

ORIGINAL ARTICLE

Relationship between airway neutrophilia and ageing in asthmatics and non-asthmatics

COLLIN R. BROOKS,¹ PETER G. GIBSON,^{2,3} JEROEN DOUWES,¹ CHRISTINE J. VAN DALEN¹ AND JODIE L. SIMPSON^{2,3}

¹Centre for Public Health Research, Massey University Wellington Campus, Wellington, New Zealand, ²Respiratory and Sleep Medicine, School of Medicine and Public Health, University of Newcastle and ³Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle New South Wales, Australia

ABSTRACT

Background and objective: Increased sputum neutrophilia has been observed in asthma, but also during normal ageing in asthmatics and non-asthmatics. It remains unclear what constitutes 'normal' neutrophil levels in different age groups.

Methods: We assessed the relationship between age and airway neutrophils of 194 asthmatics and 243 non-asthmatics (age range: 6–80 years). Regression analyses were used to assess this relationship adjusted for confounders including asthma status, atopy, gender, smoking and current use of inhaled corticosteroids (ICS). Age-corrected reference values for different age groups were determined using the 95th percentile of non-asthmatic participants.

Results: Age was positively associated with sputum neutrophils in both asthmatic and non-asthmatic adults (0.46% neutrophil increase/year (95% confidence interval (CI) 0.18, 0.73) and 0.44%/year (0.25, 0.64, respectively), but no association was found in the <20-year age category. Individuals with high sputum neutrophil counts (>95th percentile of non-asthmatic counts for any given age group) were significantly more likely to be asthmatic (odds ratio = 2.5; 95% CI: 1.3, 5.0), with the greatest effect observed in the older age group. Other factors that independently associated with increased sputum neutrophil levels included atopy in non-asthmatic adults, male gender and current use of ICS in asthmatic adults. Age-specific reference values for neutrophil percentage were under 20 years-76%, 20–40 years-62%, 40–60 years-63% and over 60 years-67%.

Conclusions: Airway neutrophilia is related to age in adults, with a neutrophilic asthma phenotype present in older adults. The use of appropriate age-specific

SUMMARY AT A GLANCE

The association between airway neutrophilia and ageing was assessed in a large population. Similar neutrophil increases with age were observed in asthmatics and non-asthmatics, with neutrophilic asthma present in older adults. Use of age-specific reference values for sputum neutrophils may be helpful in future studies assessing neutrophilic inflammation in asthma.

reference values is recommended for future studies aimed at elucidating the role of neutrophils in asthma.

Key words: ageing, asthma, neutrophil, induced sputum.

Abbreviation: ICS, inhaled corticosteroids.

INTRODUCTION

While asthma is classically characterized by eosinophilic airway inflammation, increased neutrophil numbers have been shown in fatal status asthmaticus,¹ occupational asthma² and during severe asthma exacerbations.³ Moreover, airway neutrophilia is evident in asthmatics in the absence of eosinophilia, and associated with both decreased lung function and poor response to inhaled corticosteroids (ICS).^{4,5} Nonetheless, the importance of neutrophils in the immunopathogenesis of asthma remains largely unclear.

A critical requirement for identifying increased airway neutrophilia is to determine what are 'normal' neutrophil numbers. Some studies designed to provide reference values for differential leukocyte counts in induced sputum include few older subjects,^{6,7} limiting their applicability. Recent studies in both asthmatics^{8,9} and non-asthmatics^{10,11} have shown that differential sputum neutrophils increase with advancing age. This is particularly relevant when

Correspondence: Collin R. Brooks, Centre for Public Health Research, Massey University Wellington Campus, Private Box 756, Wellington, New Zealand. Email: c.r.brooks@massey.ac.nz

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comparisons are being made among different age groups. For example, some studies of chronic obstructive pulmonary disease showed increased neutrophilic airway inflammation compared with controls.^{12,13} However, the reference population was significantly younger than the study population, and part of the difference in neutrophil levels may therefore have been explained by age differences alone. Similarly, we have proposed four inflammatory asthma phenotypes, with two phenotypes classified based on a fixed cut-point of >61% sputum neutrophils (designated neutrophilic asthma and mixed granulocytic asthma). However, those with high neutrophil levels were significantly older than those with neutrophil levels <61% (eosinophilic and paucigranulocytic asthma).¹⁴ Thus, at least part of the difference in neutrophils observed between both phenotypes may be due to age. Nonetheless, there is evidence that neutrophil increases are associated with persistent airway obstruction in asthmatics even after adjusting for age,¹⁵ and also with an increase in sputum levels of neutrophil products implicated in airway remodeling, such as neutrophil elastase, interleukin-8 and matrix metalloproteinase-9.^{9,16,17} This suggests that neutrophils may play an active role in asthma pathogenesis.

