



# Blood eosinophil level and lung function trajectories: cross-sectional and longitudinal studies in European cohorts

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## ABSTRACT

**Background:** Elevated blood eosinophils have been associated with lower lung function and are believed to be associated with accelerated lung function decline.

**Method:** Blood eosinophils were measured in four cohorts: <45 years cohort within the Vlagtwedde–Vlaardingen (V&V) study, the Uppsala cohort of the European Community Respiratory Health Survey (ECRHS-Uppsala; <45 years), ≥45 years cohort within the V&V study, and the Rotterdam study (≥45 years). Blood eosinophils at baseline were classified as normal (<300 cells·μL<sup>-1</sup>) or elevated (≥300 cells·μL<sup>-1</sup>). Lung function was measured at baseline and follow-up with spirometry: forced expiratory volume in 1 s (FEV<sub>1</sub>), vital capacity (VC) and their ratio FEV<sub>1</sub>/VC. The association between blood eosinophils and lung function was tested cross-sectionally using linear regression and longitudinally using a mixed model, both adjusted for age, sex, height, pack-years smoking and smoking status. Stratified analyses were done for asthma.

**Results:** Elevated blood eosinophils were associated with lower FEV<sub>1</sub> (regression coefficient –147 mL (95% CI –188 to –105 mL)), VC (–120 mL (–165 to –75 mL)) and FEV<sub>1</sub>/VC (–1.3% (–1.9% to –0.6%)) at baseline in the two <45 years cohorts, and with lower FEV<sub>1</sub> (–70 mL (–112 to –27 mL)) and FEV<sub>1</sub>/VC (–1.8% (–2.6% to –1.0%)) in the two ≥45 years cohorts. Elevated blood eosinophils were associated with an accelerated decline in FEV<sub>1</sub> (–5.5 mL·year<sup>-1</sup> (95% CI –10.5 to –0.5 mL·year<sup>-1</sup>)) and VC (–6.4 mL·year<sup>-1</sup> (–11.26 to –1.5 mL·year<sup>-1</sup>)) compared to normal blood eosinophils in the younger asthmatic subjects in the longitudinal studies.

**Conclusion:** Elevated blood eosinophils are associated with lower lung function in the general population and with an accelerated lung function decline among asthmatic individuals.



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**Elevated blood eosinophils (≥300 cells per μL) associate with lower lung function in an adult general population and with a worse lung function trajectory in adult asthmatic individuals**

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## Introduction

Lung function volumes increase in most subjects until around the age of 15–20 years and starts to decline around the age of 25 years [1]. An impaired peak lung function as well as an accelerated decline can contribute to low lung function at a later age [2, 3]. A common cause of accelerated lung function decline is cigarette smoking, which can lead to chronic obstructive pulmonary disease (COPD) with persistently reduced forced expiratory volume in 1 s (FEV<sub>1</sub>) [4] and FEV<sub>1</sub>/forced vital capacity (FVC) ratio [5]. Accelerated lung function decline is also observed in some asthmatic individuals and has been related to airway remodelling, with basal membrane thickening, excess mucus production, airway smooth muscle hyperplasia and epithelial shedding [6], ultimately leading to fixed airflow obstruction with persistently reduced FEV<sub>1</sub> and FEV<sub>1</sub>/FVC ratio. In COPD, the mechanisms associated with accelerated lung function decline are probably different from those in asthma and associated to a lesser extent with eosinophil inflammation [7].

In asthma and COPD, eosinophilic airway inflammation is seen as a specific phenotype with elevated levels of sputum eosinophils and/or blood eosinophils affecting one-third [8, 9] to half of the patients [10]. Blood eosinophils are associated with sputum eosinophils [11, 12]. Anti-interleukin (IL)-5 therapies, which almost deplete blood eosinophil levels, reduce exacerbations in patients with severe refractory asthma with high levels of blood eosinophils [13].

In previous population-based investigations, elevated blood eosinophils have been found to be associated with lower lung function in the general population [14–16]. In asthmatic populations, elevated blood eosinophils are likewise associated with lower FEV<sub>1</sub>, especially when both local and systemic eosinophil inflammation is present [10, 17]. Over time, some studies have found that a higher blood eosinophil level is associated with an accelerated lung function decline in the general population [14, 18] and a lower maximally attained lung function [1]; however, this finding is not consistent across studies [19]. Also in studies among asthmatic subjects, the associations between blood eosinophil levels and lung function development have been diverse [18–22]. Hence, it is still unclear whether eosinophilic inflammation is a driving factor in lung function decline, and whether this association is specific or not for persons with asthma.

Therefore, we analysed the relation between blood eosinophils and lung function level and decline in four different population-based cohorts. The cohorts were followed during different decades (from the 1950s to 2010s) both before and after introduction of inhaled corticosteroids (ICS) and with a wide age span to identify patterns of lung function impairment. Our hypothesis was that elevated blood eosinophils are associated with a lower lung function level (cross-sectional analysis) and with an accelerated lung function decline during the follow-up time (longitudinal analysis). Moreover, we stratified our analyses according to the presence or absence of asthma to see whether it was a common cause of both the accelerated lung function decline and higher blood eosinophil levels.

