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Title: Blood Eosinophil Count as Predictor of Asthma Exacerbation. A Meta-analysis

Short title: Blood Eosinophil Count and Asthma Exacerbation

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Authors' Contributions: NM conducted the literature review, extracted, analyzed, and interpreted the data, and designed and wrote the manuscript. SR-S participated in the studies localization. NM and BT extracted the data. FJG-B conceived the research idea and resolved disagreements about articles' selection. BT designed the study and supervised the data analysis and interpretation. All authors reviewed and revised the manuscript and approved it for publication.

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ABSTRACT

Background: Evidence about the association of high blood eosinophil count with asthma exacerbation is inconsistent and unclear. The objective of this meta-analysis is to determine whether elevated blood eosinophils count predicts asthma exacerbation.

Methods: We searched MEDLINE, EMBASE, and additional databases, without any language restriction. We also checked the reference lists of the included studies and of relevant systematic reviews. The main outcome was the occurrence of asthma exacerbation. We calculated global pooled Odds Ratios (ORs) and their 95% Confidence Intervals (CIs) and performed predefined subgroup analyses. We appraised the quality of the studies using Newcastle-Ottawa Scale, examined the heterogeneity between studies, assessed publication bias and carried out sensitivity analyses.

Results: Among 1567 retrieved publications, 23 observational studies comprising 155,772 participants met the inclusion criteria. High blood eosinophil count was associated with higher odds of asthma exacerbation [OR: 1.31 (95%CI: 1.16, 1.49)], specifically with asthma-related outpatient visits [OR: 1.46 (95%CI: 1.25, 1.70)] and emergency department visits [OR: 1.63 (95%CI: 1.29, 2.07)]. A significant association was observed starting from an eosinophils' cut-off value of 200 cells/ μ l. The association was observed for cohort studies [OR: 1.30 (95%CI: 1.13, 1.49)], North-American studies [OR: 1.43 (95%CI: 1.31, 1.57)], Asian populations [OR: 1.67 (95%CI: 1.34, 2.08)] children [OR: 1.38 (95%CI: 1.22, 1.56)], and studies that adjusted for inhaled corticosteroids therapy [OR: 1.42 (95%CI: 1.28, 1.56)].

Conclusions: Blood eosinophil counts ≥ 200 cells/ μ l are associated with asthma exacerbation. Blood eosinophil count is a modifiable factor that could be addressed in asthma management strategies.

Keywords: asthma exacerbation, blood eosinophils, meta-analysis

Abbreviations: CCI: Charlson Comorbidity Index; CI: Confidence Interval; CRP: C-Reactive Protein; FEV1: Forced Expiratory Volume in 1 second; FeNO: Fractional Exhaled Nitric Oxide; ICS: Inhaled Corticosteroids; NOS: Newcastle-Ottawa Scale; OR: Odds Ratio; PEF: Peak Expiratory Flow; RR: Relative Risk

INTRODUCTION

Asthma is a prominent chronic disease that affects 235 million individuals worldwide and represents the most common chronic disease among children.^{1,2} When exacerbated, it requires urgent medical assistance to prevent serious outcomes including death.³ Asthma exacerbation occurs in higher frequency and with more severity when asthma is not controlled; nevertheless, it may also affect patients on asthma treatment.⁴ The global mortality rate from asthma was 0.19 per 100,000 individuals in 2012, with stalled improvements in lowering mortality over the past decade.⁵ Indeed, in 2016, asthma was classified as the 23rd leading cause of premature mortality.⁶ The Global Asthma Report 2018 estimates that 1000 people die every day from asthma worldwide.⁶ Asthma prevalence is still growing in many countries, imposing major consequences on public health, society and

economy.⁶ These observations emphasize the need to better understand predisposing factors to predict the occurrence and severity of asthma exacerbation.