To date, the association between age and airway neutrophils has only been reported in adults, with no studies including children and adolescents. In this study, we examined the relationship between age and airway neutrophils in a large population of asthmatic and non-asthmatic adults and children, and assessed which other characteristics predict airway neutrophilia. Furthermore, we determined age-specific reference values for sputum neutrophils to avoid misclassification of the neutrophilic asthma phenotype, particularly in older populations.

METHODS

Study population

This study involved a retrospective assessment of cross-sectional data obtained from 264 non-asthmatics and 231 asthmatics recruited for studies at either Massey University, Wellington, New Zealand,^{18,19} or the John Hunter Hospital, Newcastle, Australia,^{14,20–23} between 1995 and 2010. Recruitment was conducted either by advertisement, from the Respiratory Ambulatory Care Service at the John Hunter Hospital, or, for many of the adolescents studied ($n = 145$), from five schools that had participated in the International Study of Asthma and Allergies in Childhood Phase III survey.²⁴ Studies were approved by the appropriate ethics committee, and all participants (or children's parents) provided written informed consent. Participants underwent a similar core protocol involving detailed physical and questionnaire-based assessment of smoking, medication and respiratory health status, spirometry, skin prick testing and sputum induction. Sputum was analysed for inflammatory cell counts. Subjects with asthma were asked to withhold their rescue medications for at least 6 h (short-acting β -agonist) or 24 h

(long-acting β -agonist) prior to evaluation. Participants who had a respiratory infection within 4 weeks were asked to return at a later date. Assessments were conducted by a respiratory physician. Current smokers and individuals with evidence of other respiratory morbidities were excluded from further study.

Asthma definition

Asthma was defined as physician-diagnosed asthma, with asthma symptoms and/or asthma medication use within 12 months.²⁴ Non-asthmatics reported no asthma symptoms, asthma medication use, no diagnosis or evidence of respiratory disease and had normal spirometric results. In the Australian studies, physician's diagnosis of asthma was confirmed by demonstrated airway hyperreactivity to hypertonic saline.

Skin prick testing

Skin prick testing was conducted using a panel of aeroallergens. For Australian studies, these included *Aspergillus fumigatus*, *Alternaria tenuis*, house dust mite (*Dermatophagoides pteronyssinus*), and cockroach mix and grass mix (Bayer Australia Ltd, Pymble, NSW, Australia). For New Zealand studies, allergens used were house dust mite, tree mix, grass mix, cat and dog dander, *Alternaria tenuis* and *Penicillium* mix (Hollister-Stier Laboratories, Spokane, WA, USA). Positive atopy status was determined as the presence of at least one weal >3 mm. Histamine and saline were used as positive and negative controls, respectively. Atopy was not assessed in 37 of the non-asthmatics in the Australian studies.

Spirometry

Spirometric measurements were conducted according to American Thoracic Society criteria,²⁵ using a spirometer from either KoKo PD Instrumentation (Louisville, CO, USA) (Australia) or Easyone spirometer (NDD Medizintechnik AG, Zurich, Switzerland) (New Zealand). National Health and Nutrition Examination Survey III equations were used for lung function parameters.

Sputum induction, processing and analysis

Sputum induction (combined with bronchial provocation assessment to determine provocation dose of hypertonic saline resulting in a decrease in forced expiratory volume in 1 s of $\geq 15\%$), sputum processing, and differential and total cell enumeration were conducted as described previously,²⁶ except in one New Zealand study¹⁸ in which participants ($n = 54$) were premedicated with a β -2 agonist (salbutamol) prior to sputum induction as part of reversible airway obstruction assessment.

Statistical analysis

Data analyses were performed using STATA version 11.0 (STATA Corp., College Station, TX, USA) and Prism 5 (Graphpad Software Inc, La Jolla, CA, USA).

Data are described as median and interquartile ranges unless stated. The population was stratified into either adults (>20 years), children/adolescents (<20 years) or quartiles on the basis of 20-year age categories. Univariate analyses were conducted using Mann–Whitney *U*-testing, linear regression or Spearman's correlation. Fisher's exact test was used for categorical data, and Kruskal–Wallis with Dunn's post-test analysis was used for multiple group comparison. Multiple linear regression analyses were performed to assess associations between sputum neutrophils and age while controlling for potentially confounding variables. Variables were selected for multivariate analysis if they showed an association ($P < 0.10$) with sputum neutrophil percentage, or if these variables were previously shown to be related to neutrophils, and depending upon the model included gender, previous smoking status, asthma, atopy and use of ICS. Logistic regression analyses were used for dichotomous outcomes with adjustment for potential confounders (age, gender and previous smoking).

