Check for updates

MISS NUR SABRINA IDROSE (Orcid ID : 0000-0002-7079-3670)
DR BIRCAN ERBAS (Orcid ID : 0000-0001-9597-418X)
DR CAROLINE JANE LODGE (Orcid ID : 0000-0002-2342-3888)
DR SHYAMALI C DHARMAGE (Orcid ID : 0000-0001-6063-1937)

Article type : Systematic reviews and Meta-analysis

Outdoor pollen-related changes in lung function and markers of airway inflammation: A systematic review and meta-analysis

Running title: Outdoor pollen exposure and lung health

N. Sabrina Idrose, MSc[a,b], E. Haydn Walters, DM[a,c], Jingwen Zhang, MPH[a], Don Vicendese, PhD[a,d], Ed J Newbigin, PhD[e], Jo A Douglass, MD[f,g], Bircan Erbas, PhD[h], Adrian J Lowe, PhD[a], Jennifer L Perret, PhD[a], Caroline J Lodge, PhD[a]*,Shyamali C Dharmage, MD, PhD[a]*

*Equal senior

a. Allergy and Lung Health Unit, Melbourne School of Population and Global Health, The University of Melbourne, 207 Bouverie St, Carlton, Victoria, 3053, Australia.
b. Centre for Food and Allergy Research (CFAR), Murdoch Children's Research Institute, Parkville, Victoria, 3052, Australia.
c. School of Medicine, University of Tasmania, Hobart, Tasmania, 7000, Australia.
d. Department of Mathematics and Statistics, La Trobe University, Bundoora, 3086, Victoria, Australia.
e. School of Biosciences, The University of Melbourne, Victoria, 3052, Australia.
f. Department of Clinical Immunology and Allergy, Royal Melbourne Hospital, Parkville, 3050, Victoria, Australia.
g. Department of Medicine, University of Melbourne, Victoria, 3052, Australia.
h. School of Psychology and Public Health, La Trobe University, Bundoora, 3086, Victoria, Australia

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.13842

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

This article is protected by copyright. All rights reserved

Corresponding Author: Shyamali Dharmage
Centre of Epidemiology and Biostatistics,
Melbourne School of Population and Global Health,
The University of Melbourne,
207 Bouverie St, Carlton, VIC 3052
Tel: +61 3 83440737
Email: s.dharmage@unimelb.edu.au

Authors' contributions: N. Sabrina Idrose: Investigation, Writing- Original Draft, Review & Editing. **E. Haydn Walters:** Writing- Review & Editing. **Jingwen Zhang:** Investigation. **Don Vicendese:** Writing- Review & Editing. **Ed J Newbigin:** Writing- Review & Editing. **Jo Douglass:** Writing- Review & Editing. **Bircan Erbas:** Writing- Review & Editing. **Adrian Lowe:** Writing- Review & Editing. **Jennifer L Perret:** Writing- Review & Editing. **Caroline J Lodge:** Conceptualization, Writing- Review & Editing, Supervision. **Shyamali C Dharmage:** Conceptualization, Writing- Review & Editing, Supervision.

Word count (Abstract): 282
Word count (Manuscript): 4476
Table count: 2
Figure count: 2

Data Availability Statement: The data that support the results of the meta-analyses are available from the corresponding author upon reasonable request.

Abbreviations: CANO: alveolar nitric oxide concentration; EBC: exhaled breath condensate; ECP: eosinophil cationic protein; EPO: eosinophil peroxidase; FeNO: fractional exhaled nitric oxide; FEV: forced expiratory volume; FVC: forced vital capacity; HDM: house dust mite; HNL: human neutrophil lipocalin; IL: interleukin; J*á*wNO: maximal flow of nitric oxide from airways; LT: leukotrienes; MBP: major basic protein; MC_T: tryptase-only positive mast cell; MC_{TC}: chymase plus tryptase positive mast cell; MCAF/MCP-1: monocyte chemotactic and activating factor/monocyte chemoattractant protein-1; MEF: maximal expiratory flow; MIP-1α: macrophage inflammatory protein 1-alpha; NPRW+: no nasal polyps but with ragweed allergy; PEF: peak

This article is protected by copyright. All rights reserved

68 expiratory flow; PRW+: nasal polyps with ragweed allergy; PRW-: nasal polyps without ragweed
69 allergy; RANTES: CCL5; SAR: seasonal allergic rhinitis; sICAM-1: soluble intracellular adhesion
70 molecule 1; SMD: standard mean difference; T_H2: T-helper 2.

Accepted Article

This article is protected by copyright. All rights reserved

Abstract

Background: Experimental challenge studies have shown that pollen can have early and delayed effects on the lungs and airways. Here, we qualitatively and quantitatively synthesise the evidence of outdoor pollen exposure on various lung function and airway inflammation markers in community-based studies.

Methods: Four online databases were searched: Medline, Web of Science, CINAHL and Google Scholar. The search strategy included terms relating to both exposure and outcomes. Inclusion criteria were human-based studies published in English that were representative of the community. Additionally, we only considered cross-sectional or short-term longitudinal studies which investigated pollen exposure by levels or season. Study quality assessment was performed using the Newcastle-Ottawa scale. Meta-analysis was conducted using random-effects models.

Results: We included 27 of 6,551 studies identified from the search. Qualitative synthesis indicated associations between pollen exposure and predominantly type-2 inflammation in both the upper and lower airways, but little evidence for lung function changes. People with ever asthma and/or seasonal allergic rhinitis (SAR) were at higher risk of such airway inflammation. Meta-analysis confirmed a positive relationship between pollen season, eosinophilia and eosinophil cationic protein (ECP) in people with ever SAR but the results between studies were highly variable. Heterogeneity was reduced after further subgrouping by age and the forest plots indicated that eosinophilic airway inflammation to outdoor pollen exposure increased with age.

Conclusion: Among people with ever asthma and ever SAR, exposure to increased ambient pollen triggers type-2 upper and lower airway inflammation rather than a non-specific or innate inflammation. These findings can lead to the formulation of specific pollen immunotherapy for susceptible individuals. Future research should be directed towards investigating lagged associations and effect modifications using larger and more generalised populations.

Systematic review registration: CRD42020146981 (PROSPERO)

Key words: Airway inflammation, airway obstruction, lung function, lung health, pollen

Accepted Article

This article is protected by copyright. All rights reserved

Introduction

Asthma is a complex disease which involves the interplay between epithelial activation, various airway inflammatory and remodelling pathways, bronchial hyperresponsiveness, and usually variable airflow obstruction [1, 2]. People with asthma often have allergic rhinitis, which is an upper airway allergic disease [3, 4]. One of the major environmental triggers of asthma exacerbations is exposure to pollen [5, 6]. While this has been established through community-based studies, laboratory challenge studies have provided a more detailed understanding of the underlying physiological changes that take place during a pollen-induced asthma exacerbation.

Laboratory challenge studies showed that pollen can have both early- and late-phase effects on the upper and lower airways, but the reaction is more frequently early, particularly in people with seasonal allergic rhinitis (SAR) and/or asthma [7-10]. Similarly for lung function, 81% of adult asthmatics were shown to have decreased forced expiratory volume in 1 second (FEV_1) following bronchoprovocation with pollen: 40% had an early response, 25% had both early and late (i.e. up to 7 hours after challenge) responses and 16% had just a late response [10]. Two types of inflammatory responses have been observed in these studies: Type-2 immune responses leading to eosinophilic inflammation, and more non-specific inflammation likely due to activation of innate inflammatory pathways [8, 9, 11-14].

At a community level, in addition to asthma hospitalisations [15], outdoor pollen has been shown to trigger thunderstorm asthma events [16, 17]. Under extreme weather conditions, pollen is hypothesised to rupture into small inhalable particles capable of triggering asthma even in individuals without a documented clinical history of asthma [18-21]. It is suggested that the associations between pollen exposure, asthma hospitalisations and thunderstorm asthma may be the 'tip of an iceberg' of the community burden, as some studies have observed more subtle associations between outdoor pollen exposure and lung function changes and airway inflammatory responses [22-24].

Although few community-based studies have assessed the relationship between outdoor pollen exposure, lung function and airway inflammation, the findings have not been integrated

This article is protected by copyright. All rights reserved

systematically, especially at a detailed mechanistic level. Laboratory challenge studies may not be representative of outdoor pollen exposure because they involve direct introduction of allergens into the nostrils [25], do not take into account other seasonal factors or environmental co-stimulants [26] and often impose extremely high pollen concentrations that are unlikely to occur in pollen seasons [27, 28]. Here, we synthesised the available evidence to determine the strength and magnitude of the association between outdoor pollen exposure and lung function and airway inflammation at the community level. As an extension, we also aimed to classify whether the inflammatory data reflect a predominantly type-2 immune response, or alternatively a more non-specific/innate response.

Accepted Article

This article is protected by copyright. All rights reserved

Methods

Our systematic review followed the MOOSE [29] and PRISMA [30] guidelines for reporting systematic review and meta-analysis. A protocol was registered prospectively via the International Prospective Register of Systematic Reviews (PROSPERO Registration no. CRD42020146981).

Search strategy

The online databases used were Medline, CINAHL, Web of Science and the first 100 records of Google Scholar. The search strategy provided in the Online Data Supplement (Table E1) includes terms related to pollen and lung function and airway inflammation, combined. Further searches were conducted by looking through reference lists of included articles and any published reviews on similar topics. Two reviewers (NSI and JZ) independently conducted a screening process to identify eligible studies, with persisting disagreements settled by a third reviewer (SCD).

Eligibility criteria

The inclusion criteria were English language studies published until 16th of June 2020 that investigated the relationship between outdoor pollen exposure, lung function measures and airway inflammation markers. The studies must be representative of the community so only studies with no inclusion criteria or with a reference group were eligible. If the studies examined different groups (e.g. people with and without asthma) separately, the analysis results within each group must be reported. Only cross-sectional or short-term longitudinal studies were eligible. Short-term longitudinal studies were defined as studies that investigated exposure by pollen season compared to non-pollen season while cross-sectional studies were defined as studies that investigated exposure by daily average pollen levels i.e. the effects of pollen exposure on the day of clinical testing (lag 0) and/or days before (e.g. lag 1= pollen exposure the day before testing). Studies that compared participants living in high pollen load areas to those living in low pollen load areas were also considered to be cross-sectional. As cross-sectional and short-term longitudinal studies may not be comparable, the findings were reported separately.

Data extraction and quality assessment

Accepted Article

This article is protected by copyright. All rights reserved

For every included study, where available, relevant data such as study design and characteristics, exposure and outcome definitions and measurements, and findings were extracted. Airway inflammation indices in the various studies were segregated into those suggestive of a type-2, innate or non-specific inflammatory responses and whether they were derived from the upper or lower airways. The methodological quality of the included studies in the review were formally assessed using the Newcastle-Ottawa scale [31].

Assessments to conduct a meta-analysis

To be eligible for inclusion in meta-analyses, studies had to measure exposure in pollen season (of any taxon) compared to non-pollen season with comparable outcome estimates. As only eosinophils and eosinophil cationic protein (ECP) had comparable estimates, these two outcomes only were considered for the meta-analysis. To allow inclusion in meta-analyses where different outcome measures were reported, effect estimates were transformed to standardised mean difference and standard deviation by using the methods formulated by Wan et al. [32]. Meta-analysis was conducted using a random-effects model, with small-study bias assessed by funnel plots. We performed subgroup analyses defined by the presence or absence of ever SAR. Heterogeneity between included studies' results (I^2) [33] was categorised into low (0% to 40%), moderate (30% to 60%), substantial (50% to 90%) and considerable (75% to 100%) [34]. Meta-analyses were conducted using Stata IC 16.0 [35].

This article is protected by copyright. All rights reserved

Results

In total, 27 articles fulfilled the eligibility criteria (Figure 1). Approximately 80% of the 6,551 records were excluded at the initial screening stage because they were either non-human studies, not related to exposure or outcome or were immunotherapy studies (Figure 1). Four articles [23, 36-38] assessed lung function only as the outcome variable, 20 articles [24, 39-57] assessed airway inflammation only and three articles [58-60] assessed both lung function and airway inflammation. Altogether, six articles [47, 49, 51-53, 55] were included in the meta-analysis.

Study characteristics

These are summarised in Table 1. Most studies were conducted in Europe [23, 24, 36-44, 46, 47, 49, 52-55, 57-60], with some in North America [45, 50, 51, 56] and one in Asia [48].

Most studies investigated exposure by pollen season compared to non-pollen season (short-term longitudinal studies), others [24, 39] investigated exposure by daily average pollen levels (cross-sectional studies), either during the pollen season only [24, 37] or the entire study period which also included the non-pollen season [36, 39] and one [38] assessed participants living in high pollen load areas compared to those living in low pollen load areas (cross-sectional study).

Of the 27 studies, eight [24, 39, 41, 44, 47, 52, 53, 57] investigated grass pollen only, seven [23, 40, 48, 49, 55, 58, 59] investigated tree pollen only, four [46, 50, 51, 56] investigated weed pollen only, four [38, 42, 45, 54] investigated combined grass and tree pollen and one [36] investigated all three: grass, tree and weed pollen, separately. Three studies [37, 43, 60] did not state the type of pollen taxa they investigated. Eleven studies [23, 24, 38-40, 47, 49, 52, 53, 57, 59] used a Burkard volumetric spore trap and one [56] used a Rotorod sampler. Others [36, 37, 41-46, 48, 50, 51, 54, 55, 58, 60] did not state the type of instrument used to collect pollen data though they only investigated exposure by pollen season compared to non-pollen season.

The sample sizes ranged from 14 to 2063, with a median of 55. Five studies [24, 37, 38, 53, 56] investigated children only (age range: 5-12), three [23, 36, 55] investigated children and adolescents (age range: 8-16), three [44, 52, 54] investigated adolescents and adults (age range: 12-58), and sixteen

This article is protected by copyright. All rights reserved

[39-43, 45-51, 57-60] investigated adults only (age range: 17-68). In all studies, 'ever asthma' and 'ever SAR' were defined by having (i) a history of asthma or allergic rhinitis that was confined to the pollen season; plus (ii) a positive skin-prick test (SPT) and/or radioallergosorbent test (RAST) to the pollen allergen in the study. In the context of this review, the reference group were (i) participants who did not have ever asthma, ever SAR, atopy and nasal polyps; or (ii) participants living in areas with a low pollen load. Non-pollen season was defined as before and/or after pollen season.

There were 23, 11, and 8 studies that included data on type-2, innate and non-specific immune responses, respectively (Figure 2). Markers were sampled either from the upper and/or lower airways (Figure 2). The studies that sampled markers from the upper airways used either nasal lavage fluids [24, 39, 40, 45, 47-51, 53-57], nasal scrapings [46], nasal biopsy [41] or nasal incubation samples [52]. Only one study [42] did not state how the upper airway inflammation marker was obtained.