Increased clinical interest in the use of eosinophil count as a predictor of asthma worsening was observed in the recent literature. However, to reach appropriate clinical decisions, a careful interpretation of the available evidence is required. Findings concerning blood eosinophils and asthma exacerbation diverged widely across studies, as the directions and the magnitudes of measures of effect were inconsistent. While some studies did not find a correlation between increased blood eosinophil count and asthma severity,^{7,8} others reported a borderline effect,⁹⁻¹¹ or a positive association which magnitude varied greatly between studies, ranging between 30% and 150% increase in the risk of asthma worsening.¹²⁻¹⁹ On the other side, some authors reported opposite findings which associated elevated levels of blood eosinophils with a lower risk of asthma exacerbation.^{20,21} In light of these contradictory observations, and taking into account that blood eosinophil count may be lowered through adequate therapy, we considered that there was a need for a meta-analysis that assesses the association between blood eosinophil count and asthma exacerbation, and explores the threshold of blood eosinophils which potentially induces asthma worsening.

METHODS

Data sources and searches

We performed a systematic review and meta-analysis of blood eosinophil counts as predictors of the risk of asthma exacerbation. Asthma exacerbation is also sometimes referred as asthma attack.^{22,23} We defined asthma exacerbation (or asthma attack) as a deterioration in asthma requiring any of the following: treatment with an oral corticosteroid, visit to an emergency department, outpatient visit, or hospitalization. We included studies that ascertained asthma exacerbations using self-assessment tools. However we later stratified the analysis based on the mean used for outcome ascertainment.

We searched the following databases since their inception until May 2020: MEDLINE, EMBASE, Conference Proceedings Citation Index- Science, the five regional bibliographic databases of the World Health Organization; as well as the Open Access Thesis and Dissertations. We did not apply

any restriction on language or publication date. In MEDLINE we applied the search syntax: ("Asthma"[Mesh] AND "Eosinophils"[Mesh] AND (exacerbation OR worsening)), which we then adapted to other databases. We completed our search by using the terms: asthma, blood eosinophil, exacerbation, worsening, attack, as free text words, and by checking manually the reference lists of the eligible studies as well as that of other relevant systematic reviews.

Study selection

Two researchers (NM and SR-S) independently reviewed the titles and abstracts of the retrieved studies and subsequently selected the potentially eligible ones for a full-text revision. Disagreements were resolved by referring to a third researcher (FJG-B).

We included observational studies that reported Odds Ratio (OR) or Relative Risk (RR) of the association between high blood eosinophil count and asthma exacerbation. We excluded studies in which the comparison group was limited to healthy subjects. In the case of duplicated reports, we considered the most complete and updated version. The study protocol is registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42020157743).

Data extraction and quality assessment

Two epidemiologists (NM and BT) extracted data on: 1) study source: first author's name and publication year; 2) study characteristics: country, study design, blood eosinophil cut-off limit, sample size, participants' inclusion and exclusion criteria, effect measure and 95% confidence interval (CI), and adjustment variables; 3) patients' characteristics: age and sex; and 4) asthma exacerbation definitions: hospital admission, emergency department visit, outpatient visit and/or increased corticosteroids treatment. When relevant data were incomplete or not available, we contacted the corresponding author to request the missing information.^{8,9,14,15,24-28}

To assess the methodological quality of the studies, we used the Newcastle-Ottawa Scale (NOS) for cohort and case-control studies,²⁹ and an adapted NOS version for the evaluation of cross-sectional studies.³⁰ NOS is a widely used tool to evaluate the methodological aspects of observational studies such as samples' representativeness, participation rate, control for confounding factors, and

ascertainment of exposure and outcome. Quality scoring was performed independently by two reviewers and the average score between reviewers was assigned to the studies. A maximum of 8 points was attributed to each study.(e-Appendix 1) Disagreement between raters was measured by the Bland-Altman limits of agreement method.³¹

Data synthesis and statistical analysis

We weighted the study-specific adjusted measures of effect by the inverse of their variance to obtain a pooled OR. When various thresholds of blood eosinophil count were reported, we used 400 cells/ μ l as a cut-off limit.³² When effect measures were reported for different subgroups within the same study, we pooled the different estimates in order to obtain one global OR per study.