RESULTS

From a total of 495 individuals (264 non-asthmatics, 231 asthmatics), 437 (243 non-asthmatics, 194 asthmatics) were included in the final analyses. Participant exclusion was due to an inadequate sputum sample (no sample or excessive contamination with squamous epithelial cells) ($n = 21$; 9 asthma, 12 no asthma), current smoking ($n = 36$; 27 asthma, 9 no asthma) or unrecorded asthma status ($n = 1$). Demographics and clinical characteristics are described in Table 1.

Sputum neutrophil percentage and absolute neutrophils/mL of sputum increased with increasing age (Table 1), which equated to 0.27%/year (95% confidence interval (CI): 0.17, 0.38; $P \leq 0.0001$) (Fig. 1a) for all participants. For asthmatics and non-asthmatics separately these estimates were 0.37%/year (95% CI: 0.20, 0.53; $P \leq 0.0001$) and 0.19%/year (95% CI: 0.05–0.32; $P \leq 0.09$) (Fig. 1b). Exclusion of the <20-year group, which showed considerable variation and a non-significant reduction in neutrophil percentage per year (Fig. 1c), suggested that there was no difference in estimated change of neutrophil percentage per year when all adults were analysed (0.47%/year (95% CI: 0.31, 0.63)), or divided into asthmatics (0.46%/year (95% CI: 0.18, 0.73)) and non-asthmatics (0.44%/year (95% CI: 0.25, 0.64)) (all $P \leq 0.0001$) (Fig. 1d). Similarly, no difference was observed when adults were stratified into males (0.46%/year (95% CI: 0.17, 0.75)) and females (0.45%/year (95% CI: 0.25, 0.64)), or if previous smokers were excluded from analyses (data not shown). Significant associations were also observed when neutrophils were considered as absolute number per mL/sputum rather than percentages, but both univariate analyses and analyses controlling for potential confounders showed weaker associations than when using percentages (data not shown).

In support of previous findings,¹⁵ we observed in univariate analysis that neutrophil percentage was

inversely associated with a reduction in both % predicted forced expiratory volume in 1 s and forced expiratory volume in 1 s/forced vital capacity. This was particularly significant when only adults were included in analysis (Spearman's $r = -0.21$; $P = 0.0007$ and $r = -0.26$; $P \leq 0.0001$, respectively).

Controlling for asthma status, atopy, gender, smoking and current ICS use in multivariate analysis did not significantly alter the observed association between neutrophil percentage and age in adults (Table 2). In addition to age, factors independently associated with increased neutrophils in multivariate analysis included atopy in non-asthmatic adults ($P = 0.05$), and male gender and current use of ICS in asthmatic adults ($P = 0.01$ and 0.02 , respectively; Table 2). In a multivariate model including all adults, asthma was associated with a lower percentage of sputum neutrophils, although this did not reach significance ($P = 0.07$; Table 2).

There was no significant difference in neutrophil percentages within age groups between asthmatics and non-asthmatics (Table 1, Fig 2), although the asthma group had increased total cell counts as well as slightly significantly higher absolute numbers of neutrophils (Mann–Whitney *U*-test; $P = 0.046$; Table 1).

Interestingly, despite the lack of difference in neutrophil percentages between asthmatics and non-asthmatics, those with high sputum neutrophil differential counts (greater than 95th percentile of non-asthmatic values for any given age group) were significantly more likely to be asthmatic, with the strongest association for the oldest age group (Table 3). When using cut-off values derived from 95th percentile of absolute neutrophils (rather than neutrophil percentage), we found a similar association (Table 4).

Although 61% has been previously been determined as the 95th percentile of airway neutrophil percentage in non-asthmatic populations (and thus indicative of neutrophilic inflammation), an overall 95th percentile value for sputum neutrophils in non-asthmatics of 69.43% was observed. Using a similar 95th percentile cut-off approach, we observed age-specific reference values of 75.57% for those <20 years, 61.61% for 20–40 years, 63.2% for 40–60 years and 67.25% for those >60 years (Table 1, Fig. 2).