## Material and methods

### Populations

The Vlagtwedde–Vlaardingen study, which began in 1965, took its study population from the two communities of Vlagtwedde and Vlaardingen in the Netherlands and involved >8000 participants [23, 24]. The study followed up the participants approximately every 3 years, with the maximum number of visits per participant being eight (at most 25 years of follow-up data). In total, 6684 participants were included in the cross-sectional analysis, and 3642 were included in the longitudinal analysis (subjects had at least two spirometry measurements over a period of at least 5 years). The study population was divided into two separate cohorts, based on their age at baseline: 18–44 years (<45 years) *versus* 45–74 years (≥45 years).

The European Community Respiratory Health Survey (ECRHS) is a multinational European cohort with three visits: 1991–1992, 1999–2000 and 2011–2012. In this study, eligible participants from the study centre in Uppsala, Sweden (ECRHS-Uppsala) were included. They were randomly selected and aged 20–44 years, and were included for a questionnaire in 1990. A random sample of 800 subjects was selected for clinical examination during 1991–1992, and in addition to them, the subjects who had reported asthma symptoms in the initial questionnaire [25]. In total, 836 subjects were examined, 676 of whom performed a spirometry test and had blood eosinophils measured at baseline. Of these 676 individuals, 487 subjects participated in at least one of the two follow-ups.

The Rotterdam study is a population-based cohort study that started in 1989 comprising almost 20 000 participants aged ≥45 years, which aimed to assess the occurrence of, and risk factors for, chronic diseases in the elderly [26]. Every 3–4 years, participants underwent a home interview and clinical examinations at the research centre. The current study contains participants who visited the Rotterdam research centre

between 2014 and 2016 (RS-I-6 and RS-II-4) and had an interpretable spirometry and a measured blood eosinophil count. This population was aged 70–95 years at this follow-up. At that time, 2857 participants were invited for examination, 1737 of whom were eligible (figure 1).

In all cohorts, smoking status and smoking history were self-reported, and the number of pack-years (one pack-year corresponding to one pack of 20 cigarettes smoked daily during 1 year) was calculated. Smoking status was divided into never-, ex- or current smokers.

In the Vlagtvedde–Vlaardingen study, asthma was defined as present when the participant answered yes to the question “Have you ever had attacks of shortness of breath with wheezing at rest?” In the ECRHS-Uppsala, current asthma at baseline was defined as present if the subject self-reported a doctor’s diagnosis of asthma, used asthma medication and/or had an asthma attack in the past 12 months. In the Rotterdam study, current asthma was defined as asthma diagnosis by a doctor and asthma symptoms and/or medication in the last 6 months [27].

No information on medication was available in the Vlagtvedde–Vlaardingen study. In the ECRHS-Uppsala, ICS use or oral corticosteroid (OCS) use was reported for the past 12 months. In the Rotterdam study, medication prescription data were available through automated pharmacy records. ICS or OCS use was defined as presence of a prescription within 90 days prior to the date of blood analysis.

### Lung function

Pre-bronchodilation values were used for all analyses. In the Vlagtvedde–Vlaardingen study, a water-sealed spirometer (Lode Spirograph D52, Lode, Groningen, the Netherlands) was used [1]. In the ECRHS-Uppsala, a dry rolling seal spirometer system (Sensor Medics 2130, Sensor Medics, Anaheim, CA, USA) was used in the first and second survey, while the NDD Easy One spirometer (NDD Medizintechnik, Zurich, Switzerland) was used in the third survey. In the Rotterdam study, a Master