Quality assessment

The quality assessment of included studies is summarised in Table E2. The quality scores ranged from 5 (i.e. satisfactory) to 9 (i.e. very good), with a median score of 8 (i.e. good). The main limitations and biases arose from limited power and/or lack of justification on sample sizes [23, 24, 36-60], lack of information on pollen data collection [36], the use of a Rotorod sampler [56] (lower collection efficiency compared to Burkard [61]), lack of information on outcome measurements [42] (measurement bias), lack of information on non-respondents [36, 37, 40-51, 54, 56-60] (potential selection bias) and lack of adjustment for *a priori* key confounders such as temperature and humidity [24, 36, 37, 39] (confounding bias). Some studies [42, 43, 45, 47, 53, 54] did not state whether statistical methods were used for within-group paired samples when it was appropriate to do so (appropriateness of statistical testing).

Outcome: Lung function

All seven studies which assessed lung function by pollen season [23, 58-60], pollen levels on the same day of testing (lag 0) [36, 37] and pollen load [38] did not find evidence of an association (Table E3). Only the study [36] that also investigated lagged effects of grass pollen exposure found a significant reduction in FEV_1 and the forced vital capacity (FVC) at lag day 1, cumulative lag 3 and

This article is protected by copyright. All rights reserved

cumulative lag 7. This study [36] analysed 1838 8-year-old children and 2063 16-year-olds in the BAMSE cohort, separately. The significant association with lung function was only observed among 8-year-olds, with pollen sensitization as an effect modifier.

Outcome: Airway inflammation

Type-2 immune response

This inflammatory profile was categorised into three groups: (i) IL-4 and IgE-mediated, (ii) eosinophilic and (iii) mast cell-mediated inflammation. Altogether, 23 studies [24, 39-60] included data on this group of outcomes (Figure 2). The studies that examined upper airway samples identified 18 inflammatory markers (Table E4) but only 13 showed a positive association with pollen exposure i.e. IL-4, IgE, eosinophils, eosinophil cationic protein (ECP), major basic protein (MBP) ("total eosinophils"), EG2 ("activated eosinophils"), eosinophil peroxidase (EPO), TAME-esterase, leukotriene C_4 (LTC_4), metachromatic (mast) cells, tryptase-only positive mast cells (MC_T) and mast cell-derived mediators such as histamine and tryptase. All four of the lower airway markers identified from studies that examined lower airway samples (Table E4) showed a positive association with pollen exposure i.e. nitric oxides (FeNO and JáwNO), leukotriene (LTE_4), eosinophils and ECP. These are summarised below, according to their study designs. Further details are provided in Table 2.

Short-term longitudinal studies (By pollen season) (n=21)

IL-4 and IgE-mediated inflammation in the upper airways

For IL-4, both studies [48, 54] found the levels to be higher when exposed to grass [54] and tree [48, 54] pollen seasons compared to non-pollen seasons among adolescents [54] and adults [48, 54] with ever SAR. For IgE, an Italian study [52] found significantly higher levels pre-peak compared to non-pollen season among adolescents and adults with ever SAR (n=14), but among those with both ever SAR and asthma (n=17), significantly higher levels were observed during pollen peak compared to non-pollen season [52].

Eosinophilic inflammation in the upper airways

For eosinophils, the numbers were higher in grass [45, 47, 53], tree [45, 49, 55] and weed [46] pollen seasons compared to non-pollen season in pollen sensitised children (n=22) [53], adults with ragweed allergy

This article is protected by copyright. All rights reserved

but without nasal polyps (n=16) [50], and people of all ages with ever SAR [45-47, 49, 55]. For ECP, the levels were higher inside the grass [42, 47, 52, 53], tree [42, 55] and weed [51] pollen seasons compared to non-pollen season among pollen-sensitised children (n=22) [53] and people of all ages with ever SAR and/or asthma [42, 47, 51, 52, 55]. Only one study each investigated MBP [41], EG2 [41], EPO [57], [TAME]-esterase [40] and LTC_4 [56]; the studies found higher levels in the grass [41, 57], tree [40] and weed [56] pollen seasons compared to non-pollen season among adults [40, 41, 57] and children (n=16) [56] with ever SAR.

Mast cell-mediated inflammation in the upper airways

One study each investigated metachromatic (mast) cells [46] and MC_T [41]; they found higher numbers in weed [46] and grass [41] pollen seasons compared to non-pollen season among adults with ever SAR. Of the two studies [47, 51] that investigated histamine, only one [51] found higher levels in weed pollen season compared to non-pollen season among adults with ever SAR (n=18). Both studies [47, 51] that investigated tryptase reported higher levels among adults with ever SAR in grass [47] and weed [51] pollen seasons compared to non-pollen season.

Eosinophilic inflammation in the lower airways

All five studies [42, 43, 53, 58, 59] that investigated FeNO found higher levels in grass [42, 53], tree [42, 58, 59], and unspecified [43] pollen seasons compared to non-pollen season in adults with ever asthma [58, 59] and ever SAR [42, 43] and in pollen-sensitised children [53]. One study each investigated JáwNO [58] and LTE_4 [44] from exhaled breath; they found higher levels in birch [58] and grass [44] pollen seasons compared to non-pollen season in adults with ever asthma (n=13) [58] and adolescents and adults (n=28) [44] with ever SAR.

The one study [60] that quantified eosinophils directly from sputum found a higher percentage during the pollen season (species unspecified) compared to non-pollen season in adults with ever SAR (n=52). The two studies [49, 60] that investigated ECP from sputum reported higher levels in tree [49] and unspecified [60] pollen seasons compared to non-pollen season in adults with ever SAR [49, 60] or ever asthma [49].

Cross-sectional studies (By the average daily pollen levels) (n=2)

Eosinophilic inflammation in the upper airways

This article is protected by copyright. All rights reserved

Both studies showed a positive association with ECP [24, 39]. Higher grass pollen exposure at cumulative lag 3 was associated with a higher ECP in (n=62) adults (unclear whether this was found in the reference group, adults with ever SAR only or both) [39] but not at lag 0 [24, 39] and cumulative lag 7 [39].

Eosinophilic inflammation in the lower airways

One of the studies showed a positive association with FeNO [24]. Higher grass pollen exposure was associated with a higher FeNO in all groups of children (n=119) at lag 0 but not at cumulative lag 7.

Innate immune response

This inflammatory profile was categorised into neutrophilic or macrophage inflammation. Altogether, 11 studies [24, 41, 44, 46, 48, 49, 51, 53, 55, 57, 60] investigated indices related to these outcomes derived from upper and/or lower airways (Figure 2). Of the studies that examined upper airway samples, 8 inflammatory markers were identified (Table E4) but only four showed a positive association with pollen exposure i.e. neutrophils, CD45/ICAM-1, MCAF/MCP-1 and IL-8. Of those studies that examined lower airway samples, four inflammatory markers were identified (Table E4) but only one showed a positive association with pollen exposure i.e. LTB_4. These are summarised below, according to their study designs. Further details are provided in Table 2.

Short-term longitudinal studies (By pollen season) (n=10)

Neutrophilic inflammation in the upper airways

Two studies [46, 55] investigated neutrophils but only one [46] found higher numbers among adults with ever SAR (n=10) during weed pollen season compared to non-pollen season. Both studies [46, 48] that investigated CD45/ICAM-1, a neutrophil endothelial adhesion molecule, found higher levels in weed [46] and tree [48] pollen seasons compared to non-pollen season among adults with ever SAR.

Macrophage inflammation in the upper airways

This study [51] found MCAF/MCP-1 to be higher levels in weed pollen season compared to non-pollen season among adults with ever SAR (n=18).

Neutrophilic inflammation in the lower airways

This article is protected by copyright. All rights reserved

The one study [44] that investigated LTB_4, a neutrophil chemoattractant, in exhaled breath condensate found the levels to be higher in grass pollen season compared to non-pollen season among adolescents and adults with ever SAR (n=28).

Cross-sectional studies (By the average daily pollen levels) (n=1)

Neutrophilic inflammation in the upper airways

This study [24] found the levels of IL-8, a neutrophil chemoattractant, to be higher with increased grass pollen exposure at lag 0 among the reference group (n=29) and pollen-sensitised children (n=23) but not at cumulative lag 7.

Non-specific immune response

Altogether, eight studies [24, 41, 50, 51, 53-55, 59] investigated such outcomes derived from upper and/or lower airways (Figure 2). Of studies that examined upper airway samples, 12 inflammatory markers were identified (Table E4) but only two showed a positive association with pollen exposure i.e. IgA and IFN-γ. Of the one study that examined lower airway samples, "total particles" was identified (Table E4) and it showed a positive association with pollen exposure. All positive associations were observed in short-term longitudinal studies only and are summarised below. Further details are provided in Table 2.

IgA and IFN-γ in the upper airways

In the one study [55] that investigated IgA, the levels of birch pollen allergen (Bet v 1)-specific IgA and the ratio of Bet v 1-specific IgA to total IgA were significantly higher in tree pollen season compared to the non-pollen season among children and adolescents with ever SAR (n=30). The one study [54] that investigated IFN-γ reported higher levels in grass and tree pollen seasons compared to non-pollen season only among the reference group of adolescents and adults (n=19).

Total particles in the lower airways

The one study [59] that investigated total mass of exhaled particles found lower levels in tree pollen season compared to non-pollen season among adults with ever asthma (n=13).

This article is protected by copyright. All rights reserved

Meta-analysis

We were able to perform limited meta-analyses for eosinophils and ECP sampled from the upper airways in pollen season versus out of pollen season, subgrouped by the presence or absence of ever SAR (Figures E1-E2). In the pollen season, people with ever SAR were observed to have relatively higher numbers/levels of eosinophilic inflammatory markers compared to people without. However, given there was substantial heterogeneity within the ever SAR group for both outcomes (I^2=86.5% for eosinophils and I^2=79.5% for ECP), we conducted a sensitivity analysis by age group but, only within the ever SAR group. For eosinophils, the heterogeneity remained substantial after this subgrouping (I^2=77.9%, Figure E3). For ECP, the heterogeneity became considerably lower after subgrouping by age (I^2=0%, Figure E4). Both forest plots (Figures E3-E4), nonetheless, showed a greater increase in the numbers/levels of inflammatory markers with increased age among people with ever SAR in pollen season compared to out of the pollen season. The asymmetrical funnel plots for both outcomes may indicate small-study bias (Figures E5-E6).

Accepted Article

This article is protected by copyright. All rights reserved

Discussion

To our knowledge, this is the first systematic review of the associations between outdoor pollen exposure, lung function changes and airway inflammation markers. There was substantial evidence to suggest an association between pollen exposure and both upper and lower airway inflammation. The data from the studies included in this review strongly reflected a type-2 inflammation rather than a more innate immune response, though some studies had non-specific features. The evidence for an association between outdoor pollen exposure and lung function was limited to lag effects.

People with ever asthma and/or ever SAR were more susceptible to increased airway inflammation during pollen seasons. This may indicate a "priming" phenomenon, whereby susceptible individuals have an increased sensitivity to allergens with repeated or persistent exposure [40, 62], or they may have an increased barrier permeability at the mucosal surface [63]. There was little evidence of a lagged effect on airway inflammation among people with ever asthma and/or ever SAR, but only two studies [24, 39] investigated this. The study [24] that did not find a positive association only considered cumulative pollen exposure over 7 days in children aged 7 to 12. This may have been too long because eosinophils are believed to peak only 24 to 72 hours after exposure takes place [64]. This hypothesis is further supported by Klimek et al. [39] who found a positive association with ECP when they investigated cumulative pollen exposure 3 days prior.

Furthermore, published evidence suggests that pollen exposure may increase the risk of both upper and lower airway inflammation, consistent with those of experimental studies [65]. One study included in this review [40] found TAME-esterase levels to increase in pollen season. This biochemical marker was shown to correlate strongly with nasal symptoms in a laboratory setting among people with ever SAR [66, 67]. Moreover, leukotrienes taken from both the exhaled breath condensate (LTE_4 and LTB_4) and the nasal lavage fluids (LTC_4) were significantly higher in pollen season among people with ever SAR [44, 56]. To further support this, some studies [49, 60] reported increased sputum eosinophils and ECP, which are good markers of lower airway inflammation [60].

This article is protected by copyright. All rights reserved

The meta-analyses showed higher numbers of eosinophils and ECP levels in the upper airways in pollen season compared to non-pollen season among people with ever SAR, but variability between studies limited interpretability. This could be due to a number of methodological factors such as, but not limited to, the different pollen taxon investigated between countries, the year the studies were conducted and publication bias. Nevertheless, further subgrouping by age within the ever SAR group reduced the heterogeneity considerably for ECP and slightly for eosinophils. The funnel plots also showed that adults had a greater increase in the numbers of eosinophils and levels of ECP compared to children and adolescents in the pollen season, suggesting that eosinophilic upper airway inflammation to pollen exposure increased with age. This provides a biological plausibility for the recent findings on higher thunderstorm-asthma related hospitalizations among adults aged 20 to 59 [68]. Another explanation could be the length of time with asthma/SAR. For example, adults who have had these conditions since childhood may be more sensitive towards outdoor pollen exposure. However, none of the studies included provided information on this.

For lung function, there were some evidence to suggest a delayed response to pollen exposure. This Swedish study [36] of the BAMSE cohort found a significant decrease in FEV_1 and FVC associated with increased grass pollen exposure at lag day 1 and up to seven days before in 8-year-olds but not in 16-year-olds, indicating that adolescents may be less susceptible possibly as a result of asthma remission, physical maturation, time spent outdoors and/or development of tolerance [36, 69]. The lack of associations found for FEV_1 from studies that investigated pollen seasons may leave the possibility that pollen induces obstruction in the more peripheral airways (<2mm) instead of the more central airways (>2mm) as has been suggested by the impact on mid-forced expiratory flow ($FEF_{25-75\%}$) [70]. None of the studies investigated $FEF_{25-75\%}$ so we were unable to synthesise any evidence on this outcome. Nevertheless, a fall in FVC would also be consistent with small airway obstruction with co-existent gas trapping [71], and this was reflected in the Swedish study [36] that showed some associations with pollen exposure. This hypothesis was further supported by the elevation of NO derived from peripheral airways in one study [58].