We computed fixed and random effect models but considered only the latter when heterogeneity was detected. We checked for heterogeneity using DerSimonian and Laird's Q test and quantified it using R_i , the proportion of total variance due to between-study variance.³³ We interpreted R_i values as follows: low heterogeneity ($R_i < 0.4$), moderate heterogeneity ($0.4 \leq R_i \leq 0.75$), and high heterogeneity ($R_i > 0.75$). Finally, we stratified the analysis according to study design, type of cohort study, quality score, age category, geographical location, blood eosinophils' cut-off limit, asthma exacerbation definitions (indicators), methods of asthma exacerbation ascertainment, and frequency of asthma attacks. We planned *a priori* all these subgroup analyses.

We explored the presence of publication bias, first visually using a funnel plot and then, more formally, using Egger's regression test. We also carried out a sensitivity analysis assuming that cross-sectional studies are the least likely to be published when they show a null association. We recalculated the pooled OR assuming that: 1) the cross-sectional studies included in our meta-analysis represent only half of the studies that have ever been conducted on this topic, 2) the unpublished cross-sectional studies found a null association of OR = 1 and 3) the prevalence of asthma exacerbation in the unpublished studies was equal to the average prevalence of the published ones.

In addition, to further assess publication bias, we performed a "trim and fill" analysis.³⁴

We carried out the analyses using the software HEpiMA version 2.1.3,³⁵ and STATA version 12 (Stata Corp, College Station, TX, USA).

RESULTS

Literature search and study characteristics

We identified 1567 studies in our search (Figure 1). Twenty-three studies with a total population of 155772 patients met our inclusion criteria (Table 1 and Figure 2). Four studies were not considered eligible for this meta-analysis because they did not use any specific cut-off value for blood eosinophil count.^{9,24,26,27} One of the four studies measured the association using percentage of blood eosinophils instead of counts,⁹ and 3 other studies provided continuous effect measures.^{24,26,27}

We merged data from different studies carried out in the same population as explained in eAppendix 2.

We classified one cohort study as cross-sectional given that the blood eosinophil test was performed at admission to the hospital, and hence no follow-up stage was carried out.²⁰

Asthma exacerbation

Sixteen cohort studies,^{10-16,18,19,21,28,36-40} 6 cross-sectional studies,^{7,8,20,25,41,42} and one case-control study,¹⁷ examined the association of elevated blood eosinophil count with asthma exacerbation. The overall pooled OR showed a moderate association [OR: 1.31 (95%CI: 1.16, 1.49)] with a substantial amount of heterogeneity between studies ($R_i=0.90$) (Table 2).

When the analysis was stratified by study design, the pooled estimates of cohort studies showed a significant association between high blood eosinophil count and asthma exacerbation [OR: 1.30 (95%CI: 1.13, 1.49); $R_i=0.92$], whereas no association was observed in cross-sectional studies [OR: 1.27 (95%CI: 0.94, 1.72); $R_i=0.73$] (Table 2). One case-control study measured the association between blood eosinophil count and asthma exacerbation and reported an OR of 15.30 (95%CI: 3.90, 60.00).

Subgroup analysis by the type of cohort studies showed that only pooled OR estimate from prospective cohorts indicated a significant association between blood eosinophil count and asthma

exacerbation [OR_{prospective cohort}: 1.35 (95%CI: 1.17, 1.56); OR_{retrospective cohort}: 1.29 (95%CI: 0.95, 1.76)].

Asthma exacerbation and age

The age characteristic of the patients differed between studies. Elevated blood eosinophil count was associated with asthma exacerbation in children [OR: 1.38 (95%CI: 1.22, 1.56)] and no heterogeneity was found in the 4 studies of this age group ($R_i = 0.00$, Q-test p-value=0.64) (Table 2).

Asthma exacerbation and geographical location

A substantial association of blood eosinophil count with asthma exacerbation was detected in studies undertaken in North-America and Asia exclusively [OR_{North-America}: 1.43 (95%CI: 1.31, 1.57) and OR_{Asia}: 1.67 (95%CI: 1.34, 2.08)], with no heterogeneity between studies in these subgroups. No association was observed among the 12 European studies OR_{Europe}: 1.19 (0.95, 1.50).