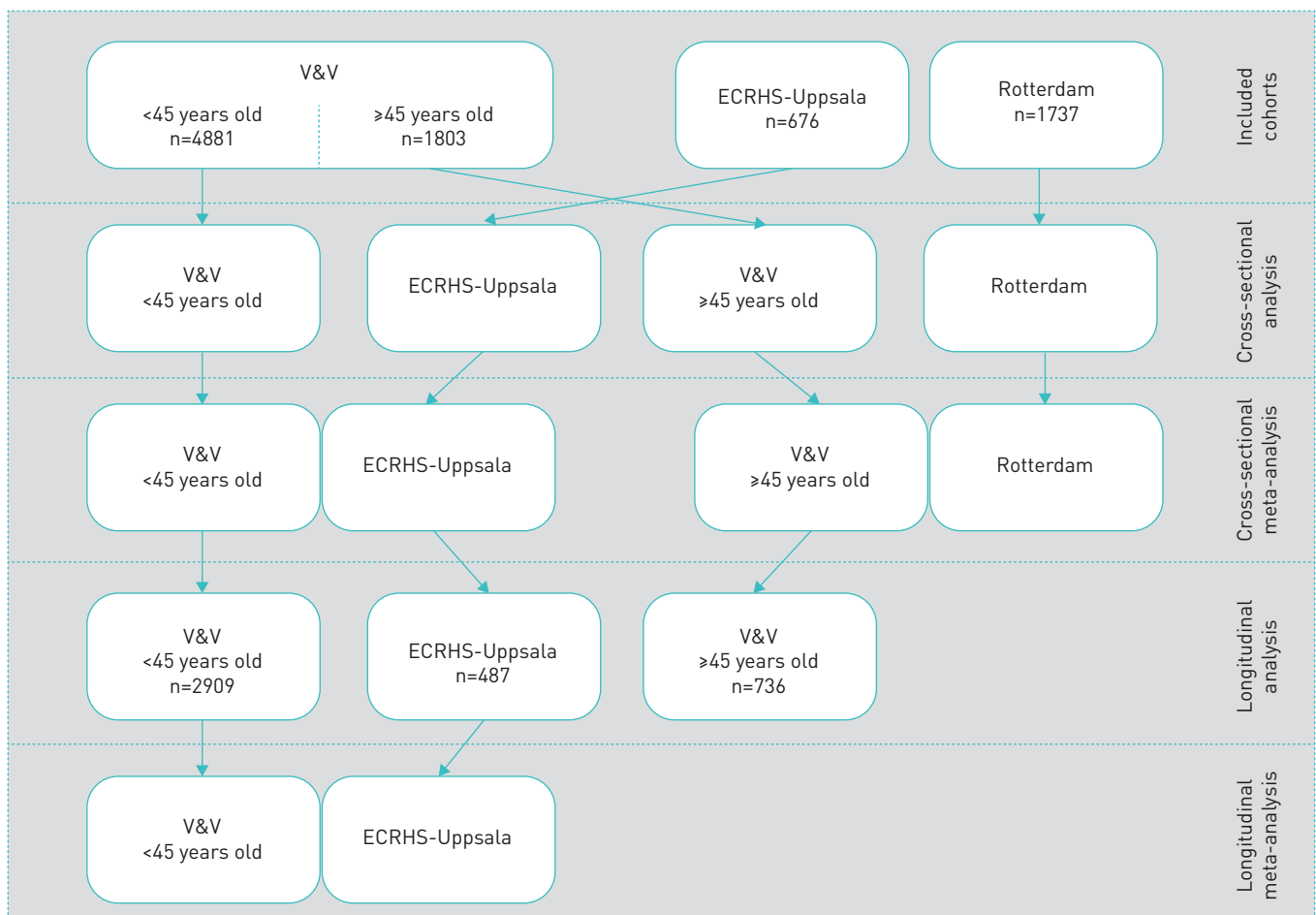


FIGURE 1 Study model. V&V: Vlagtvedde–Vlaardingen; ECRHS: European Community Respiratory Health Survey.

Screen PFT Pro (Care Fusion Netherlands, Houten, the Netherlands) was used. In the Vlagtwedde–Vlaardingen study, the FEV<sub>1</sub> and inspiratory vital capacity (IVC) was measured and FEV<sub>1</sub>/IVC was calculated at all visits. In the ECRHS-Uppsala and Rotterdam studies, FVC was measured. The term “VC” in the paper refers to IVC in the Vlagtwedde–Vlaardingen study and FVC in the ECRHS or Rotterdam studies. Predicted FEV<sub>1</sub> and FVC values were calculated using the Global Lung Initiative reference equations [28]. In all cohorts, spirometry was conducted according to the American Thoracic Society/European Respiratory Society guidelines present at the time of examination.

### **Blood eosinophils**

In the Vlagtwedde–Vlaardingen study, blood eosinophil counts were assessed at baseline in a 1:11 dilution of peripheral blood with a Bürker counting chamber [29]. In the ECRHS, blood eosinophils were analysed at baseline on a Hemalog 2R (Technicon Chemicals Company, Tournai, Belgium), in 5 mL blood supplemented with EDTA (0.34 mol·L<sup>-1</sup>) [25]. In the Rotterdam study, blood eosinophils were measured with a Sysmex XS-800 Hematology analyser (Sysmex, Norderstedt, Germany) in blood supplemented with EDTA. Blood eosinophils were analysed dichotomised (elevated if  $\geq 300$  cells· $\mu\text{L}^{-1}$  or normal if  $< 300$  cells· $\mu\text{L}^{-1}$  [30]).

### **Statistical methods**

#### *Cross-sectional analyses*

The four cohorts were cross-sectionally analysed separately in multivariate linear regression analyses with elevated blood eosinophils compared to normal blood eosinophils as the independent variable and FEV<sub>1</sub>, VC and FEV<sub>1</sub>/VC as outcomes. Inverse variance weighted meta-analyses were conducted on results from the  $< 45$  years Vlagtwedde–Vlaardingen cohort and the ECRHS-Uppsala cohort, and from the  $\geq 45$  years Vlagtwedde–Vlaardingen cohort and the Rotterdam cohort. The analyses were adjusted for age, sex, height, weight, smoking and pack-years of cigarette smoking.

#### *Longitudinal analyses*

The longitudinal analyses were performed with mixed models, having elevated blood eosinophils as an independent variable and lung function decline over time, in mL per year (FEV<sub>1</sub> and VC) and change in percent FEV<sub>1</sub>/VC per year as outcome. Results from the  $< 45$  years Vlagtwedde–Vlaardingen cohort and ECRHS-Uppsala cohort were further meta-analysed using an inverse variance weighted meta-analysis. Analyses were adjusted for age, sex, height, weight, smoking and pack-years of cigarette smoking. In the Rotterdam study, blood eosinophils were measured at the most recent study visit, and this cohort was not longitudinally analysed.

#### *Asthma*

To evaluate effect modification by asthma, known to be associated with higher blood eosinophils and potentially related to an accelerated lung function decline, asthma-stratified regression analyses were performed both cross-sectionally and longitudinally.

#### *Sensitivity analyses*

Analyses for the association between blood eosinophils as a continuous variable (logarithmised) and lung function were performed both cross-sectionally and longitudinally to evaluate the association, independent of the chosen blood eosinophil cut-off. Also, analyses with additional adjustments for OCS and ICS use in the ECRHS-Uppsala and Rotterdam cohorts were performed to evaluate the potential confounding effect of steroid treatment on the association between blood eosinophils and lung function.