There are a number of limitations that need to be considered when interpreting the results of our systematic review. There were some studies [37, 43, 60] that did not report the pollen type. Of those that did, most classified pollen to the family level but some reported pollen at the genus or species

This article is protected by copyright. All rights reserved

level. Furthermore, the baseline prevalence of asthma and allergies and the types of allergenic pollen differ between countries. Of the studies that investigated people with ever SAR, some included information on whether the participants also had asthma while others did not. The definitions for 'non-pollen season' also varied between studies. There could also be variation in pollen levels between studies dependent on the geographic location and the year the study was conducted. It is possible that the pollen concentration in a particular year and region may differ to another region in the same year as there are many region-specific factors that impact on pollen seasons. Climatic conditions have also changed resulting in increased pollen levels over the years [72]. Higher levels during one year may provide more power for detectable human biological effects and, therefore could affect such associations published. These issues provided some difficulty in comparing some of the study findings. Moreover, none of the studies allowed for possible non-linear associations, which is common in environmental exposure modelling [73, 74]. Taken together these issues, some associations could potentially be missed and it could cause some bias, but this would have biased the associations towards the null. Similarly, most studies also had small sample sizes, so there may have been insufficient power to detect real associations. Only one study [36] tested for interactions and found pollen sensitisation to modify the association between pollen exposure and lung function in 8-year-old children. No other study tested for interactions especially with SAR or asthma, although people with ever asthma and ever SAR were reported to have a higher risk of airway inflammation. Additionally, not all lung function and airway inflammation markers were considered in the included studies (e.g. bronchial hyperresponsiveness (BHR) and innate lymphoid cells). Lastly, meta-analysis was only possible for eosinophilic inflammatory markers related mainly to the upper airways as there was an insufficient number of studies that investigated other inflammatory markers or the lower airways. There was also high heterogeneity between the studies included in the meta-analyses, possibly due to differences in the study methods, so more homogenous studies would be needed to allow for more accurate comparison.

In summary, outdoor pollen exposure is an important risk factor for type-2 airway inflammation among people with ever asthma and/or SAR, but in general without lung function changes reflective of larger airway narrowing. Future research should consider possible non-linear associations and effect modifiers using larger, more general population-based studies. There is also a need to examine lagged effects because very few studies investigated this. Better understanding on the role of pollen on lung health can lead to precautionary measures pre-pollen

This article is protected by copyright. All rights reserved

season and improved clinical interventions that can target specific immune markers in susceptible individuals.

Potential conflicts of interest: In the past 5 years, Jo Douglass has received honoraria for educational presentations from Astra-Zeneca, GSK, Novartis, Alphapharm, Shire and CSL. She has served on advisory boards: Sanofi-Aventis, Novartis, GSK, Astra-Zeneca, Shire, Immunosis and CSL. She has undertaken contracted or investigator-initiated research on behalf of: GSK, Novartis, Immunosis, Astra-Zeneca, Sanofi-Aventis, Grifols, CSL, BioCryst & Equilium. She has a personal superannuation shareholding in CSL. Other authors have no potential conflicts of interest to declare.

Acknowledgements: NSI is supported by the Centre for Food and Allergy Research (NHMRC Centre of Research Excellence) PhD scholarship and the Melbourne Children's LifeCourse top-up scholarship (Royal Children's Hospital Foundation grant #2018-984). JZ is supported by the China Scholarship Council. SCD, AJL and JLP are funded by the Australian National Health and Medical Research Council (NHMRC).

Accepted Article

This article is protected by copyright. All rights reserved

References

1. Ishmael FT. The Inflammatory Response in the Pathogenesis of Asthma. The Journal of the American Osteopathic Association. 2011;111(11_suppl_7):S11-S7.
2. Wang Y, Bai C, Li K, Adler KB, Wang X. Role of airway epithelial cells in development of asthma and allergic rhinitis. Respiratory medicine. 2008;102(7):949-55.
3. Bergeron C, Hamid Q. Relationship between Asthma and Rhinitis: Epidemiologic, Pathophysiologic, and Therapeutic Aspects. Allergy, Asthma & Clinical Immunology. 2005;1(2):81.
4. Thomas M. Allergic rhinitis: evidence for impact on asthma. BMC Pulmonary Medicine. 2006;6(1):S4.
5. Hayden TJ, Muscatello DJ. Increased presentations to emergency departments for asthma associated with rye grass pollen season in inland NSW. N S W Public Health Bull. 2011;22(7-8):154-8.
6. Erbas B, Akram M, Dharmage S, Tham R, Dennekamp M, Newbigin E, et al. The Role of Seasonal Grass Pollen on Childhood Asthma Emergency Department Presentations. Journal of Allergy and Clinical Immunology. 2012;129(2):AB166.
7. Serrano CD, Valero A, Bartra J, Roca-Ferrer J, Munoz-Cano R, Sanchez-Lopez J, et al. Nasal and bronchial inflammation after nasal allergen challenge: assessment using noninvasive methods. J Investig Allergol Clin Immunol. 2012;22(5):351-6.
8. Scadding GW, Calderon MA, Bellido V, Koed GK, Nielsen NC, Lund K, et al. Optimisation of grass pollen nasal allergen challenge for assessment of clinical and immunological outcomes. J Immunol Methods. 2012;384(1-2):25-32.
9. Christodoulopoulos P, Cameron L, Durham S, Hamid Q. Molecular pathology of allergic disease: II: Upper airway disease. Journal of Allergy and Clinical Immunology. 2000;105(2):211-23.
10. Park HS, Lee MK, Hong C-S. Bronchial challenge responses in asthmatic patients sensitized to Artemisia spp. pollen. Yonsei Med J. 1989;30(2):173-9.
11. Canonica GW, Compalati E. Minimal persistent inflammation in allergic rhinitis: implications for current treatment strategies. Clin Exp Immunol. 2009;158(3):260-71.

This article is protected by copyright. All rights reserved

12. Wang D, Clement P, Smitz J, De Waele M, Derde MP. Correlations between complaints, inflammatory cells and mediator concentrations in nasal secretions after nasal allergen challenge and during natural allergen exposure. Int Arch Allergy Immunol. 1995;106(3):278-85.
13. Gelfand EW. Inflammatory mediators in allergic rhinitis. Journal of Allergy and Clinical Immunology. 2004;114(5):S135-S8.
14. Scadding G, Eifan A, Penagos M, Koed GK, Wurtzen PA, Shamji M, et al. Grass pollen nasal challenge is associated with increases in Th2 cytokines, Eotaxin, MDC and IL-6 in nasal fluid. Clinical and Translational Allergy. 2013;3(2):P29.
15. Erbas B, Jazayeri M, Lambert KA, Katelaris CH, Prendergast LA, Tham R, et al. Outdoor pollen is a trigger of child and adolescent asthma emergency department presentations: A systematic review and meta-analysis. Allergy. 2018;73(8):1632-41.
16. Idrose NS, Dharmage SC, Lowe AJ, Lambert KA, Lodge CJ, Abramson MJ, et al. A systematic review of the role of grass pollen and fungi in thunderstorm asthma. Environmental Research. 2020;181:108911.
17. Silver JD, Sutherland MF, Johnston FH, Lampugnani ER, McCarthy MA, Jacobs SJ, et al. Seasonal asthma in Melbourne, Australia, and some observations on the occurrence of thunderstorm asthma and its predictability. PLOS ONE. 2018;13(4):e0194929.
18. D'Amato G, Liccardi G, Frenguelli G. Thunderstorm-asthma and pollen allergy. Allergy. 2007;62(1):11-6.
19. D'Amato G, Vitale C, D'Amato M, Cecchi L, Liccardi G, Molino A, et al. Thunderstorm-related asthma: what happens and why. Clinical & Experimental Allergy. 2016;46(3):390-6.
20. Suphioglu C, Singh MB, Taylor P, Bellomo R, Holmes P, Puy R, et al. Mechanism of grass-pollen-induced asthma. Lancet. 1992;339(8793):569-72.
21. Clayton-Chubb D, Con D, Rangamuwa K, Taylor D, Thien F, Wadhwa V. Thunderstorm asthma: revealing a hidden at-risk population. Internal Medicine Journal. 2019;49(1):74-8.
22. Baraldi E, Carrá S, Dario C, Azzolin N, Ongaro R, Marcer G, et al. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med. 1999;159(1):262-6.

This article is protected by copyright. All rights reserved

548 23. Ferdousi HA, Munir AKM, Zetterström O, Dreborg SKG. Seasonal differences of peak
549 expiratory flow rate variability and mediators of allergic inflammation in non-atopic adolescents.
550 Pediatric Allergy and Immunology. 2001;12(5):238-46.
551 24. Steerenberg PA, Bischoff EW, de Klerk A, Verlaan AP, Jongbloets LM, van Loveren H, et al.
552 Acute effect of air pollution on respiratory complaints, exhaled NO and biomarkers in nasal
553 lavages of allergic children during the pollen season. Int Arch Allergy Immunol. 2003;131(2):127-
554 37.
555 25. Bonay M, Neukirch C, Grandsaigne M, Leçon-Malas V, Ravaud P, Dehoux M, et al.
556 Changes in airway inflammation following nasal allergic challenge in patients with seasonal
557 rhinitis. Allergy. 2006;61(1):111-8.
558 26. Tenn MW, Steacy LM, Adams DE, Walker TJ, Ellis AK. Comparison of allergic rhinitis
559 outcomes of the environmental exposure unit and nasal allergen challenge model. Ann Allergy
560 Asthma Immunol. 2019;123(1):105-6.e1.
561 27. Badorrek P, Dick M, Emmert L, Schaumann F, Koch W, Hecker H, et al. Pollen starch
562 granules in bronchial inflammation. Ann Allergy Asthma Immunol. 2012;109(3):208-14.e6.
563 28. Okuma Y, Okamoto Y, Yonekura S, Iinuma T, Sakurai T, Hamasaki S, et al. Persistent nasal
564 symptoms and mediator release after continuous pollen exposure in an environmental challenge
565 chamber. Annals of Allergy, Asthma & Immunology. 2016;117(2):150-7.
566 29. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of
567 observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational
568 Studies in Epidemiology (MOOSE) group. Jama. 2000;283(15):2008-12.
569 30. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred
570 reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement.
571 Systematic Reviews. 2015;4(1):1.
572 31. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the
573 quality of nonrandomized studies in meta-analyses. European Journal of Epidemiology.
574 2010;25(9):603-5.
575 32. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from
576 the sample size, median, range and/or interquartile range. BMC Medical Research Methodology.
577 2014;14(1):135.

Accepted Article

This article is protected by copyright. All rights reserved

33. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed). 2003;327(7414):557-60.
34. Collaboration C. 9.5. 2 Identifying and measuring heterogeneity. Cochrane Handbook for Systematic Reviews of Interventions (Version 502) Oxford, UK: Cochrane Collaboration. 2009.
35. StataCorp L. Stata statistical software: release 16. College Station, TX. 2019.
36. Gruzieva O, Pershagen G, Wickman M, Melén E, Hallberg J, Bellander T, et al. Exposure to grass pollen – but not birch pollen – affects lung function in Swedish children. Allergy. 2015;70(9):1181-3.
37. Scarlett JF, Abbott KJ, Peacock JL, Strachan DP, Anderson HR. Acute effects of summer air pollution on respiratory function in primary school children in southern England. Thorax. 1996;51(11):1109-14.
38. Zwick H, Popp W, Jager S, Wagner C, Reiser K, Horak F. Pollen sensitization and allergy in children depend on the pollen load. Allergy. 1991;46(5):362-6.
39. Klimek L, Riechelmann H, Amedee R. Eosinophil Cationic Protein in Nasal Secretions and Blood Serum in Grass-Pollen Allergic Rhinitis. American Journal of Rhinology. 1996;10(5):319-22.
40. Andersson M, Svensson C, Andersson P, Pipkorn U. Objective monitoring of the allergic inflammatory response of the nasal mucosa in patients with hay fever during natural allergen exposure. The American review of respiratory disease. 1989;139(4):911-4.
41. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. The Journal of allergy and clinical immunology. 1992;89(4):877-83.
42. Bergmann-Hug K, Wirth R, Henseler M, Helbling A, Pichler WJ, Schnyder B. Effect of natural seasonal pollen exposure and repeated nasal allergen provocations on elevation of exhaled nitric oxide. Allergy. 2009;64(11):1629-34.
43. Bozek A, Krajewska J, Jarzab J. Nasal nitric oxide and other diagnostic procedures in seasonal allergic rhinitis: elderly vs juvenile patients. American Journal of Otolaryngology. 2011;32(2):105-8.

Accepted Article

This article is protected by copyright. All rights reserved

606 44. Cap P, Maly M, Pehal F, Pelikan Z. Exhaled leukotrienes and bronchial responsiveness to
607 methacholine in patients with seasonal allergic rhinitis. Ann Allergy Asthma Immunol.
608 2009;102(2):103-9.
609 45. Choi CH, Poroyko V, Watanabe S, Jiang D, Lane J, deTineo M, et al. Seasonal allergic
610 rhinitis affects sinonasal microbiota. American journal of rhinology & allergy. 2014;28(4):281-6.
611 46. Ciprandi G, Pronzato C, Ricca V, Bagnasco M, Canonica GW. Evidence of intercellular
612 adhesion molecule-1 expression on nasal epithelial cells in acute rhinoconjunctivitis caused by
613 pollen exposure. The Journal of allergy and clinical immunology. 1994;94(4):738-46.
614 47. Di Lorenzo G, Mansueto P, Melluso M, Candore G, Colombo A, Pellitteri ME, et al. Allergic
615 rhinitis to grass pollen: measurement of inflammatory mediators of mast cell and eosinophils in
616 native nasal fluid lavage and in serum out of and during pollen season. The Journal of allergy and
617 clinical immunology. 1997;100(6 Pt 1):832-7.
618 48. Kato M, Liu W, Hattori T, Nakashima I. Evidence of potential regulation by interleukin-4 of
619 the soluble intercellular adhesion molecule 1 level in patients with seasonal allergic rhinitis under
620 provocation by a small amount of natural allergen. Ann Otol Rhinol Laryngol. 1998;107(3):232-5.
621 49. Kämpe M, Stålenheim G, Janson C, Stolt I, Carlson M. Systemic and local eosinophil
622 inflammation during the birch pollen season in allergic patients with predominant rhinitis or
623 asthma. Clinical and Molecular Allergy. 2007;5(1):4.
624 50. Keith PK, Conway M, Evans S, Wong DA, Jordana G, Pengelly D, et al. Nasal polyps: effects
625 of seasonal allergen exposure. The Journal of allergy and clinical immunology. 1994;93(3):567-74.
626 51. Kuna P, Lazarovich M, Kaplan AP. Chemokines in seasonal allergic rhinitis. J Allergy Clin
627 Immunol. 1996;97(1 Pt 1):104-12.
628 52. Marcucci F, Sensi LG, Migali E, Coniglio G. Eosinophil cationic protein and specific IgE in
629 serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. Allergy.
630 2001;56(3):231-6.
631 53. van Amsterdam JG, Bischoff EW, de Klerk A, Verlaan AP, Jongbloets LM, van Loveren H, et
632 al. Exhaled NO level and number of eosinophils in nasal lavage as markers of pollen-induced
633 upper and lower airway inflammation in children sensitive to grass pollen. Int Arch Occup Environ
634 Health. 2003;76(4):309-12.