DISCUSSION

In this cross-sectional study, we found a positive association between age and sputum neutrophils in both asthmatic and non-asthmatic adults. Overall, there was little difference in sputum neutrophil levels between asthmatics and non-asthmatics within age groups, although individuals with high sputum neutrophil differential counts (greater than 95th percentile) were significantly more likely to be asthmatic, with this effect being most pronounced for older age groups.

To the authors' knowledge, this is the first report comprehensively comparing the association of sputum neutrophil levels in both asthmatics and

Table 1 Demographics and sputum analysis data stratified on the basis of age, expressed as median (interquartile range (IQR)) or number (percentage) unless stated

	Group							
	Less than 20 years		20–40 years		40–60 years		Greater than 60 years	
	No asthma	Asthma	No asthma	Asthma	No asthma	Asthma	No asthma	Asthma
Number	111	72	59	34	41	40	32	48
Age	15.0 (12.8–16.1)	15.8 (15.0–16.6)	27.6 (23.2–34.2)	27.7 (24.8–35.3)	50.6 (43.3–55.2)	52.5 (47.7–56.4)	65.6 (62.3–70.2)	66.12 (66.2–70.1)
Gender (female/male)	42/69	13/59**	46/13	22/12	29/12	26/14	15/17	28/20
FEV ₁ % predicted	101.7 (93.2–107.4)	94.7 (86.4–103.2)***	102.6 (96–111.3)	94 (83.5–104.4)***	103.7 (93.9–114)	75 (53.1–85.6)***	100.4 (92.6–111.7)	71.4 (54.5–87.1)***
FEV ₁ /FVC (%)	81.9 (75–87.5)	81.4 (75.5–84.8)	85.9 (83–88.3)	79.9 (71.2–82)***	82 (79.1–85)	68 (57.3–76)***	78 (74–80)	65 (56.1–73)***
Smoking status (prev%)	0	0	9 (15.3)	5 (14.7)	12 (29.3)	5 (12.5)	18 (56.3)	14 (29.2)
ICS use (%)	–	28 (41.8)	–	24 (70.6)	–	34 (85)	–	43 (89.6)
Atopy/no atopy (%)	43/37 (53.8)	60/12 (83.3)***	23/31 (42.6)	31/3 (91.2)***	21/19 (52.5)	25/15 (62.5)	8/24 (25)	38/10 (79.2)***
TCC/mL	2.79 (1.4–5.1)	4.0 (1.6–8.2)	1.7 (1.3–2.4)	1.8 (1–4.1)	1.9 (0.9–2.7)	2.9 (1.2–7.4)*	2.0 (0.9–3.8)	2.5 (1.3–6.8)
Viability (%)	78 (68–88)	79.5 (70.7–89.1)	71.4 (56.4–78.9)	63.6 (55.1–78.7)	66.7 (50–85)	72.1 (58–82.7)	79.3 (66.9–91)	81.7 (66.5–88.9)
% neutrophils (%N)	21.2 (11.5–44.1)	16.1 (5.8–33.9)	18.3 (10.1–30.4)	25.3 (15.4–32.8)	29.7 (20.4–51.4)	29.1 (16.1–52.1)	38.6 (23.1–58.2)	35.4 (15.2–67.1)
95th percentile (%N)	75.57	89.46	61.61	70.34	63.25	79.63	67.25	94.75
Neutrophils ×10 ⁴ mL (TN)	57.2 (13.3–154.5)	42.1 (10.2–182.2)	36.3 (14.7–58.4)	35.3 (18.8–94)	40.9 (18.7–111.8)	86.7 (29.1–224.1)	56.41 (28–217.1)	93 (29.1–357.1)
95th percentile (TN)	735	1751	162	589	319	1587	442	931
% eosinophils	0 (0–0.6)	1.31 (0–8)***	0 (0–0.3)	1.71 (0.4–3.8)***	0 (0–0.3)	1.3 (0–4.5)***	0.3 (0–0.5)	1 (0.1–2.4)***
Eosinophils ×10 ⁴ mL	0 (0–2.2)	2.6 (0–47.8)***	0 (0–0.6)	2.5 (0.5–10.1)***	0 (0–0.4)	4.8 (0.1–20.8)***	0.4 (0–1.1)	1.6 (0.1–9.8)**

Mann-Whitney test * $P < 0.05$, ** $P \leq 0.01$, *** $P < 0.0001$ compared with corresponding non-asthmatic group.FEV₁, forced expiratory volume in 1 s; FEV₁%, percent of predicted forced expiratory volume in 1 s; FVC, forced vital capacity; %N, neutrophil percentage; TCC/mL, total non-squamous cells per mL sputum, ×10⁶; TN, total neutrophils ×10⁴ per mL of sputum; Viability, percentage viability of non-squamous cells.