The statistical analyses in the Vlagtwedde–Vlaardingen study were done in SPSS version 23 (released 2015; IBM SPSS Statistics for Windows, Version 23.0; IBM Corp., Armonk, NY, USA) and in the ECRHS-Uppsala study in Stata/IC 15.1 (StataCorp, College Station, TX, USA). The analyses in the Rotterdam study and the meta-analyses were done in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

### **Ethics**

The Vlagtwedde–Vlaardingen study protocol was approved by the Medical Ethics Committee of the University Medical Centre Groningen, Groningen, The Netherlands. All participants provided informed written consent.

ECRHS-Uppsala was approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr. 1991/33; 1999/313; 2010/068), and all participants signed the informed consent.

The Rotterdam study was approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Centre and by the review board of The Netherlands Ministry of Health, Welfare and Sports (Dnr. MEC 02.115).

## Results

The baseline characteristics of the four cohorts are shown in table 1. The blood eosinophil level was lowest in the Vlagtwedde–Vlaardingen study and highest in the Rotterdam study. The FEV<sub>1</sub> (% pred) was close to 100% at baseline in the ECRHS and Rotterdam studies; 86% in the <45 years Vlagtwedde–Vlaardingen cohort and 80% in the ≥45 years Vlagtwedde–Vlaardingen cohort. The mean age was highest in the Rotterdam study (78 years). The Vlagtwedde–Vlaardingen <45 years cohort had the highest prevalence of current smokers, while the ECRHS-Uppsala and ≥45 years Vlagtwedde–Vlaardingen cohort had the highest prevalence of never-smokers (50%). The prevalence of asthma was highest in the ECRHS cohort, which was enriched with asthmatic subjects, at 13%, and lowest in the Vlagtwedde–Vlaardingen cohorts at 3–4% (table 1). The Rotterdam study had 7.7% asthmatic participants. The use of ICS among the asthmatic individuals was 33% in the ECRHS study and 49% in the Rotterdam study. The baseline characteristics stratified on asthma are presented in supplementary table S1a and b, and of the subjects included in the longitudinal analysis in supplementary table S2.

### Cross-sectional analysis

In the cross-sectional analysis, elevated blood eosinophils were associated with a lower FEV<sub>1</sub> in all cohorts, with borderline statistical significance ( $p=0.057$ ) in the Rotterdam study (table 2) and a significant association in the meta-analyses (figure 2). Moreover, elevated blood eosinophils were associated with a lower VC in the <45 years cohorts but not in the ≥45 years cohorts. Elevated blood eosinophils were associated with a lower FEV<sub>1</sub>/VC in all cohorts (table 2) and this was also seen in the meta-analyses (figure 2). Stratified for asthma, the <45 years Vlagtwedde–Vlaardingen cohort showed an association between elevated blood eosinophils, compared to normal blood eosinophils, and a lower lung function, in both asthmatic and non-asthmatic individuals, but with larger estimates among asthmatic individuals. In the ECRHS-Uppsala, there was a significant association between elevated blood eosinophils compared to normal blood eosinophils and lower lung function only among the non-asthmatic individuals. In the ≥45 years cohorts, there were associations between elevated blood eosinophils and lower VC among the asthmatic patients from Vlagtwedde–Vlaardingen, and a lower ratio among the non-asthmatic individuals from Vlagtwedde–Vlaardingen and a trend in Rotterdam (table 3). In the meta-analyses elevated blood eosinophils in general were associated with lower lung function estimates in the asthma group; however, the smaller group size yielded larger confidence intervals (figure 2).

TABLE 1 Characteristics of the cross-sectional study populations

	V&V 18–44 years of age	ECRHS-Uppsala 20–45 years of age	V&V 45–74 years of age	Rotterdam 70–95 years of age
Subjects	4881	676	1803	1737
Female	2348 (48%)	341 (50%)	987 (55%)	952 (55%)
Age years	30.3±8.0	33±7.3	53±5.9	78±5.1
Never-smokers	1559 (32%)	338 (50%)	896 (50%)	589 (34%)
Previous smokers	490 (10%)	154 (23%)	146 (8%)	1036 (60%)
Current smokers	2832 (58%)	182 (27%)	761 (42%)	111 (6%)
Pack-years <sup>#</sup>	4.2 (0.9–11.3)	8.5 (3.2–17)	23.1 (12.9–36.0)	18.1 (6.8–35.0)
Asthma	176 (3%)	90 (13%)	65 (3.6%)	127 (7.5%)
ICS use		38 (6%)		155 (9%)
ICS use, asthmatic individuals		30 (33%)		79 (48%)
OCS use		2 (0.3%)		123 (7%)
OCS use, asthmatic individuals		2 (2%)		26 (16%)
BMI kg·m <sup>-2</sup>	24.6±3.5	23.3±3.5	27.4±4.0	27.4±4.1
FEV <sub>1</sub> % pred	85.9±12.1	102±13	79.5±15.2	97±19
Blood eosinophils <sup>†</sup> cells·μL <sup>-1</sup>	98±2.7	152±2.1	109±2.6	182±1.9
Blood eosinophils ≥300 cells·μL <sup>-1</sup>	412 (8%)	119 (18%)	158 (9%)	369 (21%)

Data are presented as n, n (%), mean±SD or median (interquartile range), unless otherwise stated. V&V: Vlagtwedde–Vlaardingen study; ECRHS: European Community Respiratory Health Survey; ICS: inhaled corticosteroids; OCS: oral corticosteroids; BMI: body mass index; FEV<sub>1</sub>: forced expiratory volume in 1 s; % pred: % predicted. <sup>#</sup>: among the participants with a history of smoking; <sup>†</sup>: geometric mean±exp.sd.