Accepted Article

This article is protected by copyright. All rights reserved

54. Benson M, Strannegard IL, Wennergren G, Strannegard O. Cytokines in nasal fluids from school children with seasonal allergic rhinitis. Pediatr Allergy Immunol. 1997;8(3):143-9.
55. Keen C, Johansson S, Reinholdt J, Benson M, Wennergren G. Bet v 1-specific IgA increases during the pollen season but not after a single allergen challenge in children with birch pollen-induced intermittent allergic rhinitis. Pediatr Allergy Immunol. 2005;16(3):209-16.
56. Volovitz B, Osur SL, Bernstein JM, Ogra PL. Leukotriene C4 release in upper respiratory mucosa during natural exposure to ragweed in ragweed-sensitive children. The Journal of allergy and clinical immunology. 1988;82(3 Pt 1):414-8.
57. Nielsen LP, Bjerke T, Christensen MB, Skamling M, Peterson CG, Mygind N, et al. Eosinophil markers in seasonal allergic rhinitis. Intranasal fluticasone propionate inhibits local and systemic increases during the pollen season. Allergy. 1998;53(8):778-85.
58. Bake B, Viklund E, Olin AC. Effects of pollen season on central and peripheral nitric oxide production in subjects with pollen asthma. Respiratory medicine. 2014;108(9):1277-83.
59. Larsson P, Larstad M, Bake B, Hammar O, Bredberg A, Almstrand AC, et al. Exhaled particles as markers of small airway inflammation in subjects with asthma. Clin Physiol Funct Imaging. 2017;37(5):489-97.
60. Panzner P, Malkusova I, Vachova M, Liska M, Brodska P, Ruzickova O, et al. Bronchial inflammation in seasonal allergic rhinitis with or without asthma in relation to natural exposure to pollen allergens. Allergol Immunopathol (Madr). 2015;43(1):3-9.
61. Crisp HC, Gomez RA, White KM, Quinn JM. A side-by-side comparison of Rotorod and Burkard pollen and spore collections. Ann Allergy Asthma Immunol. 2013;111(2):118-25.
62. Erbas B, Lowe AJ, Lodge CJ, Matheson MC, Hosking CS, Hill DJ, et al. Persistent pollen exposure during infancy is associated with increased risk of subsequent childhood asthma and hayfever. Clin Exp Allergy. 2013;43(3):337-43.
63. Choi CH, Poroyko V, Watanabe S, Jiang D, Lane J, deTineo M, et al. Seasonal allergic rhinitis affects sinonasal microbiota. Am J Rhinol Allergy. 2014;28(4):281-6.
64. Boldogh I, Bacsi A, Choudhury BK, Dharajiya N, Alam R, Hazra TK, et al. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. J Clin Invest. 2005;115(8):2169-79.

Accepted Article

This article is protected by copyright. All rights reserved

65. Yonekura S, Okamoto Y. Allergic reaction induced by pollen in the upper respiratory tract affects eosinophils and basophils in peripheral blood as well as in the lower respiratory tract. Journal of Allergy and Clinical Immunology. 2017;139(2):AB159.
66. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr., Meyers DA, Norman PS, et al. Mediator release after nasal airway challenge with allergen. The American review of respiratory disease. 1983;128(4):597-602.
67. Subratty AH, Yan MY, Shun LH, Hebe A, Soomaree V. TAME-esterase activity during a prolonged attack of asthma. Int J Clin Pharmacol Ther. 1994;32(11):598-9.
68. Thien F, Beggs PJ, Csutoros D, Darvall J, Hew M, Davies JM, et al. The Melbourne epidemic thunderstorm asthma event 2016: an investigation of environmental triggers, effect on health services, and patient risk factors. The Lancet Planetary Health. 2018;2(6):e255-e63.
69. Withers AL, Green R. Transition for Adolescents and Young Adults With Asthma. Frontiers in pediatrics. 2019;7:301-.
70. Mead J. The lung's "quiet zone". N Engl J Med. 1970;282(23):1318-9.
71. McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. Eur Clin Respir J. 2014;1:10.3402/ecrj.v1.25898.
72. Frei T, Gassner E. Climate change and its impact on birch pollen quantities and the start of the pollen season an example from Switzerland for the period 1969–2006. International Journal of Biometeorology. 2008;52(7):667.
73. Lee SW, Yon DK, James CC, Lee S, Koh HY, Sheen YH, et al. Short-term effects of multiple outdoor environmental factors on risk of asthma exacerbations: Age-stratified time-series analysis. The Journal of allergy and clinical immunology. 2019;144(6):1542-50.e1.
74. Erbas B, Chang JH, Dharmage S, Ong EK, Hyndman R, Newbigin E, et al. Do levels of airborne grass pollen influence asthma hospital admissions? Clin Exp Allergy. 2007;37(11):1641-7.

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Table 1. Characteristics of included studies.

No.	Author (Year), Country	Study design	Sample size	Age range	Study population	Exposure definition
1.	Gruzieva et al. [36] (2015), Sweden	Cross-sectional	1838 children at the 8-year follow-up 2063 adolescents at the 16-year follow-up	8 and 16	All children from the Swedish birth cohort BAMSE	**Grass, tree and weed pollen (*Corylus, Alnus, Ulmus, Betula, Pinus, Salix, Quercus, Artemisia and Poaceae*)**: Pollen levels per increase in 3 grains/m^3 at lag 0, lag 1, lag 0-3 and lag 0-6
2.	Scarlett et al. [37] (1996), United Kingdom	Case-crossover	154 children	7-11	None, all children were included.	Pollen measured per grains/m^3 *Species not specified -Pollen data obtained from local authority monitoring site (6 miles away).
3.	Bake et al. [58] (2014), Sweden	Short-term longitudinal study	25 adults	• 26-61 (Subjects) • 34-67 (Controls)	• 13 pollen allergic, non-smokers with ever asthma • 12 healthy non-smokers	**Tree pollen (birch):** During and after pollen season
4.	Ferdousi et al. [23] (2001), Sweden	Short-term longitudinal study	55 children & adolescents *Total was 56 but one child was excluded in PEF statistical	11-16	• All children were healthy (i.e. asymptomatic and non-allergic)	**Tree pollen (birch):** All children were evaluated for 4 weeks during pollen season and 44 were evaluated for 4 weeks during autumn season -Collected using Burkard pollen trap

This article is protected by copyright. All rights reserved

			comparison			
5.	Larsson et al. [59] (2017), Sweden	Short-term longitudinal study	26 adults	• 22-61 (Subjects) • 17-63 (Controls)	• 13 patients with ever asthma (Subjects) • 13 healthy, non-smokers	**Tree pollen (birch):** During and out of season -Pollen collected using Burkard 7-day recording volumetric spore trap
6.	Panzner et al. [60] (2015), Czech Republic	Short-term longitudinal study	113 adults	• Mean age of 32.3 (Subjects with SAR and asthma) • Mean age of 38.8 (Subjects with SAR but no asthma) • Mean age of 43.3 (Controls)	• 38 patients with ever SAR and asthma • 52 patients with ever SAR but no asthma • 23 healthy controls	Before, during and after pollen season *Species not specified
7.	Zwick et al.[38] (1991), Austria	Cross-sectional	400 children	Mean age of 11.5	No inclusion criteria were applied, all children were included.	**Grass and tree (birch) pollen:** Children living in high pollen load vs. low pollen load -Collected using Burkard pollen trap

Accepted Article

This article is protected by copyright. All rights reserved

8.	Klimek et al. [39] (1996), Germany	Cross-sectional	62 adults	• 18-49 (Subjects) • 19-43 (Controls)	• 43 patients with ever SAR (did not state whether the patients also had asthma) (Subjects) • 19 healthy volunteers with no history of rhinitis (Controls)	**Grass pollen** at cumulative lag 3: 5 classes • Class 1: 0-3 grains/m^3 • Class 2: 4-20 grains/m^3 • Class 3: 21-50 grains/m^3 • Class 4: 50-100 grains/m^3 • Class 5: >100 grains/m^3 *Collected using Burkard trap
9.	Steerenberg et al. [24] (2003), Netherlands	Cross-sectional	119 children	7-12	No inclusion criteria were applied, all children were included. However, they were categorised into subgroups in the analysis: • Non-atopic (n=29) • Pollen-sensitised (n=23) • HDM-sensitised (n=34) • Pollen and HDM-sensitised (n=29)	**Grass pollen:** Mean levels of from 0 to 24 hours (acute exposure) and the week before (cumulative lag 7) *Collected using Burkard pollen sampler
10.	Andersson et al. [40] (1989), Sweden	Short-term longitudinal study	14 adults	• 18-31 (Subjects) • 20-39 (Controls)	• 9 patients with ever SAR (did not state whether the patients also had asthma)	**Tree pollen (birch):** Before and during pollen season *Collected using Burkard pollen trap

Accepted Article

This article is protected by copyright. All rights reserved

					• 5 healthy, non-atopic controls	
11.	Bentley et al. [41] (1992), London, United Kingdom	Short-term longitudinal study	20 adults (pre-season) 8 adults (during season)	• 20-48 (Subjects) • 20-34 (Controls)	• 13 patients with ever SAR (did not state whether the patients also had asthma) • 7 normal healthy controls	**Grass pollen:** In and out-of-pollen season
12.	Bergmann-Hug et al. [42] (2009), Switzerland	Short-term longitudinal study	20 adults	>18 years old	• 13 patients with ever SAR without symptomatic asthma • 7 non-atopic controls	**Tree (birch) and grass pollen:** Before, during and after pollen exposure
13.	Bozek et al. [43] (2011), Poland	Short-term longitudinal study	733 adults	• Mean age of 64.1 (Elderly subjects) • Mean age of 23.7 (young SAR patients) • Mean age of 65 (non-allergic elderly patients)	• 248 elderly patients with ever SAR but without asthma • 289 young patients with ever SAR without asthma • 196 non-allergic elderly controls	During and after pollen season *Species not stated

Accepted Article

This article is protected by copyright. All rights reserved

14.	Čáp et al. [44] (2009), Prague, Czech Republic	Short-term longitudinal study	78 adolescents & adults	• 17-58 (Subjects) • 16-55 (Controls)	• 28 patients with ever SAR but no asthma • 50 healthy, non-smokers	**Grass pollen:** During and after 4 months after pollen season
15.	Choi et al. [45] (2014), United States of America	Short-term longitudinal study	39 adults	• 18-39 (Subjects) • 18-48 (Controls)	• 19 patients with ever SAR (did not state whether the patients also had asthma) • 20 healthy, non-allergic controls	**Tree and grass pollen:** Before and during pollen season *Pollen count obtained from Chicago National Allergy Bureau station
16.	Ciprandi et al. [46] (1994), Italy	Short-term longitudinal study	20 adults	• 18-43 (Subjects) • 19-35 (Controls)	• 10 patients with ever SAR (did not state whether the patients also had asthma). • 10 healthy controls	**Weed pollen (*Parietaria Judaica*)**: Before and during season
17.	Di Lorenzo et al. [47] (1997), Italy	Short-term longitudinal study	30 adults	• 23-38 (Subjects) • 22-35 (Controls)	• 20 patients with ever SAR but without asthma • 10 non-atopic controls	**Grass pollen:** During and out of pollen season *Collected using Burkard volumetric pollen trap.
18.	Kato et al. [48] (1998),	Short-term	19 adults	• Mean age 36	• 12 patients with ever SAR (did	**Tree (Japanese cedar) pollen:** Pre-,

This article is protected by copyright. All rights reserved

	Japan	longitudinal study		(Subjects) • Mean age 35 (Controls)	not state whether the patients also had asthma) • 7 controls with no history of nasal or allergic diseases	early-, mid-, late and post-season *Pollen count obtained from the Aichi Prefecture Department of Health
19.	Kämpe et al. [49] (2007), Sweden	Short-term longitudinal study	21 adults	• 24-66 (Allergic rhinitis subjects) • 27-56 (Allergic asthma subjects) • 27-58 (Controls)	• 9 patients with ever SAR but without asthma • 7 ever asthma patients • 5 healthy non-atopic, non-smokers	**Tree pollen (birch):** During and out of season -In season visit was made two to three weeks after pollen counts had reached 4000 grains/m^3 -Pollen collected using Burkard 7-day recording volumetric spore trap
20.	Keith et al. [50] (1994), Canada	Short-term longitudinal study	64 adults	• 23-63 (patients with nasal polyps and ragweed allergy) • 21-61 (patients with nasal	• 16 patients with nasal polyps and ragweed allergy (did not state whether the patients also had asthma) – PRW+ • 16 patients with nasal polyps but without ragweed allergy (did not state whether the	**Weed (Ragweed) pollen:** Before and during pollen season

Accepted Article

This article is protected by copyright. All rights reserved

				polyps but without ragweed allergy) • 23-68 (patients without nasal polyps but with ragweed allergy) • 23-66 (Controls without nasal polyps and ragweed allergy)	patients also had asthma)- PRW- • 16 patients without nasal polyps but with ragweed allergy (did not state whether the patients also had asthma)- NPRW+ • 16 healthy controls without nasal polyps and ragweed allergy- NPRW-	
21.	Kuna et al. [51] (1996), United States of America	Short-term longitudinal study	27 adults	>18 years old	• 18 patients with ever SAR but no asthma • 9 healthy, non-atopic controls	**Weed (Ragweed) pollen:** During and out of pollen season
22.	Marcucci et al. [52] (2001), Italy	Short-term longitudinal	36 adolescents & adults	12-42	• 14 patients with ever SAR but without asthma (Subject group	**Grass pollen:** Out of pollen season, before pollen peak, during pollen peak and

Accepted Article

This article is protected by copyright. All rights reserved

		study			1) • 17 patients with ever SAR and asthma (Subject group 2) • 10 age-matched, healthy, non-allergic controls	after pollen peak *Collected using Hirst pollen trap
23.	Van Amsterdam et al. [53] (2003), Netherlands	Short-term longitudinal study	504 children (before pollen season) 119 children (during pollen season)	7-12	No inclusion criteria were applied, all children were included. However, they were categorised into subgroups in the analysis: • Non-atopic (n=31) • Pollen-sensitised (n=22) • HDM-sensitised (n=34) • Pollen and HDM-sensitised (n=32)	**Grass pollen:** Before and during pollen season *Collected using Burkard pollen sampler
24.	Keen et al. [55] (2005), Sweden	Short-term longitudinal study	60 children and adolescents	• Median (range): 12.5 (11-16) (Subjects) • Median (range): 13.4 (12-16) (Controls)	• 30 patients with ever SAR but without asthma • 30 healthy, non-atopic controls	**Tree pollen (birch):** Before and during pollen season *Pollen counts obtained from Department of Botany at Göteborg University

Accepted Article

This article is protected by copyright. All rights reserved

25.	Volovitz et al. [56] (1988), United States of America	Short-term longitudinal study	28 children	• 5-12	• 16 patients with ever SAR (did not state whether the patients also had asthma) • 12 healthy, non-atopic controls	**Weed (ragweed) pollen:** Before, during and after pollen season *Pollen collected using a Rotorod sampler
26.	Nielsen et al. [57] (1998), Denmark	Short-term longitudinal study	69 adults	• 19-59 (Subjects) • 31-45 (Controls)	• 63 patients with ever SAR • 6 healthy, non-atopic controls	**Grass pollen:** Before and during pollen season *Pollen collected using a Burkard trap
27.	Benson et al. [54] (1997), Sweden	Short-term longitudinal study	57 adolescents and adults	• 12-20 (Subjects) • 13-19 (Controls)	• 38 patients with ever SAR • 19 healthy, non-atopic controls	**Tree (birch) and grass pollen:** Before and during pollen season *Pollen counts obtained from Department of Botany at Göteborg University

Table 2. Findings of included airway inflammation studies.