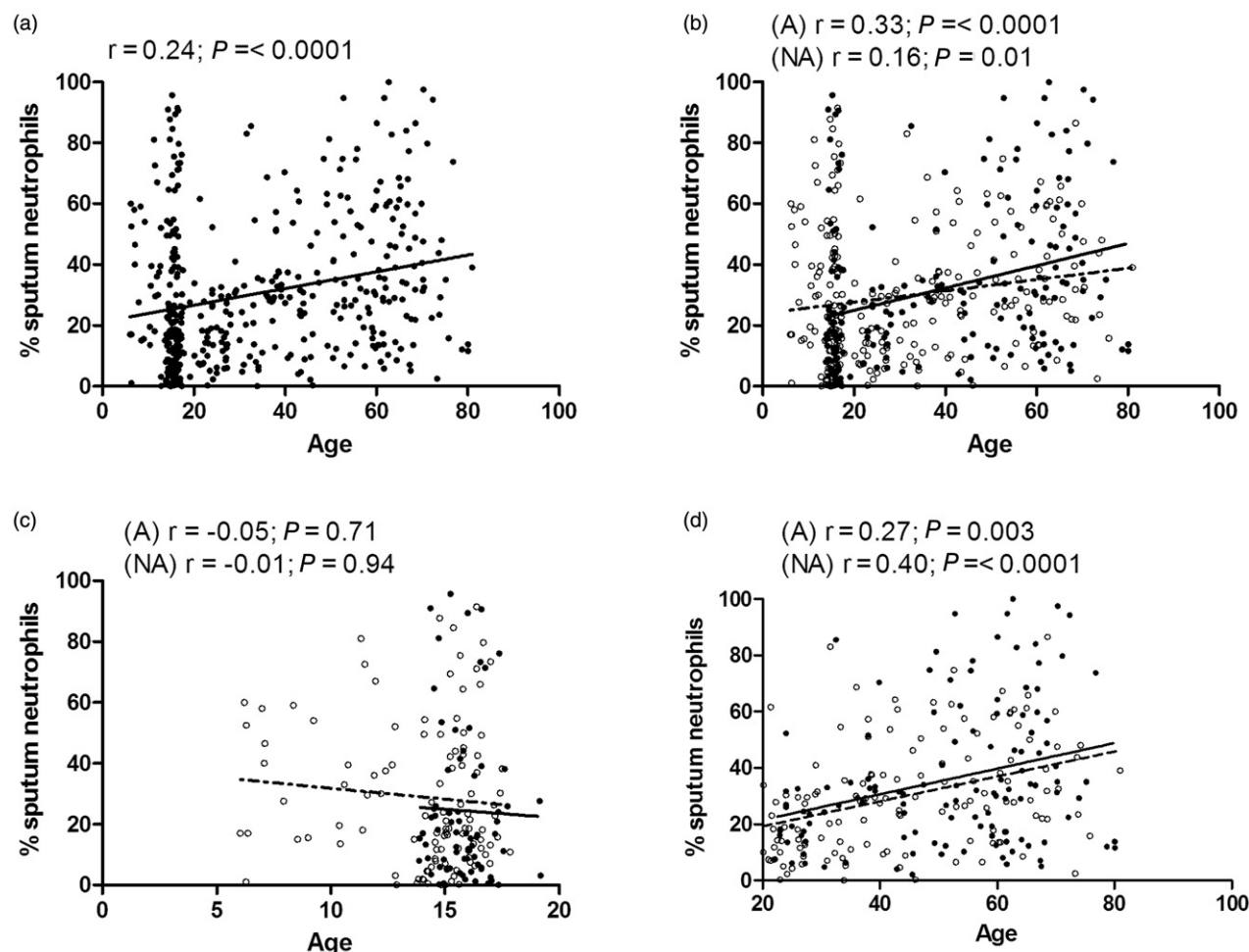


Figure 1 Correlations and linear regression of sputum neutrophil percentages against age for: (a) all study participants, (b) all study participants divided on the basis of asthma/no asthma, (c) participants under 20 years divided on the basis of asthma/no asthma (d) and all adult participants divided on the basis of asthma/no asthma (for b, c and d, filled circle/solid line = asthma, empty circle/dashed line = no asthma). A, asthmatic, NA, non-asthmatic.