TABLE 2 Associations between elevated blood eosinophils ( $\geq 300$  cells- $\mu\text{L}^{-1}$ ) and lung function in a cross-sectional analysis

	FEV <sub>1</sub> <sup>#</sup> mL β (95% CI)	VC <sup>#,†</sup> mL β (95% CI)	FEV <sub>1</sub> /VC <sup>#,†</sup> % β (95% CI)
Vlagtwedde-Vlaardingen <45 years of age, n=4881	-142 [-188 to -96]	-120 [-169 to -71]	-1.1 [-1.9 to -0.3]
ECRHS-Uppsala, n=676	-167 [-265 to -67]	-119 [-231 to -6.5]	-1.7 [-3.0 to -0.36]
Vlagtwedde-Vlaardingen $\geq 45$ years of age, n=2139	-108 [-184 to -32]	-40 [-119 to 39]	-2.9 [-4.4 to -1.5]
Rotterdam, n=1686	-52 [-104 to 0.4]	-12 [-73 to 50]	-1.3 [-2.3 to -0.4]

Significant associations in bold. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity; β: regression coefficient; CI: confidence interval; ECRHS: European Community Respiratory Health Survey. <sup>#</sup>: adjusted for previous or current smoking, pack-years, age, sex, height and weight; <sup>†</sup>: forced VC in the Rotterdam and ECRHS studies, or inspiratory VC in the Vlagtwedde-Vlaardingen study.

### Longitudinal analysis

In the longitudinal analysis, no statistically significant associations were seen between elevated blood eosinophils at baseline and an accelerated lung function decline in the two Vlagtwedde-Vlaardingen cohorts or in the meta-analyses (figure 3). In the ECRHS-Uppsala, elevated blood eosinophils were associated with an accelerated FEV<sub>1</sub> and VC decline, while FEV<sub>1</sub>/VC was not significantly affected (table 4). Stratified for asthma, elevated compared to normal blood eosinophils at baseline was associated with an accelerated VC decline among asthmatic individuals in the <45 years Vlagtwedde-Vlaardingen cohort, and with an accelerated FEV<sub>1</sub> decline among asthmatic individuals and an accelerated VC decline among the non-asthmatic individuals in the ECRHS-Uppsala cohort (table 5). The  $\geq 45$  years Vlagtwedde-Vlaardingen cohort had too few asthmatic subjects to analyse (n=19). In the meta-analyses, an accelerated decline in FEV<sub>1</sub> and VC was seen among the asthmatic individuals, but not the non-asthmatic individuals (figure 3).

### Sensitivity analyses

There were only minor differences when blood eosinophils were analysed as a continuous variable in the cross-sectional (supplementary table S3) and longitudinal analyses (supplementary table S4). In the ECRHS-Uppsala, adjustment for ICS/OCS resulted in a non-significant association between elevated blood eosinophils and a lower VC and FEV<sub>1</sub>/VC in the cross-sectional analysis and a non-significant association with accelerated VC decline among non-asthmatic individuals in the longitudinal analysis. The association between elevated blood eosinophils and lower FEV<sub>1</sub> became significant (-45 mL, 95% CI -82 to -8 mL) among the non-asthmatic individuals with adjustment for ICS/OCS in the Rotterdam study.

### Discussion

In this study, we investigated the association between elevated blood eosinophils (*i.e.*  $\geq 300$  cells- $\mu\text{L}^{-1}$ ) and lung function parameters in cross-sectional and longitudinal analyses in four European cohorts. The main results show an association between elevated blood eosinophils and lower lung function cross-sectionally in the entire population. Longitudinally, the results were more scattered. Meta-analysed, an association between elevated blood eosinophils and an accelerated FEV<sub>1</sub> and VC decline was found in asthmatic