No.	Author (Year), Country	Outcome measures	Findings	Confounders	Effect modifiers	Statistical tests
1.	Klimek et al. [39] (1996), Germany	ECP taken from nasal secretions	• Nasal ECP was significantly correlated to the cumulative pollen concentration at lag 3 ($r=0.87$; $p<0.001$). -graphical analysis • There was no correlation with grass pollen count at lag 0 (data	None	None tested	• Spearman's rank correlation coefficient

This article is protected by copyright. All rights reserved

			not shown).			
2.	Steerenberg et al.[24] (2003), Netherlands	Exhaled NO; and eosinophils, ECP, IL-6 and IL-8 taken from nasal lavage fluid	• Pollen exposure at lag 0 was positively associated with exhaled NO in all groups (i.e. non-atopic, pollen sensitised, HDM sensitised, pollen + HDM sensitised). • Pollen exposure at cumulative lag 7 was not associated with exhaled NO in all groups (i.e. non-atopic, pollen sensitised, HDM sensitised, pollen + HDM sensitised). • Pollen exposure at lag 0 and cumulative lag 7 was not associated with eosinophils in any of the groups. • Pollen exposure at lag 0 was negatively associated with ECP in pollen sensitised children. No association in other groups. • Pollen exposure at cumulative lag 7 was not associated with ECP in any of the groups. • Pollen exposure at lag 0 was positively associated with IL-8 in non-atopic children. No association in other groups. • Pollen exposure at lag 0 was not associated with IL-6 in any of the groups. • Pollen exposure at cumulative lag 7 was negatively associated with IL-6 in mite sensitised as well as pollen and mite sensitised children. No association in other groups. • Pollen exposure at cumulative lag 7 was negatively associated	Age, gender, environmental tobacco smoke exposure, having a cold during sampling, unvented water heater and gas cooking	None tested	• Linear and logistic regression models

Accepted Article

This article is protected by copyright. All rights reserved

			with IL-8 in non-atopic children and positively associated in pollen-sensitised children. No association in other groups.			
3.	Andersson et al. [40] (1989), Sweden	Nasal N-alpha-Tosyl L-arginine methyl ester [TAME]-esterase levels	• There was a significant ($p<0.05$) increase in TAME-esterase activity in allergic rhinitis patients during pollen exposure despite pollen count being low (graphical evidence). • There was no significant change in TAME-esterase activity in the healthy control group during pollen exposure (graphical evidence).	None	None	• Spearman's rank sum test • ANOVA • Wilcoxon's signed rank sum test (intragroup analysis) • Wilcoxon's rank sum test (intergroup analysis)
4.	Bake et al. [58] (2014), Sweden	$FeNO_{0.05}$, $FeNO_{0.1}$, $FeNO_{0.27}$, maximal flow of NO from airways (J*á*wNO) and alveolar NO concentration (CANO)	• Pollen exposure was associated with increased FeNO among asthmatics. • Pollen exposure had no effect on FeNO among controls. • Pollen exposure was associated with increased J*á*wNO and unchanged CANO among asthmatics. • Pollen exposure had no effect on J*á*wNO and CANO among controls.	None	None tested	• Wilcoxon exact test • Wilcoxon signed rank test • Two-sided paired t-test
5.	Bentley et al. [41] (1992), London	Nasal CD45, CD3, CD4, CD8, IL-2R, major basic protein (MBP), CD68, neutrophil elastase, EG2, HLA-DR, tryptase-only	**Nasal submucosa** • In rhinitis patients, there was a significant increase in total (i.e. MBP) and "activated" (i.e. EG2) eosinophils during season compared to out of season and this increase was significant when compared to controls. • In rhinitis patients, seasonal change did not have an effect on	None	None tested	• Wilcoxon's matched-pairs signed-ranks test (intragroup analysis) • Mann-Whitney U test (intergroup analysis)

Accepted Article

This article is protected by copyright. All rights reserved

		positive mast cell (MC_T), Chymase plus tryptase positive mast cell (MC_{TC}) taken from nasal biopsy	MC_T and MC_{TC}. • No significant change in any of the other inflammatory mediators among rhinitis patients between seasons. • No significant change in any of the inflammatory mediators among controls between seasons. **Nasal epithelium** • In rhinitis patients, there is a significant increase in total (i.e. MBP) and "activated" (i.e. EG2) eosinophils during season compared to out of season. This increase was only significant for EG2 when compared to controls. • In rhinitis patients, there is a significant increase in MC_T during season compared to out of season and this increase was significant when compared to controls. • No significant change in any of the other inflammatory mediators among rhinitis patients between seasons. • No significant change in any of the inflammatory mediators among controls between seasons.			
6.	Bergmann-Hug et al. [42] (2009), Switzerland	FeNO, ECP	• Pollen exposure was associated with a significant increase in ECP (p=0.03) in both groups. • In rhinitis patients, there was significant increase in FeNO (mean difference=14.8ppb; p=0.017) during season compared to before season.	None	None tested	• Non-parametric method of Brunner and Langer for analysing longitudinal data • Wilcoxon rank sum test

Accepted Article

This article is protected by copyright. All rights reserved

			• In controls, there was a slight but non-significant decrease in FeNO (mean difference=-0.1ppb; p=0.69) during season compared to before season.			
7.	Bozek et al. [43] (2011), Poland	FeNO (ppb)	• In elderly rhinitis patients, there was a significant decrease in FeNO after season compared to during season. • In young rhinitis patients, there was a significant decrease in FeNO after season compared to during season. • No significant difference in FeNO between seasons in the young and elderly control group.	None	None tested	• Chi-squared test • Mann-Whitney U test
8.	Čáp et al. [44] (2009), Prague	Leukotrienes (LTB_4 and LTE_4) in exhaled breath condensate	• In rhinitis patients, there was a significant decrease in LTB_4 and LTE_4 after season compared to during season. • In controls, there was no significant difference in LTB_4 and LTE_4 between seasons.	None	None tested	• Analysis of variance model (ANOVA) • Wilcoxon matched-pair signed-rank test • Mann-Whitney test
9.	Choi et al. [45] (2014), United States of America	Nasal eosinophils	• No significant change in percentage of nasal eosinophil during pollen season compared to outside of season ($p>0.05$) among healthy controls. • There was a significant increase in median nasal eosinophils during pollen season compared to outside of season ($p<0.05$) among seasonal allergic rhinitis patients.	Age, sex, race	None	• Mann-Whitney nonparametric test
10.	Ciprandi et al. [46] (1994), Italy	Nasal neutrophil, eosinophil,	• In healthy controls, the results are the same as pre-seasonal values.	None	None tested	• Wilcoxon signed rank test (paired data)

Accepted Article

This article is protected by copyright. All rights reserved

		metachromatic cells and CD54 expression	• In rhinoconjunctivitis patients, there was a significant ($p<0.001$) increase in neutrophils, eosinophils and metachromatic cells during season compared to before season. This increase was significant when compared to controls examined during season ($p<0.001$). -graphical analysis • In rhinoconjunctivitis patients, there was a mild (i.e. positivity score of 1 or 2) significant increase ($p<0.001$) in CD54 positive expression on nasal epithelial cells during season compared to before season. This increase was significant when compared to controls examined during season ($p<0.001$). -graphical analysis			• Mann Whitney test (intergroup analysis) • Spearman test
11.	Di Lorenzo et al. [47] (1997), Italy	Eosinophils taken from nasal scrapings; histamine, tryptase, ECP taken from nasal fluids	• In healthy controls, there was no significant difference in the levels of histamine, tryptase, ECP and eosinophils during season compared to out of season. • In rhinitis subjects, there was a significant increase in tryptase, ECP and eosinophils during season compared to out of season. No significant difference found in histamine between seasons.	None	None tested	• Kruskal-Wallis non-parametric test • Spearman's correlation coefficient
12.	Kato et al. [48] (1998), Japan	Nasal IL-4 and soluble intracellular adhesion molecule 1 (sICAM-1)	• The levels of nasal sICAM-1 during early and mid-season were significantly higher compared to pre-season in the allergic rhinitis group ($p<0.05$) (graphical evidence). • The levels of nasal IL-4 were significantly higher post-season compared to pre-season ($p<0.02$) in the allergic rhinitis group	None	None	• Wilcoxon's matched-pairs signed-rank test (intragroup analysis) • Mann-Whitney U test (intergroup analysis)

Accepted Article

This article is protected by copyright. All rights reserved

			(graphical evidence). • There was no significant difference in both nasal sICAM-1 and IL-4 throughout all seasonal periods in the control group (graphical evidence).			• Spearman's rank correlation coefficient
13.	Kämpe et al. [49] (2007), Sweden	Eosinophils, ECP and human neutrophil lipocalin (HNL) taken from nasal lavage; sputum ECP and HNL	• There was a significant ($p<0.05$) increase in sputum ECP among rhinitis and asthma patients during season compared to out of season. No change in sputum ECP among controls between seasons. • No change in other inflammatory mediators between seasons.	None	None tested	• Kruskal-Wallis test, ANOVA, Mann-Whitney U test (intergroup analysis) • Friedman's ANOVA, Wilcoxon's matched pairs test (intragroup analysis)
14.	Keith et al. [50] (1994), Canada	Nasal ECP, eosinophils and albumin concentrations	• Only the patients without nasal polyp but with ragweed allergy (NPRW+) were associated with a significant increase in nasal eosinophils during season compared to pre-season ($p=0.02$) (graphical evidence). • No significant change observed in nasal ECP or albumin for any of the groups.	None	None	• Variance analysis for repeated measurements • Student's t tests (paired & non-paired) • Intraclass correlation coefficient
15.	Kuna et al. [51] (1996), United States of America	Nasal histamine, ECP, tryptase, monocyte chemotactic and activating factor/monocyte	• Seasonal allergic rhinitis patients had a significant increase in nasal ECP, histamine and tryptase levels. • Seasonal allergic rhinitis patients had a significant increase in MCAF/MCP-1 and a significant decrease in IL-8 during season compared to out of season.	None	None	• Student's paired t test and Mann-Whitney U test (paired data) • Student's t test (unpaired data)

Accepted Article

This article is protected by copyright. All rights reserved

		chemoattractant protein-1 (MCAF/MCP-1), IL-8, CCL5 (RANTES), macrophage inflammatory protein 1-alpha (MIP-1α)	• Most subjects (controls & SAR) did not have detectable quantities of CCL5 (RANTES) and MIP-1α • No significant difference in the controls for any of the outcomes during season compared to out of season.			• Spearman's rank correlation coefficient
16.	Larsson et al. [59] (2017), Sweden	Exhaled particles, exhaled NO	**Exhaled particles** • In asthmatics, the total mass of exhaled particles decreased significantly during pollen season compared to out of season (median [25th percentile, 75th percentile]: 900 pg l^{-1} [510, 1280] vs. 1250 pg l^{-1} [790, 1790]) • In both asthmatics and controls, the concentrations of albumin and surfactant protein A (i.e. SP-A) in exhaled particles did not differ significantly between seasons. **FeNO** • In asthmatics, there was a significant increase in season compared to out of season. • In controls, there was no significant change in season compared to out of season.	None	None tested	• Wilcoxon signed rank-sum test (intragroup analysis) • Wilcoxon-Mann-Whitney U test (intergroup analysis) • Spearman's correlation coefficient

Accepted Article

This article is protected by copyright. All rights reserved

17.	Marcucci et al. [52] (2001), Italy	IgE specific to timothy grass and ECP taken from nasal secretions	**Before pollen peak vs. out of season** • In the rhinitis only and rhinitis and asthma patients, there was a slight increase in nasal IgE, but the increase was only significant for the rhinitis only group (p=0.02). • In both groups, there was a significant increase in nasal ECP (p=0.02 for rhinitis only; p=0.005 for asthma and rhinitis group). - graphical analysis • Controls were not assessed before peak. **Pollen peak vs. out of season** • In the rhinitis only and rhinitis and asthma patients, there was an increase in nasal IgE, but the increase was only significant for the rhinitis and asthma group (p=0.0076). • In both groups, there was a significant increase in nasal ECP (p=0.004 for rhinitis only; p=0.00065 for rhinitis and asthma group). However, for the rhinitis and asthma group, the median values were lower compared to before pollen peak. • No difference in controls for IgE and ECP.	None	None tested	• Non-parametric tests for non-gaussian distribution of values • Mann Whitney test (intergroup analysis) • Wilcoxon's rank sum test (intragroup analysis)
18.	Panzner et al. [60] (2015), Czech Republic	ECP, eosinophils, neutrophils and macrophages taken from sputum	• There was a significant increase in ECP levels during season compared to out of season only in seasonal allergic rhinitis only patients (Geometric means [95% CI]): 52.4 ng/ml [31.3, 88] vs. 19 [11.9, 30]; p=0.006).	None	None tested	• Variance analysis for repeated measurements • Sidak's test • Pearson's chi-squared test

Accepted Article

This article is protected by copyright. All rights reserved

			• There was a significant increase in eosinophils during season compared to out of season only in seasonal allergic rhinitis only patients (Arithmetic means [95% CI]): 1.0% [0, 2.2] vs. 0.7 [0, 1.6]; p=0.073). • No difference in macrophages and neutrophils in any of the groups between seasons.			• Spearman's correlation coefficient
19.	Van Amsterdam et al. [53] (2003), Netherlands	Exhaled NO; and IL-6, IL-8, albumin, uric acid, urea, ECP, eosinophils taken from nasal lavage fluid	• Pollen-sensitised children had significantly higher eosinophils (mean [SD]: 7.3% [8.6] vs. 2.4% [6.8]; p<0.05) and ECP (mean [SD]: 15.3ng/ml [21.3] vs. 4.4ng/ml [4.6]; p<0.05) levels during season compared to before season. • Pollen-sensitised children had significantly higher exhaled NO during season compared to before season (64.5ppb vs. 35.8 ppb; p<0.05). • No significant change for other inflammatory markers in other groups (i.e. non-atopic, HDM-sensitised and pollen + HDM-sensitised).	None	None tested	One-way ANOVA
20.	Keen et al. [55] (2005), Sweden	Eosinophils, albumin, ECP, neutrophils, total IgA and Bet v 1-specific IgA taken from nasal lavage	• In SAR children, the percentage of eosinophils, levels of ECP, Bet v 1-specific IgA and ratio of Bet v 1-specific IgA to total IgA significantly increased during pollen season compared to out of season. Total IgA decreased during pollen season compared to out of season. • In healthy children, no significant changed was observed in any	None	None tested	• Wilcoxon's signed-rank test (intragroup analysis) • Wilcoxon-Mann-Whitney U-test (intergroup analysis)

Accepted Article

This article is protected by copyright. All rights reserved

		fluid	of the inflammatory markers.			
21.	Volovitz et al. [56] (1988), United States of America	Leukotriene C_4 (LTC_4) taken from nasopharyngeal secretions	• In SAR children, the individual and mean concentrations of LTC_4 significantly increased during pollen season compared to out of season. • In healthy children, there was no significant changes in the individual and mean concentrations of LTC_4 during pollen season compared to out of season.	None	None tested	• Two-tailed t test for dependent measures
22.	Nielsen et al. [57] (1998), Denmark	ECP, eosinophil peroxidase (EPO) and human neutrophil lipocalin (HNL) taken from nasal lavage fluid	• In SAR patients, there was a significant increase in EPO ($p<0.05$) but not ECP and HNL during season compared to out of season (graphical analysis). • In healthy controls, no significant change was observed in any of the markers during pollen season compared to out of season.	None	None tested	• ANOVA • Wilcoxon rank sum test
23.	Benson et al. [54] (1997), Sweden	IL-4, IFN-γ, IL-4sR, IL-5 and IL-10 taken from nasal lavage fluid	• In SAR patients, there was a significant increase in IL-4 but not the other inflammatory cytokines during pollen season compared to out of season. • In healthy controls, there was a significant increase in IFN-γ and IL-5 but not the other inflammatory cytokines during pollen season compared to out of season	None	None tested	• Kruskal-Wallis test (intergroup analysis)

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Figure legends

Figure 1. PRISMA flow diagram

Figure 2. Summary of articles investigating airway inflammation markers.