non-asthmatics in a large population, ranging from childhood to old age. Our findings are consistent with those previously described separately for asthmatics and non-asthmatic adults. For example, Thomas *et al.* reported a significant correlation ($r = 0.58$; $P < 0.001$) between sputum neutrophils and age in healthy volunteers ($n = 66$),¹⁰ while in a study investigating older (50–70 years; $n = 6$) and younger (20–40 years; $n = 12$) asthmatics,¹⁶ a trend was shown for an increased percentage of sputum neutrophils in the older group, and a corresponding increase in interleukin-8, matrix metalloproteinase-9 and neutrophil elastase.⁹ The correlation observed for adults in our study ($r = 0.35$; $P \leq 0.0001$) was not as strong as that reported by Thomas *et al.*, but was similar to those observed previously in asthmatics ($r = 0.27$ to $r = 0.38$).^{8,27,28} No studies reporting on age and neutrophil levels in children are available.

Gender and ICS use had a significant effect on sputum neutrophil differential independent of age in asthmatics, while atopy had a significant effect on neutrophil percentage in non-asthmatics. In a recent lon-

gitudinal study, ICS use was shown to increase sputum neutrophils in asthmatics,²⁷ possibly through mechanisms leading to impairment of apoptosis.²⁹ Woodruff *et al.*⁸ and Shaw *et al.*¹⁵ have previously established that decreased lung function was independently associated with sputum neutrophil percentage, alongside age, male gender, ethnicity and use of ICS, although to our knowledge, a direct relationship between male gender and sputum neutrophils has not previously been described. A similar gender effect was observed with serum matrix metalloproteinase-9 levels (produced by neutrophils), which were significantly increased in elderly men (not women) with decreased forced expiratory volume in 1 s.³⁰ However, the aforementioned study did not specifically assess this in asthma, or determine if it was associated with neutrophilic airway inflammation. The association between atopy and airway neutrophilia in non-asthmatic adults was unexpected. However, one cross-sectional study comparing food allergy patients (without respiratory symptoms), asthmatics and controls found a significantly increased proportion of

Table 2 Multiple linear regression models with sputum neutrophil percentage as dependent variable (unstandardized coefficients)

Variable	All individuals (n = 395 ¹)		Individuals <20 years (n = 147 ¹)		All adults (n = 248 ¹)		Non-asthmatic adults (n = 126 ¹)		Asthmatic adults (n = 106 ¹)	
	Neutrophil change (% per variable unit (95% CI))	P	Neutrophil change (% per variable unit (95% CI))	P	Neutrophil change (% per variable unit (95% CI))	P	Neutrophil change (% per variable unit (95% CI))	P	Neutrophil change (% per variable unit (95% CI))	P
Age	0.33 (0.2, 0.47)	<0.001	3.14 (−0.66, 6.94)	0.11	0.43 (0.26, 0.61)	<0.001	0.48 (0.26, 0.69)	<0.001	0.40 (0.021, 0.76)	0.04
Gender	−3.86 (−8.56, 0.84)	0.11	−3.01 (12.36, 6.34)	0.53	−3.88 (−9.61, 1.83)	0.18	3.67 (−3.43, 10.77)	0.31	−13.96 (−24.57, −3.36)	0.01
Smoking status (never/previous)	−0.22 (−7.06, 6.61)	0.95	—	—	0.59 (−5.77, 6.95)	0.85	3.21 (−4.41, 10.83)	0.41	−1.25 (−14.04, 11.54)	0.85
Atopy (yes/no)	1.18 (−3.87, 6.23)	0.65	−6.68 (−15.85, 2.49)	0.15	5.63 (−0.23, 11.48)	0.06	6.56 (0.07, 13.05)	0.05	3.94 (−8.17, 16.05)	0.52
Asthma (yes/no)	−2.65 (−9.46, 4.16)	0.44	−0.57 (−10.49, 9.35)	0.91	−9.37 (−19.38, 0.63)	0.07	—	—	—	—
ICS use (yes/no)	4.68 (−2.84, 12.20)	0.22	−4.60 (16.74, 7.55)	0.46	12.50 (2.09, 22.90)	0.02	—	—	20.38 (3.93, 36.84)	0.02
Constant	22.89 (14.00, 31.77)	<0.001	−14.37 (−74.28, 45.54)	0.64	14.53 (0.28, 28.78)	0.05	−1.40 (−18.56, 15.76)	0.87	12.83 (−15.17, 40.83)	0.37

All individuals—model-adjusted $R^2 = 0.09$; $P \leq 0.0001$. All adults—model-adjusted $R^2 = 0.15$; $P \leq 0.0001$. Individuals <20 years—model-adjusted $R^2 = 0.01$; $P \leq 0.28$. All non-asthmatic adults—model-adjusted $R^2 = 0.16$; $P \leq 0.0001$. All asthmatic adults—model-adjusted $R^2 = 0.13$; $P \leq 0.002$.