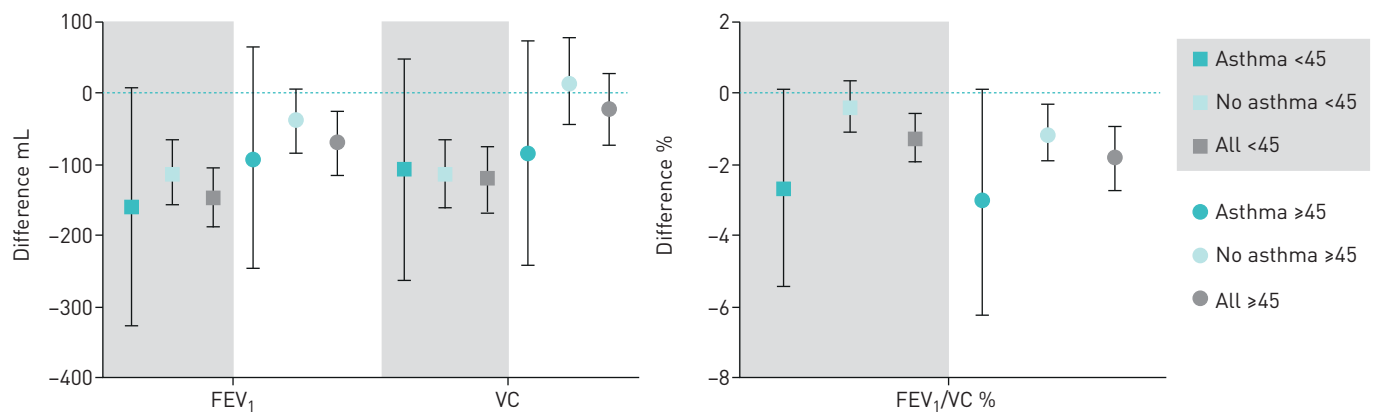


FIGURE 2 Cross-sectional meta-analyses, presenting results for the whole cohort and stratified for asthma. Grey-shaded areas represent the meta-estimates and 95% confidence intervals for the two <45 years cohorts. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity (forced VC in the Rotterdam and European Community Respiratory Health Survey studies, or inspiratory VC in the Vlagtwedde-Vlaardingen study).



TABLE 3 Asthma-stratified analysis, cross-sectional associations between elevated blood eosinophils ( $\geq 300$  cells- $\mu\text{L}^{-1}$ ) and lung function

	FEV <sub>1</sub> adjusted <sup>#</sup> mL $\beta$ (95% CI)	VC adjusted <sup>#†</sup> mL $\beta$ (95% CI)	FEV <sub>1</sub> /VC adjusted <sup>#†</sup> % $\beta$ (95% CI)
<b>Asthma</b>	<b>-385 (-610 to -159)</b>	<b>-215 (-414 to -16)</b>	<b>-6.1 (-10.3 to -1.8)</b>
Vlagtwedde-Vlaardingen <45 years of age, n=176			
<b>No asthma</b>	<b>-102 (-150 to -55)</b>	<b>-106 (-158 to -55)</b>	-0.3 (-1.1 to 0.6)
Vlagtwedde-Vlaardingen <45 years of age, n=4703			
<b>Asthma</b>	125 (-129 to 381)	67 (-187 to 321)	-0.12 (-3.8 to 3.6)
ECRHS-Uppsala, n=90			
<b>No asthma</b>	<b>-158 (-269 to -48)</b>	<b>-156 (-290 to -23)</b>	-0.58 (-2.0 to 0.80)
ECRHS-Uppsala, n=585			
<b>Asthma</b>	-309 (-687 to 69)	<b>-382 (-663 to -102)</b>	-5.2 (-14.1 to 3.7)
Vlagtwedde-Vlaardingen $\geq 45$ years of age, n=65			
<b>No asthma</b>	-53 (-131 to 25)	111 (-32 to 254)	<b>-1.8 (-3.2 to -0.3)</b>
Vlagtwedde-Vlaardingen $\geq 45$ years of age, n=1738			
<b>Asthma</b>	-44 (-217 to 128)	64 (-131 to 260)	-2.7 (-6.1 to 0.7)
Rotterdam, n=127			
<b>No asthma</b>	-31 (-87 to 25)	-3 (-69 to 64)	-0.8 (-1.8 to 0.1)
Rotterdam, n=1571			

Significant associations in bold. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity;  $\beta$ : regression coefficient; CI: confidence interval; ECRHS: European Community Respiratory Health Survey. #: adjusted for age, height, weight, sex, pack-years, previous and current smoking; †: forced VC in the Rotterdam and ECRHS studies, or inspiratory VC in the Vlagtwedde-Vlaardingen study.

individuals in the younger cohorts; the non-asthmatic individuals did not have an accelerated lung function decline either among the younger or older participants.

Cross-sectionally, an association was found between elevated blood eosinophil counts and lower lung function. The association with the obstructive pattern (lower FEV<sub>1</sub> and FEV<sub>1</sub>/VC ratio) was found in both the younger and older cohorts. The association between elevated blood eosinophils and lower VC was only seen in the younger cohorts, suggesting an additional relation with lower lung volumes in younger adults, as opposed to the older cohorts. This might be explained to some extent by the fact that in young adults who are still in their growth phase, a higher blood eosinophil level has been linked to a lower peak lung function [1].

Ageing affects both level of lung function and level of blood eosinophils. Lung function increases with age until around 15–20 years of age, after which it plateaus until ~20–25 years of age [1, 31], after which it decreases, although higher cut-off points have been reported [32]. JACINTO *et al.* [33] reported that blood eosinophil levels decreased from 6 years until ~35 years of age, and then slightly increased with higher age. The increase of blood eosinophils with age, together with the decline in pulmonary function, might