Figure E1. Forest plot of studies investigating nasal eosinophils by the presence of ever SAR.

Figure E2. Forest plot of studies investigating nasal ECP by the presence of ever SAR.

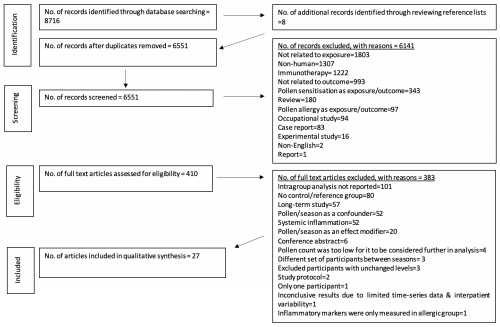
Figure E3. Forest plot of studies investigating nasal eosinophils among people with ever SAR by age group.

Figure E4. Forest plot of studies investigating nasal ECP among people with ever SAR by age group.

Figure E5. Funnel plot of studies investigating nasal eosinophils.

Figure E6. Funnel plot of studies investigating nasal ECP.

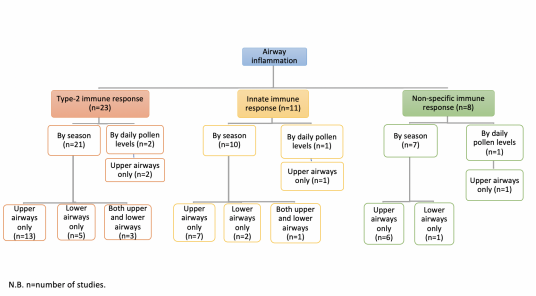
This article is protected by copyright. All rights reserved



cea_13842_f1.png

Accepted Article

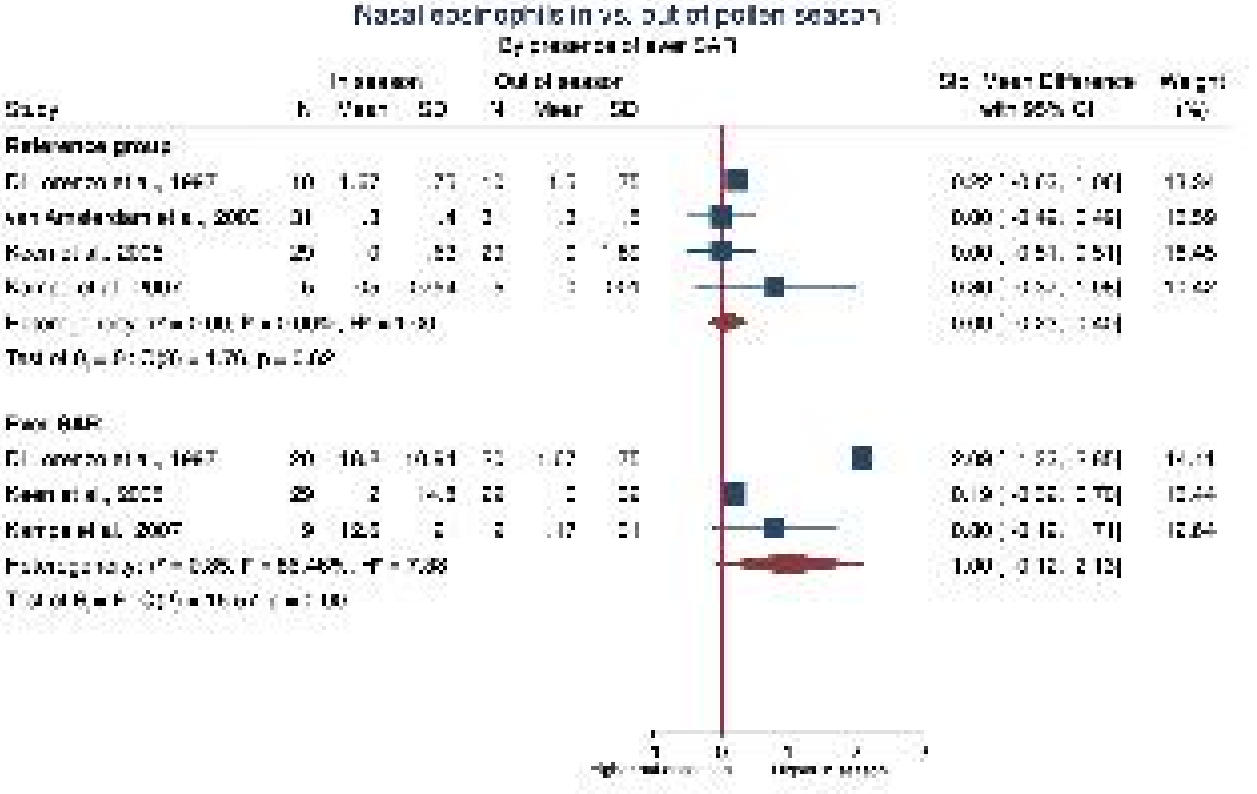
This article is protected by copyright. All rights reserved