¹Number of participants with observations available for variables included.

—, not applicable; CI, confidence interval; ICS, inhaled corticosteroids.

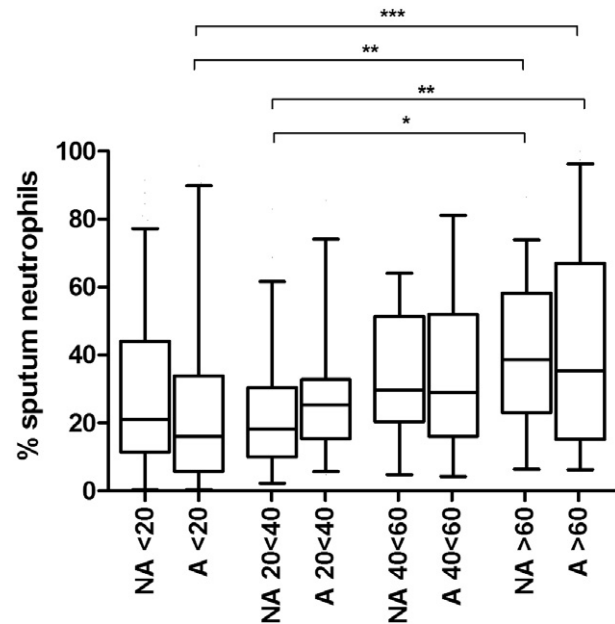


Figure 2 Distribution of sputum neutrophil percentages for asthmatics and non-asthmatics in different age groups (line = median, box = interquartile range (IQR), whiskers = 5th–95th percentile values; * $P = 0.01$ – 0.05 , ** $P = 0.001$ – 0.01 and *** $P \leq 0.0001$). A, asthmatic, NA, non-asthmatic.

airway neutrophils in the food allergy patients,³¹ suggesting that allergic/atopic mechanisms may be associated with subclinical neutrophilic airway inflammation. It is unclear if this is related to food allergy specifically, or atopy in general.

A 61% cut-off value has been commonly used to define 'normal' neutrophil numbers and is based on the 95th percentile value of sputum neutrophil percentages in mixed-age healthy populations.¹⁴ Other studies reporting sputum neutrophil reference values examined relatively young groups (mean age 34–38 years).^{6,7} As shown in the current study, airway neutrophil levels are dependent on age, and the previous 61% cut-off may not be entirely appropriate for some groups, particularly the very young or very old. The 95th percentile of neutrophil differential from non-asthmatic subjects in discrete 20-year age groups as determined in the current study (under 20 years—75.57%, 20–40 years—61.61%, 40–60 years—63.25% and over 60 years—67.25%) may guide the development of more valid age-specific reference values.

There were some limitations with this study. Firstly, it was cross-sectional, with only one assessment per participant. The variation we observed in each age group might be due, at least in part, to assessing individuals when they have particularly high or low neutrophil numbers, as intra-individual variation in sputum neutrophil percentages has previously been reported in longitudinal studies.^{32–34} Nonetheless, this would contribute to random error (independent of age) and is therefore unlikely to explain the specific trends with age, which we and others have shown.

Due to analysing data from several smaller studies, there were relatively small numbers in some age

Table 3 Number of individuals with >95th percentile of age-specific reference value of percentage sputum neutrophils/number of individuals with <95th percentile per age group

Age group	Asthma	No asthma	OR (95% CI)	Adj OR (95% CI)
<20 years	6/66 (8.3%)	6/106 (5.4%)	1.59 (0.49–5.14)	1.40 (0.40–4.93)
20–40 years	2/32 (5.9%)	3/56 (5.1%)	1.17 (0.19–7.35)	1.35 (0.18–9.94)
40–60 years	6/34 (15%)	3/38 (7.7%)	2.24 (0.52–9.64)	2.03 (0.43–9.52)
>60 years	12/36 (25%)	2/30 (6.25%)	5.00 (1.04–24.12)*	4.22 (0.84–21.01)
All	26/168 (13.4%)	14/229 (5.8%)	2.53 (1.28–4.99)**	2.12 (1.04–4.29)*

95th percentile = 75.57% for those <20 years, 61.61% for 20–40 years, 63.2% for 40–60 years and 67.25% for those >60 years.