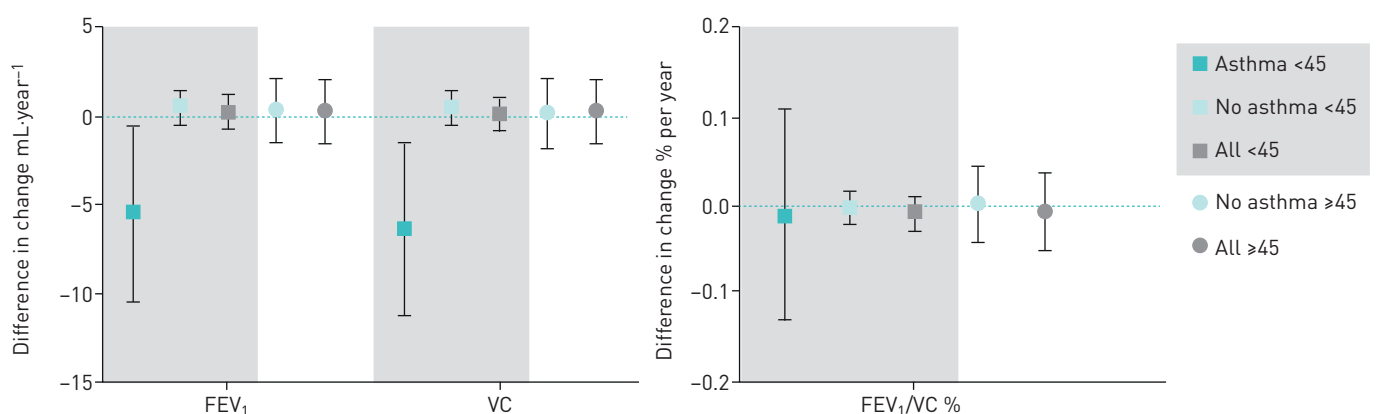


FIGURE 3 Longitudinal meta-analyses, presenting results for the whole cohort and stratified for asthma. Grey-shaded areas represent the meta-estimates and 95% confidence intervals for the two <45 years cohorts. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity (forced VC in the Rotterdam and European Community Respiratory Health Survey studies, or inspiratory VC in the Vlagtwedde-Vlaardingen study).

TABLE 4 Associations between elevated blood eosinophils ( $\geq 300$  cells· $\mu\text{L}^{-1}$ ) and excess lung function decline over time

	FEV <sub>1</sub> adjusted <sup>#</sup> mL·year <sup>-1</sup> β (95% CI)	VC adjusted <sup>#,¶</sup> mL·year <sup>-1</sup> β (95% CI)	FEV <sub>1</sub> /VC adjusted <sup>#,¶</sup> percent/year β (95% CI)
Vlagtwedde–Vlaardingen <45 years of age, n=2909	0.6 [−0.4 to 1.5]	0.4 [−0.5 to 1.4]	−5.7×10 <sup>-3</sup> [−2.5×10 <sup>-2</sup> to 1.3×10 <sup>-2</sup> ]
ECRHS-Uppsala, n=487	<b>−6.4 [−10.5 to −2.3]</b>	<b>−7.2 [−11.9 to −2.4]</b>	−3.4×10 <sup>-2</sup> [−1.1×10 <sup>-1</sup> to 4.2×10 <sup>-2</sup> ]
Vlagtwedde–Vlaardingen ≥45 years of age, n=737	0.3 [−1.4 to 2.1]	0.3 [−1.6 to 2.1]	−3.4×10 <sup>-3</sup> [−4.7×10 <sup>-2</sup> to 4.0×10 <sup>-2</sup> ]

Significant associations in bold. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity; β: regression coefficient; CI: confidence interval; ECRHS: European Community Respiratory Health Survey. #: adjusted for age, height, weight, sex, pack-years, all in interaction with time; ¶: forced VC in the Rotterdam and ECRHS studies, or inspiratory VC in the Vlagtwedde–Vlaardingen study.

account for some of the associations found in the older cohort. However, we tried to account for this by adjusting for age.

In the longitudinal analyses, only the ECRHS-Uppsala cohort showed an accelerated lung function decline among the participants with elevated blood eosinophils. The ECRHS-Uppsala cohort had a prevalence of 14% asthmatic individuals who contributed to the longitudinal analysis due to enrichment with subjects with respiratory symptoms, according to the ECRHS protocol. Asthma is associated with both higher blood eosinophil levels and an accelerated lung function decline. Indeed, in the stratified analysis for asthma, the significant association between an elevated blood eosinophil level and an accelerated FEV<sub>1</sub> decline was only present among the asthmatic individuals in the ECRHS-Uppsala cohort. Also in the <45 years Vlagtwedde–Vlaardingen cohort, elevated blood eosinophil levels were associated with an accelerated VC decline among asthmatic individuals, but not among non-asthmatic individuals. Surprisingly, no association was seen between elevated blood eosinophils and lower lung function among the asthmatic individuals in the ECRHS or the Rotterdam cohorts in the cross-sectional analyses, possibly a consequence of availability of better treatments for inflammation in this era with greater access to ICS.

Earlier studies were inconclusive. In a 20-year study of young adults, HANCOX *et al.* [18] found higher blood eosinophil levels to be associated with lower FEV<sub>1</sub> and FEV<sub>1</sub>/FVC, but not with a lower FVC. These associations were found in both asthmatic and non-asthmatic individuals. However, other investigations

TABLE 5 Asthma-stratified analysis, associations between elevated blood eosinophils ( $\geq 300$  cells· $\mu\text{L}^{-1}$ ) and excess lung function decline over time