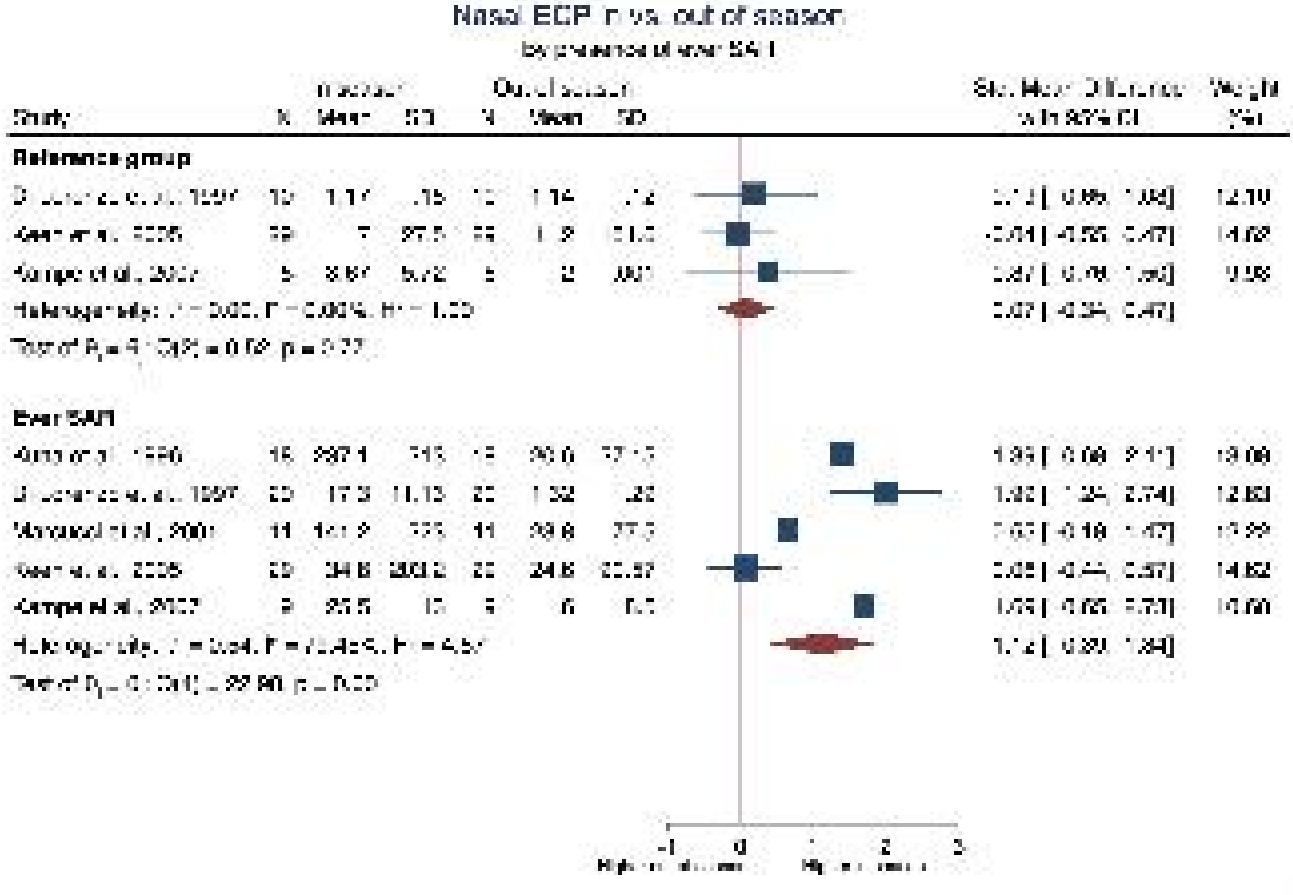


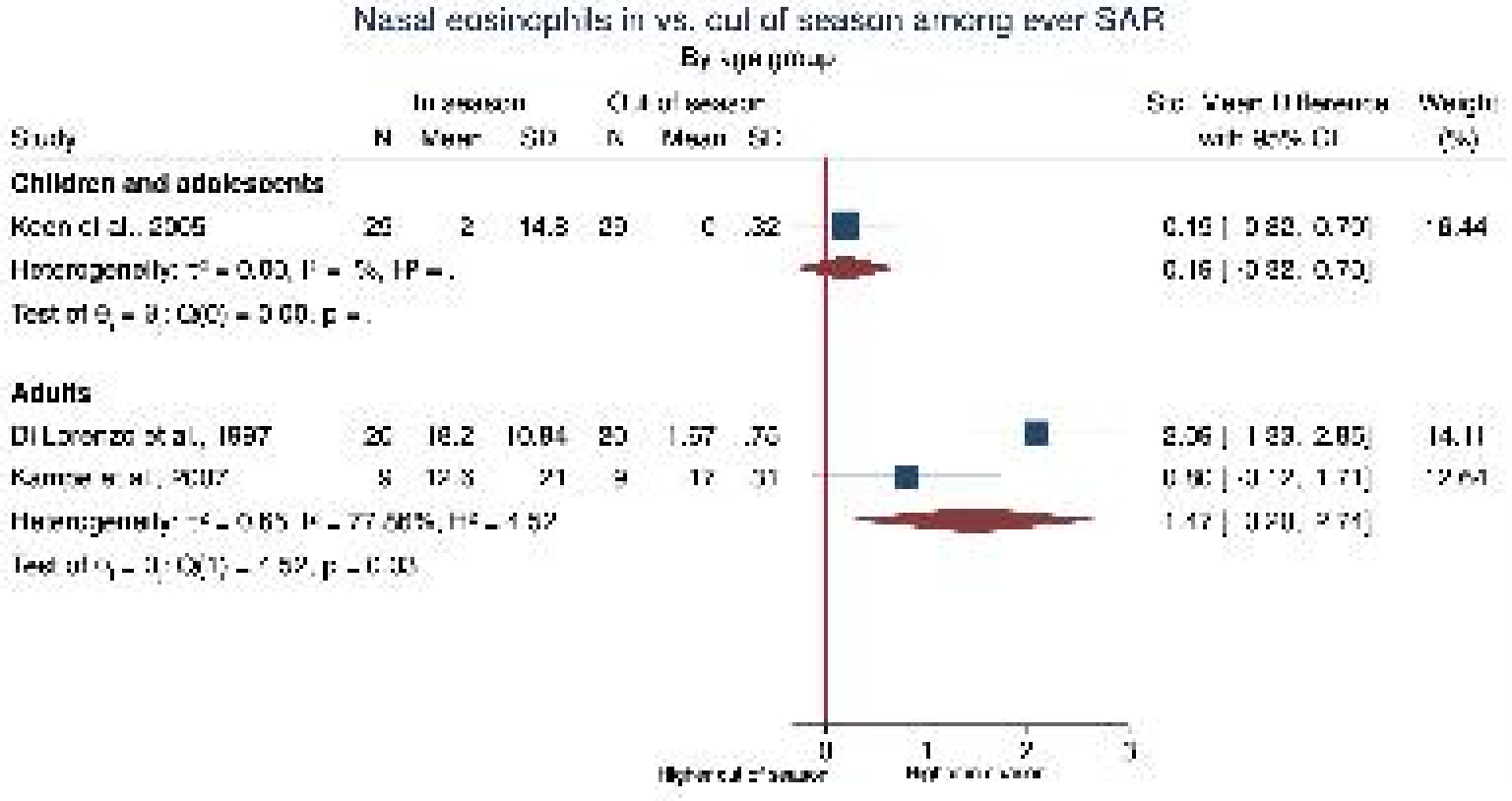
cea_13842_f2.png

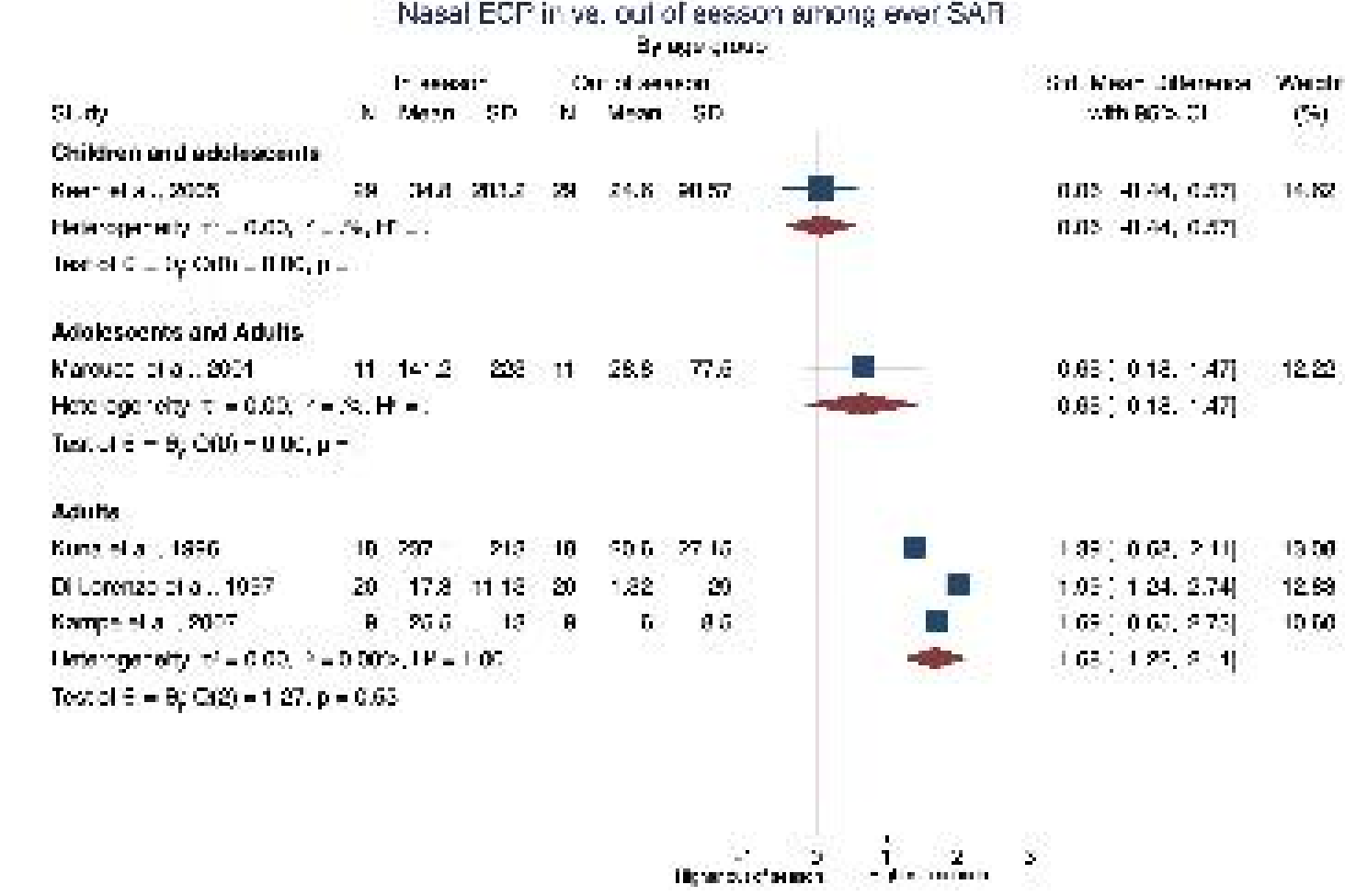
Accepted Article

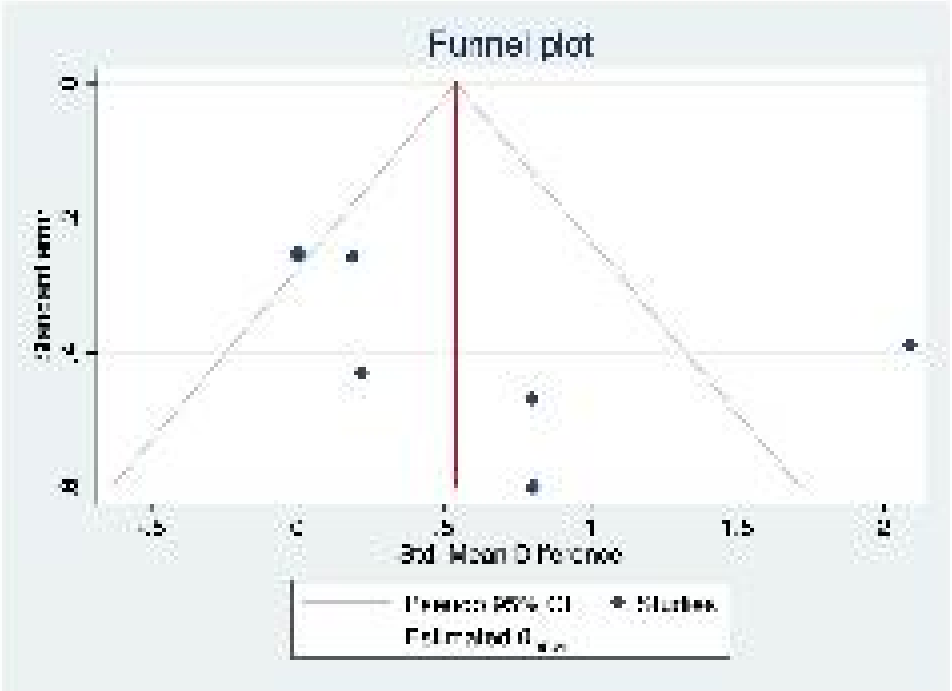
This article is protected by copyright. All rights reserved

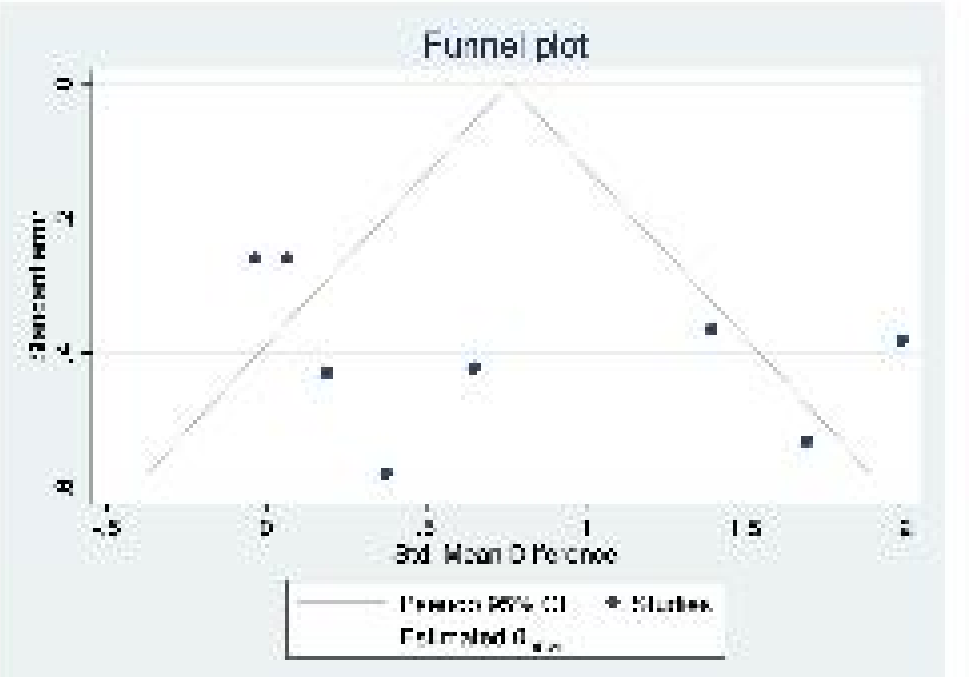

Nasal eosinophils in vs. out of pollen season
By presence of year SAT
In season
Out of season
Std. Mean Difference with 95% CI
Weight (%)
Study
N
Mean
SD
N
Mean
SD
Reference group
Di Lorenzo et al., 1997
10
1.22
.77
10
1.1
.72
0.09 [-0.73, 0.91]
11.94
van Amsterdam et al., 2009
31
.3
.4
31
.3
.5
0.00 [-0.46, 0.46]
12.59
Kaan et al., 2009
29
0
.32
29
0
.82
0.00 [-0.51, 0.51]
12.45
Kare et al., 1997
6
-0.5
0.84
6
0
0.63
-0.60 [-1.64, 0.44]
11.07
Heterogeneity: τ² = 0.00, I² = 0.00%, H² = 1.00
-0.00 [-0.33, 0.33]
Test of θi = θj: Q(3) = 1.70, p = 0.64
Pan BAP
Di Lorenzo et al., 1997
20
10.1
10.61
20
1.72
.72
2.09 [1.03, 3.15]
11.11
Kaan et al., 2009
29
2
1.43
29
0
.32
0.19 [-0.32, 0.70]
12.44
Kare et al., 2007
9
12.3
2
9
.17
.21
0.00 [-0.10, .71]
12.64
Heterogeneity: τ² = 0.00, I² = 99.46%, H² = 7.69
1.00 [-0.12, 2.13]
Test of θi = θj: Q(3) = 15.07, p = 1.00
-1
0
1
2
3
Higher in season
Higher out of season


Nasal ECP in vs. out of season
by presence of ever SAR
In season
Out of season
Study
N
Mean
SD
N
Mean
SD
Std. Mean Difference with 95% CI
Weight
Reference group
Ever SAR
-1
0
1
2
3


Nasal eosinophils in vs. out of season among ever SAR
By age group
In season
Out of season
Std. Mean Difference
Weight
Study
N
Mean
SD
N
Mean
SD
with 95% CI
(%)
Children and adolescents
Keen et al., 2005
23
2
14.8
23
0
.32
0.19 [0.82, 0.70]
18.44
Heterogeneity: τ² = 0.00, I² = %, H² = .
0.19 [0.82, 0.70]
Test of θi = θj: Q(0) = 0.00, p = .
Adults
Di Lorenzo et al., 1997
20
16.2
10.84
23
1.57
.70
2.06 [1.33, 2.80]
14.11
Kamra et al., 2007
9
12.3
21
9
17
31
0.98 [-0.12, 1.71]
12.64
Heterogeneity: τ² = 0.65, I² = 77.86%, H² = 1.52
1.17 [0.20, 2.71]
Test of θi = θj: Q(1) = 1.52, p = 0.22
0
1
2
3
Higher out of season
Higher in season


Nasal ECP in ve. out of season among ever SAR
By age group
In season
Out of season
Std. Mean Difference with 95% CI
Weight (%)
Study
N
Mean
SD
N
Mean
SD
Children and adolescents
Adolescents and Adults
Adults
Test of θ = θ; Q(2) = 1.27, p = 0.53


Funnel plot
0
-.2
-.4
-.6
Std. Mean Difference
-.5
0
.5
1
1.5
2
Studies


Funnel plot
0
-2
-4
-6
-.5
0
.5
1
1.5
2
Std. Mean Difference
Studies

Online Data Supplement (Text)

Table E1. Search strategy

Exposure	**Outcome**
pollen **OR** grass **OR** Poaceae **OR** ryegrass **OR** "secale cereale" **OR** timothy **OR** "Phleum pratense" **OR** tree **OR** birch **OR** Betula* **OR** weed **OR** ragweed **OR** Ambrosia **OR** nettle **OR** Urticaceae **OR** season	"lung function" **OR** "lung volume measurement" **OR** "respiratory function test" **OR** "pulmonary function test" **OR** "breathing test" **OR** spirometry **OR** FEV1 **OR** "forced expiratory volume in 1 second" **OR** "FEV1/FVC" **OR** FVC **OR** "forced vital capacity" **OR** FEF **OR** "mid forced expiratory flow" **OR** "peak expiratory flow" **OR** PEF **OR** PEFR **OR** "peak expiratory flow rate" **OR** "airway inflammation" **OR** "airway obstruction" **OR** "airway resistance" **OR** FeNO **OR** "fractional exhaled nitric oxide" **OR** EBC **OR** "exhaled breath condensate" **OR** eosinophil* **OR** neutrophil* **OR** "mast cell*" **OR** basophil* **OR** lavage

Table E2. Quality assessment of included studies using the Newcastle-Ottawa scale.

Studies	**Selection**				**Comparability**	**Outcome**		Total
	Representativeness of the sample	Sample size	Non-respondents	Ascertainment of the exposure		Assessment of outcome	Statistical test	
Andersson et al. [1]	*	-	-	**	**	**	*	8 stars
Bake et al. [2]	*	-	-	**	**	**	*	8 stars
Bentley et al. [3]	*	-	-	**	**	**	*	8 stars
Bergmann-Hug et al. [4]	*	-	-	**	**	*	-	6 stars
Bozek et al. [5]	*	-	-	**	**	**	-	7 stars
Čáp et al. [6]	*	-	-	**	**	**	*	8 stars
Choi et al. [7]	*	-	-	**	**	-	-	5 stars
Ciprandi et al. [8]	*	-	-	**	**	**	*	8 stars
Di Lorenzo et al. [9]	*	-	-	**	**	**	-	7 stars
Ferdousi et al. [10]	*	-	*	**	**	**	*	9 stars
Gruzieva et al. [11]	*	-	-	*	*	**	*	6 stars
Kato et al. [12]	*	-	-	**	**	**	*	8 stars
Kämpe et al. [13]	*	-	-	**	**	**	*	8 stars
Keith et al. [14]	*	-	-	**	**	**	*	8 stars
Klimek et al. [15]	*	-	*	**	-	**	*	7 stars
Kuna et al. [16]	*	-	-	**	**	**	*	8 stars
Larsson et al. [17]	*	-	-	**	**	**	*	8 stars
Marcucci et al. [18]	*	-	*	**	**	**	*	9 stars
Panzner et al. [19]	*	-	-	**	**	**	*	8 stars
Scarlett et al. [20]	*	-	-	**	-	**	*	6 stars
Steerenberg et al. [21]	*	-	*	**	*	**	*	8 stars
Van Amsterdam et al. [22]	*	-	*	**	**	**	-	8 stars
Zwick et al. [23]	*	-	*	**	**	**	*	9 stars
Keen et al. [24]	*	-	*	**	**	**	*	9 stars
Volovitz et al. [25]	*	-	-	*	**	**	*	7 stars
Nielsen et al. [26]	*	-	-	**	**	**	*	8 stars
Benson et al. [27]	*	-	-	**	**	**	-	7 stars

Quality of studies: 0-4 points= unsatisfactory; 5-6 points=Satisfactory; 7-8 points= Good; 9-10= Very good

Table E3. Findings of included lung function studies.