* $P < 0.05$; ** $P < 0.005$.

Adj, adjusted for age, gender and smoking; CI, confidence interval; OR, odds ratio.

Table 4 Number of individuals with >95th percentile of age-specific reference value of absolute number of sputum neutrophils/number of individuals with <95th percentile per age group

Age group	Asthma	No asthma	OR (95% CI)	Adj OR (95% CI)
<20 years	4/45 (8.2%)	5/92 (5.2%)	1.26 (0.47–3.37)	0.94 (0.37–5.94)
20–40 years	5/27 (15.6%)	2/52 (3.7%)	3.63 (0.32–41.54)	7.21 (0.37–141.73)
40–60 years	9/29 (23.7%)	1/33 (2.9%)	7.06 (0.81–61.56)	4.82 (0.51–45.99)
>60 years	11/37 (28.9%)	1/29 (3.3%)	8.16 (0.99–67.26)	6.83 (0.79–58.91)
All	29/138 (17.4%)	9/206 (4.2%)	2.74 (1.37–5.49)**	2.27 (1.10–4.67)*

95th percentile = $735 \times 10^4/\text{mL}$ for those <20 years, $162 \times 10^4/\text{mL}$ for those 20–40 years, $319 \times 10^4/\text{mL}$ for those 40–60 years and $442 \times 10^4/\text{mL}$ for those >60 years.

* $P < 0.05$; ** $P < 0.005$.

Adj, adjusted for age, gender and smoking; CI, confidence interval; OR, odds ratio.

groups and limited age variance in the youngest group in particular. Furthermore, individual studies that provided data for this study were conducted over several years in two different centres, and it is possible that there may be some variation due to slightly different procedures. However, training and sputum-processing methodology was identical in both centres. The definition of asthma used (physician diagnosis) was the same in both centres, and although bronchial hyperreactivity measurement was used to confirm diagnosis only in Australia, the questionnaire-based approach used in New Zealand has previously been validated against bronchial hyperreactivity.³⁵ We were unable to control for the different centres due to different demographic groups studied (predominantly younger and male in New Zealand, and older in Australia). Although we adjusted for gender in multivariate models, and observed no significant differences in univariate analysis when we stratified the population into males and females, we cannot exclude the possibility that the significantly higher number of males in the youngest age group compared with other groups may have affected our results. Furthermore, we were unable to assess asthma severity, bronchodilator response or number of exacerbations for all asthmatic participants due to study variations and the current use of ICS in the majority (66.5%) of asthmatics studied. Finally, it is possible that some of the older asthmatic subjects may have asthma/chronic obstructive pulmonary disease overlap, as although individuals with a diagnosis or evidence of chronic

obstructive pulmonary disease were excluded at assessment, the component of irreversible airway obstruction in some elderly asthmatics is well characterised.³⁶ Despite this, when we conducted analyses excluding participants with forced expiratory volume in 1 s/forced vital capacity ratio of less than 0.7 (based on Global Initiative for Chronic Obstructive Pulmonary Disease guidelines), we observed a similar significant association between neutrophilia and ageing (data not shown).

There are various immunological changes observed with ageing in the airways,³⁷ and these occur in parallel with structural and physiological changes, such as reduced elastic recoil of the lungs and increased alveolar size.³⁸ It is currently unclear as to whether increased neutrophils are actually a cause or effect of this process, previously described as 'inflammaging'.³⁹ There is evidence that neutrophilia may occur in response to bacterial colonization in asthma⁴⁰ and chronic obstructive pulmonary disease,⁴¹ and macrolide antibiotics have been shown to reduce both interleukin-8 levels and neutrophil numbers in severe refractory asthma.²² Although it remains unclear as to exactly how important airway neutrophilia is in some types of asthma, the consistent relationship between airway obstruction, age and airway neutrophilia observed in this study and previously^{8,15,28,42} especially in older asthmatic populations warrants further investigation. Furthermore, we cannot explain the considerable variation, and lack of association observed between neutrophil percentage and age in the youngest age group. Indeed, inclusion of this

group weakened the association between airway and neutrophilia and ageing observed when adults were analyses alone. We believe this requires further attention.

In conclusion, this study shows similar increases in sputum neutrophil percentages with ageing in both asthmatics and non-asthmatic adults, while the under 20-year-old age group showed substantial variation and no clear relationship with age. Our study suggests that the use of appropriate age-adjusted reference values may be useful when describing respiratory diseases characterized by airway neutrophilia.

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