	FEV <sub>1</sub> adjusted <sup>#</sup> mL·year <sup>-1</sup> β (95% CI)	VC adjusted <sup>#,¶</sup> mL·year <sup>-1</sup> β (95% CI)	FEV <sub>1</sub> /VC adjusted <sup>#,¶</sup> percent/year β (95% CI)
<b>Asthma</b> Vlagtwedde–Vlaardingen <45 years of age, n=109	−3.8 [−9.4 to 1.9]	<b>−5.8 [−11.2 to −0.4]</b>	2.2×10 <sup>-2</sup> [−1.3×10 <sup>-1</sup> to 1.7×10 <sup>-1</sup> ]
<b>No asthma</b> Vlagtwedde–Vlaardingen <45 years of age, n=2797	0.8 [−0.2 to 1.8]	0.7 [−0.2 to 1.7]	−3.3×10 <sup>-3</sup> [−2.2×10 <sup>-2</sup> to 1.6×10 <sup>-2</sup> ]
<b>Asthma</b> ECRHS-Uppsala, n=70	<b>−12.3 [−23.6 to −0.9]</b>	−8.9 [−20.6 to 2.8]	−8.2×10 <sup>-2</sup> [−3.0×10 <sup>-1</sup> to 1.4×10 <sup>-1</sup> ]
<b>No asthma</b> ECRHS-Uppsala, n=416	−4.0 [−8.5 to 0.5]	<b>−6.1 [−11.8 to −0.5]</b>	2.3×10 <sup>-2</sup> [−5.5×10 <sup>-2</sup> to 1.0×10 <sup>-1</sup> ]
<b>Asthma</b> Vlagtwedde–Vlaardingen ≥45 years of age, n=19	Too few	Too few	Too few
<b>No asthma</b> Vlagtwedde–Vlaardingen ≥45 years of age, n=717	0.4 [−1.4 to 2.2]	0.2 [−1.8 to 2.1]	1.3×10 <sup>-3</sup> [−4.3×10 <sup>-2</sup> to 4.5×10 <sup>-2</sup> ]

Significant associations in bold. FEV<sub>1</sub>: forced expiratory volume in 1 s; VC: vital capacity; β: regression coefficient; CI: confidence interval; ECRHS: European Community Respiratory Health Survey. #: adjusted for age, height, weight, sex, pack-years, all in interaction with time; ¶: forced VC in the Rotterdam and ECRHS studies, or inspiratory VC in the Vlagtwedde–Vlaardingen study.



have not found a clear association between higher blood eosinophil levels and accelerated FEV<sub>1</sub> decline [19, 20] or even a slower decline [21, 22]. This is in contrast to the findings in this investigation, where the accelerated lung function decline was mainly seen in asthmatic individuals with elevated blood eosinophils, and there was also an effect on the VC.

A strength of this investigation is the inclusion of four large cohorts with both young and old individuals with and without asthma. For three of the cohorts, we also had follow-up measurements with a follow-up time of up to 25 years. Interestingly, when analysed with the same method, we found a fairly similar association between blood eosinophils and lung function in the cohorts analysed cross-sectionally in the respective age strata, despite differences in composition, time frame and exposures. However, when stratified for asthma, the associations were heterogeneous. One explanation could be the differences in asthma diagnosis between Vlagtwedde–Vlaardingen, ECRHS-Uppsala and Rotterdam. Another important difference is the use of ICS in both the ECRHS-Uppsala and Rotterdam cohorts, but not in the Vlagtwedde–Vlaardingen cohorts, which were followed up before ICS were commonly used, although some patients might have received systemic treatment with corticosteroids. Hence, better treatment of inflammation in the two later studies (ECRHS-Uppsala and Rotterdam) could be the reason for the better lung function measures among the asthmatic individuals with elevated blood eosinophils in these cohorts in the cross-sectional analyses. Interestingly, despite this, the <45 years old cohorts had an accelerated lung function decline among asthmatic individuals with elevated blood eosinophil levels, compared with asthmatic individuals with normal blood eosinophil levels, with the largest decline in the ECRHS-Uppsala cohort. In other words, we did not find a reduced lung function decline despite ICS use in about 36% of the participants with asthma in the ECRHS-Uppsala cohort, as compared to the Vlagtwedde–Vlaardingen cohort with no ICS use. However, the accelerated decline in VC also among non-asthmatic individuals with elevated blood eosinophil levels in the ECRHS-Uppsala cohort suggests that this association is not limited to persons with asthma.

There are some limitations to our study. The blood eosinophil measurements were taken only at one time-point, but it is known that blood eosinophils vary over time [34]. Further, only pre-bronchodilator lung function was measured. In subjects with asthma, a low pre-bronchodilator FEV<sub>1</sub> could be a sign of uncontrolled disease and undertreatment associated with temporarily higher blood eosinophil levels. This would possibly lead to large fluctuations in lung function measurements between the visits. However, the long follow-up time should to some degree attenuate for the effect of temporal fluctuations. The cohorts were also followed during different decades and in different countries (Vlagtwedde–Vlaardingen and Rotterdam studies in the Netherlands and ECRHS-Uppsala in Sweden) with different exposures to environmental factors that could possibly affect the results. The definitions of asthma and lung function measures (FVC and IVC) varied among the studies and are further limitations.

In conclusion, this study observed an association between elevated blood eosinophil levels and lower lung function cross-sectionally. Moreover, elevated blood eosinophils in the younger cohorts were associated with an overall lower lung function, while in the older cohorts an association with an obstructive pattern was mainly found. We could not consistently show an accelerated lung function decline related to elevated blood eosinophil levels, and our stratified analyses for asthma suggested that the association between elevated blood eosinophils and an accelerated FEV<sub>1</sub> decline is generally present in asthmatic individuals, but not in the general population.

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