No.	Author (Year), Country	Outcome measures	Findings	Confounders	Effect modifiers	Statistical tests
1.	Gruzieva et al. [11] (2015), Sweden	FEV_1, FVC, FEV_1/FVC ratio	**8-year-olds** • Increased grass pollen exposure at lag 1 were associated with lower FEV_1 (Coef. [95% CI]: -32.4ml [-50.6, -14.2]) and FVC (data not shown). Similar was observed for exposure periods up to a week. • No significant association was observed for FEV_1/FVC ratio at all lags. • Significant interaction found between grass pollen exposure and pollen sensitization (p-int= 0.05 for FEV_1 and p-int=0.01 for FVC). Children who were sensitized to only one pollen had a less pronounced effect. • No significant association was found between other pollen types and lung function. **16-year-olds** • No significant association was found between any pollen type and lung function.	Sex, height, age, allergic heredity	Pollen sensitization	Linear regression analysis
2.	Scarlett et al. [20] (1996), United Kingdom	$FEV_{0.75}$, FVC, $FEV_{0.75}$/FVC ratio. All presented as percentage change.	• Pollen at lag 0 is not significantly associated with $FEV_{0.75}$, FVC and $FEV_{0.}$75/FVC ratio.	None	None tested	• Multiple regression analysis

						• Cochrane-Orcutt regression
3.	Bake et al. [2] (2014), Sweden	FEV_1, FVC	• No significant difference in both FEV_1 and FVC between seasons (in/out) in either groups.	None	None tested	• Wilcoxon exact test • Wilcoxon signed rank test • Two-sided paired t-test
4.	Ferdousi et al. [10] (2001), Sweden	PEF	• The variation of PEF is higher during birch pollen season compared to autumn • There is a significant decrease in PEF variability from birch pollen season (median [95% CI]: 8% [7.8-10.2]) to autumn (median [95% CI]: 6% [6.1-8.9]).	None	None tested	• Wilcoxon paired test • Spearman's rank correlation analysis
5.	Larsson et al. [17] (2017), Sweden	FEV_1 (% of predicted normal value), FVC (% of predicted normal value), FEV_1/FVC ratio (%), post-bronchodilator FEV_1 (% of predicted normal value)	• Normal spirometry values during and outside pollen season for both asthmatics and controls.	None	None tested	• Wilcoxon signed rank-sum test • Wilcoxon-Mann-Whitney U test • Spearman correlation
6.	Panzner et al. [19] (2015), Czech Republic	FEV_1 (% of predicted value)	• No significant changes and differences were found in any of the groups before, during and after pollen season.	None	None tested	• Variance analysis for repeated measurements • Sidak's test • Pearson's chi-squared test • Spearman's correlation coefficient

7.	Zwick et al. [23] (1991), Austria	FEV_1, FVC, FEV_1/FVC, maximal expiratory flow at 50% expiration (MEF_{50}), MEF_{50}/FVC, FEV_1<80% predicted, MEF_{50}/FVC<75%	• None of the lung function measures were significantly associated with pollen load.	Sex, age, air pollution and meteorological conditions	None tested	• Chi-squared test • Kolmogoroff-Smirnoff test

Table E4. The inflammatory markers identified from included studies, categorised by the type of inflammatory profile.

Type of inflammatory profile	Markers
Type-2 immune response [1, 28-35]	**Upper airways (n=18)** Positive association (n=13): Eosinophils, MBP ("total eosinophils"), EG2 ("activated eosinophils"), Eosinophil peroxidase (EPO), ECP, TAME-esterase, Leukotriene (LTC_4), Metachromatic (mast) cells, Tryptase-only positive mast cells (MC_T), Histamine, Tryptase, IL-4, IgE No association (n=5): Chymase plus tryptase positive mast cell (MC_{TC}), IL-4sR, IL-5, IL-10, CD4
	Lower airways (n=4) <u>Exhaled breath (n=2)</u> Positive association (n=2): Nitric oxides (FeNO, maximal flow of NO from airways (JáwNO), alveolar NO concentration (CANO)), Leukotriene (LTE_4) <u>Sputum (n=2)</u> Positive association (n=2): Eosinophils, ECP
Innate immune response [36-40]	**Upper airways (n=8)** Positive association (n=4): Neutrophils, IL-8, CD54/ICAM-1, Monocyte chemotactic and activating factor/monocyte chemoattractant protein (MCAF/MCP-1) No association (n=4): Macrophage inflammatory protein 1-alpha (MIP-1α), Neutrophil elastase, Human neutrophil lipocalin (HNL), CD68
	Lower airways (n=4) <u>Exhaled breath (n=1)</u> Positive association (n=1): Leukotriene (LTB_4)

	<u>Sputum (n=3)</u> No association (n=3): Neutrophils, Macrophages, HNL
Non-specific immune response [14, 41-48]	**Upper airways (n=12)** Positive association (n=2): Bet v 1-specific IgA, IFN-γ No association (n=10): IL-6, IL-2R, Uric acid, CD3, CD45, CCL5 (RANTES), Albumin, HLA-DR, Urea, CD8
	Lower airways (n=1) <u>Exhaled breath (n=1)</u> Positive association (n=1): Total mass of exhaled particles

N.B. n=number of markers.

References for this document

1. Andersson M, Svensson C, Andersson P, Pipkorn U. Objective monitoring of the allergic inflammatory response of the nasal mucosa in patients with hay fever during natural allergen exposure. The American review of respiratory disease. 1989;139(4):911-4.
2. Bake B, Viklund E, Olin AC. Effects of pollen season on central and peripheral nitric oxide production in subjects with pollen asthma. Respiratory medicine. 2014;108(9):1277-83.
3. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. The Journal of allergy and clinical immunology. 1992;89(4):877-83.
4. Bergmann-Hug K, Wirth R, Henseler M, Helbling A, Pichler WJ, Schnyder B. Effect of natural seasonal pollen exposure and repeated nasal allergen provocations on elevation of exhaled nitric oxide. Allergy. 2009;64(11):1629-34.
5. Bozek A, Krajewska J, Jarzab J. Nasal nitric oxide and other diagnostic procedures in seasonal allergic rhinitis: elderly vs juvenile patients. American Journal of Otolaryngology. 2011;32(2):105-8.
6. Cap P, Maly M, Pehal F, Pelikan Z. Exhaled leukotrienes and bronchial responsiveness to methacholine in patients with seasonal allergic rhinitis. Ann Allergy Asthma Immunol. 2009;102(2):103-9.
7. Choi CH, Poroyko V, Watanabe S, Jiang D, Lane J, deTineo M, et al. Seasonal allergic rhinitis affects sinonasal microbiota. American journal of rhinology & allergy. 2014;28(4):281-6.
8. Ciprandi G, Pronzato C, Ricca V, Bagnasco M, Canonica GW. Evidence of intercellular adhesion molecule-1 expression on nasal epithelial cells in acute rhinoconjunctivitis caused by pollen exposure. The Journal of allergy and clinical immunology. 1994;94(4):738-46.
9. Di Lorenzo G, Mansueto P, Melluso M, Candore G, Colombo A, Pellitteri ME, et al. Allergic rhinitis to grass pollen: measurement of inflammatory mediators of mast cell and eosinophils in native nasal fluid lavage and in serum out of and during pollen season. The Journal of allergy and clinical immunology. 1997;100(6 Pt 1):832-7.
10. Ferdousi HA, Munir AKM, Zetterström O, Dreborg SKG. Seasonal differences of peak expiratory flow rate variability and mediators of allergic inflammation in non-atopic adolescents. Pediatric Allergy and Immunology. 2001;12(5):238-46.
11. Gruzieva O, Pershagen G, Wickman M, Melén E, Hallberg J, Bellander T, et al. Exposure to grass pollen – but not birch pollen – affects lung function in Swedish children. Allergy. 2015;70(9):1181-3.
12. Kato M, Liu W, Hattori T, Nakashima I. Evidence of potential regulation by interleukin-4 of the soluble intercellular adhesion molecule 1 level in patients with seasonal allergic rhinitis under provocation by a small amount of natural allergen. Ann Otol Rhinol Laryngol. 1998;107(3):232-5.
13. Kämpe M, Stålenheim G, Janson C, Stolt I, Carlson M. Systemic and local eosinophil inflammation during the birch pollen season in allergic patients with predominant rhinitis or asthma. Clinical and Molecular Allergy. 2007;5(1):4.

14. Keith PK, Conway M, Evans S, Wong DA, Jordana G, Pengelly D, et al. Nasal polyps: effects of seasonal allergen exposure. The Journal of allergy and clinical immunology. 1994;93(3):567-74.
15. Klimek L, Riechelmann H, Amedee R. Eosinophil Cationic Protein in Nasal Secretions and Blood Serum in Grass-Pollen Allergic Rhinitis. American Journal of Rhinology. 1996;10(5):319-22.
16. Kuna P, Lazarovich M, Kaplan AP. Chemokines in seasonal allergic rhinitis. J Allergy Clin Immunol. 1996;97(1 Pt 1):104-12.
17. Larsson P, Larstad M, Bake B, Hammar O, Bredberg A, Almstrand AC, et al. Exhaled particles as markers of small airway inflammation in subjects with asthma. Clin Physiol Funct Imaging. 2017;37(5):489-97.
18. Marcucci F, Sensi LG, Migali E, Coniglio G. Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. Allergy. 2001;56(3):231-6.
19. Panzner P, Malkusova I, Vachova M, Liska M, Brodska P, Ruzickova O, et al. Bronchial inflammation in seasonal allergic rhinitis with or without asthma in relation to natural exposure to pollen allergens. Allergol Immunopathol (Madr). 2015;43(1):3-9.
20. Scarlett JF, Abbott KJ, Peacock JL, Strachan DP, Anderson HR. Acute effects of summer air pollution on respiratory function in primary school children in southern England. Thorax. 1996;51(11):1109-14.
21. Steerenberg PA, Bischoff EW, de Klerk A, Verlaan AP, Jongbloets LM, van Loveren H, et al. Acute effect of air pollution on respiratory complaints, exhaled NO and biomarkers in nasal lavages of allergic children during the pollen season. Int Arch Allergy Immunol. 2003;131(2):127-37.
22. van Amsterdam JG, Bischoff EW, de Klerk A, Verlaan AP, Jongbloets LM, van Loveren H, et al. Exhaled NO level and number of eosinophils in nasal lavage as markers of pollen-induced upper and lower airway inflammation in children sensitive to grass pollen. Int Arch Occup Environ Health. 2003;76(4):309-12.
23. Zwick H, Popp W, Jager S, Wagner C, Reiser K, Horak F. Pollen sensitization and allergy in children depend on the pollen load. Allergy. 1991;46(5):362-6.
24. Keen C, Johansson S, Reinholdt J, Benson M, Wennergren G. Bet v 1-specific IgA increases during the pollen season but not after a single allergen challenge in children with birch pollen-induced intermittent allergic rhinitis. Pediatr Allergy Immunol. 2005;16(3):209-16.
25. Volovitz B, Osur SL, Bernstein JM, Ogra PL. Leukotriene C4 release in upper respiratory mucosa during natural exposure to ragweed in ragweed-sensitive children. The Journal of allergy and clinical immunology. 1988;82(3 Pt 1):414-8.
26. Nielsen LP, Bjerke T, Christensen MB, Skamling M, Peterson CG, Mygind N, et al. Eosinophil markers in seasonal allergic rhinitis. Intranasal fluticasone propionate inhibits local and systemic increases during the pollen season. Allergy. 1998;53(8):778-85.
27. Benson M, Strannegard IL, Wennergren G, Strannegard O. Cytokines in nasal fluids from school children with seasonal allergic rhinitis. Pediatr Allergy Immunol. 1997;8(3):143-9.
28. Bandeira-Melo C, Weller PF. Eosinophils and cysteinyl leukotrienes. Prostaglandins Leukot Essent Fatty Acids. 2003;69(2-3):135-43.

29. Veien M, M.D., Szlam F, M.M.S., Holden Jeannine T, M.D., Yamaguchi K, M.D., Denson Donald D, Ph.D., Levy Jerrold H, M.D. Mechanisms of Nonimmunological Histamine and Tryptase Release from Human Cutaneous Mast Cells. Anesthesiology: The Journal of the American Society of Anesthesiologists. 2000;92(4):1074-81.
30. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respiratory research. 2001;2(2):66-70.
31. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. Respiratory research. 2001;2(2):71-9.
32. Laouini D, Alenius H, Bryce P, Oettgen H, Tsitsikov E, Geha RS. IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. The Journal of clinical investigation. 2003;112(7):1058-66.
33. MacPherson JC, Comhair SA, Erzurum SC, Klein DF, Lipscomb MF, Kavuru MS, et al. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. J Immunol. 2001;166(9):5763-72.
34. Luckheeram RV, Zhou R, Verma AD, Xia B. $CD4^+$T cells: differentiation and functions. Clin Dev Immunol. 2012;2012:925135-.
35. Caminati M, Pham DL, Bagnasco D, Canonica GW. Type 2 immunity in asthma. World Allergy Organization Journal. 2018;11(1):13.
36. Kuhns DB, Young HA, Gallin EK, Gallin JI. Ca2+-dependent production and release of IL-8 in human neutrophils. The Journal of Immunology. 1998;161(8):4332-9.
37. Woodfin A, Beyrau M, Voisin M-B, Ma B, Whiteford JR, Hordijk PL, et al. ICAM-1-expressing neutrophils exhibit enhanced effector functions in murine models of endotoxemia. Blood. 2016;127(7):898-907.
38. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-26.
39. Majumdar R, Tavakoli Tameh A, Parent CA. Exosomes Mediate LTB4 Release during Neutrophil Chemotaxis. PLOS Biology. 2016;14(1):e1002336.
40. Chistiakov DA, Killingsworth MC, Myasoedova VA, Orekhov AN, Bobryshev YV. CD68/macrosialin: not just a histochemical marker. Laboratory Investigation. 2017;97(1):4-13.
41. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol. 2011;4(6):603-11.
42. Muhl H, Pfeilschifter J. Anti-inflammatory properties of pro-inflammatory interferon-gamma. Int Immunopharmacol. 2003;3(9):1247-55.
43. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295-a.
44. Capobianco M, Cassiano G, Antônia A. Human interleukin 2 (IL-2) promotion of immune regulation and clinical outcomes: a review. J Cytokine Biol. 2016;1:109.

45. Spiga R, Marini Maria A, Mancuso E, Di Fatta C, Fuoco A, Perticone F, et al. Uric Acid Is Associated With Inflammatory Biomarkers and Induces Inflammation Via Activating the NF-κB Signaling Pathway in HepG2 Cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2017;37(6):1241-9.
46. Koya T, Takeda K, Kodama T, Miyahara N, Matsubara S, Balhorn A, et al. RANTES (CCL5) regulates airway responsiveness after repeated allergen challenge. Am J Respir Cell Mol Biol. 2006;35(2):147-54.
47. Donovan JA, Koretzky GA. CD45 and the immune response. J Am Soc Nephrol. 1993;4(4):976-85.
48. Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. Immunity. 2011;35(2):161-8.