal mucosa of patients with reflux esophagitis. The American journal of gastroenterology. 2003;98:551-6.
22. Amado VM, Costa AC, Guiot M, Viegas CA, Tavares P. Inspiratory flow-volume curve in snoring patients with and without obstructive sleep apnea. Braz J Med Biol Res. 1999;32(4):407-11.

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23. Li AM, Hung E, Tsang T, Yin J, So HK, Wong E, et al. Induced sputum inflammatory measures correlate with disease severity in children with obstructive sleep apnoea. Thorax. 2007;62:75-9.
24. Devouassoux G, Levy P, Rossini E, Pin I, Fior-Gozlan M, Henry M, et al. Sleep apnea is associated with bronchial inflammation and continuous positive airway pressure-induced airway hyperresponsiveness. The Journal of allergy and clinical immunology. 2007;119:597-603.
25. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. Thorax. 2007;62:211-8.
26. Wood LG, Simpson JL, Hansbro P, Gibson PG. Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia. Free Radic Res. 2010;44:146-54.
27. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. Thorax. 2007;62:211-8.
28. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. The Journal of allergy and clinical immunology. 2011;127:153-60
29. Simpson JL, Gibson PG, Yang IA, Upham J, James A, Reynolds PN, et al. Impaired macrophage phagocytosis in non-eosinophilic asthma. Clin Exp Allergy. 2013;43:29-35.
30. Wallace J, D'Silva L, Brannan J, Hargreave FE, Kanaroglou P, Nair P. Association between proximity to major roads and sputum cell counts. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2011;18:13-8.

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