Asthma exacerbation and cut-off values of blood eosinophil count

We observed an association between asthma exacerbation and blood eosinophil count for different cut off values. This association was observed starting at a threshold of 200 cells/ μ l [OR_{200 cells/ μ l}: 1.22 (95%CI: 1.02, 1.46)], while the strongest magnitude of association was observed at a threshold beyond 400 cells/ μ l [OR_{>400cells/ μ l}: 1.54 (95%CI: 1.46, 1.61)] (Table 2).

Asthma exacerbation definitions

The association of blood eosinophil count with different definitions of asthma exacerbation was explored. A high but statistically non-significant increased odds was observed for asthma-related hospitalization [OR: 1.40 (95%CI: 0.82, 2.40)]. A substantial increase in the odds of asthma-related emergency room visits and outpatient acute visits were observed for patients with high levels of blood eosinophils in comparison with asthma patients with lower blood eosinophil counts [OR_{emergency room}: 1.63 (95%CI: 1.29, 2.07); OR_{outpatient acute visit}: 1.46 (95%CI: 1.25, 1.70)] (Table 2). One study measured the association of blood eosinophils with time to exacerbation and reported an OR of 4.5

(95%CI: 1.8, 38.0).¹² The heterogeneity persisted only in the subgroup of asthma-related hospitalization ($R_i = 0.92$, Q-test p-value < 0.0001) (Table 2).

Quality assessment

Eighteen studies ascertained asthma exacerbation using medical records and 4 studies relied on self-reporting. Increased odds of exacerbation was observed in the two groups: [OR_{Medical records}: OR: 1.30 (95%CI: 1.13, 1.50) and OR_{self-reporting}: 1.40 (95%CI: 1.17, 1.67)]. No heterogeneity was observed across studies that used self-reporting ascertainment.

Studies that adjusted for inhaled corticosteroid (ICS) treatment showed a substantial higher pooled odds ratio than studies that did not control for this variable [OR: 1.42 (95%CI: 1.28, 1.56) and OR: 1.13 (95%CI: 0.73, 1.74), respectively] (Table 2).

Regarding the quality assessment, the average disagreement between the two raters was close to zero (-0.82; 95% CI: -3.26 to 1.62). The fact that the 95% CI is symmetric around zero suggests that there was no systematic disagreement between the raters. The significant association of blood eosinophils with asthma exacerbation was only observed for studies that scored ≥ 4 points: OR = 1.44 (95%CI: 1.33, 1.55). The heterogeneity between studies was present in the subgroup of lower quality score (< 4 points) $R_i = 0.98$, while it was absent in the subgroup of higher quality $R_i = 0.00$, Q-test p-value = 0.86 (Table 2).

Publication bias

The funnel plot is skewed to the right revealing the presence of small-study effects (Figure 3). However, Egger's test supported the absence of publication bias (p=0.679). The Trim-and-Fill method imputed two studies and suggested a corrected OR of 1.29 (95%CI: 1.13, 1.46), that is very similar to the overall OR originally obtained. The association did not subside under the extreme assumptions of our sensitivity analysis: pooled OR = 1.20 (95%CI: 1.07, 1.33).

DISCUSSION

Data about the association of the biomarker blood eosinophils as a predictor of asthma exacerbation are widely divergent. Therefore, to resolve these inconsistencies, we conducted the first meta-analysis to explore the association between blood eosinophil count and asthma exacerbation.

Our findings showed that high blood eosinophil count, starting at a threshold of 200 cells/ μ l, is associated with increased odds of asthma exacerbation, especially in children. The association was observed in North American and Asian populations and was exclusive to cohort- and case-control studies designs. No association was found in studies with a cross-sectional design, which could be due to biases inherent to this design and which are not found in cohort studies and case-control studies. We also reported that high blood eosinophil count is substantially associated with increased likelihood of outpatient and asthma-related emergency room visits.

Approximately half of patients with severe asthma present eosinophilic asthma,²³ and more than half of asthmatic patients show a blood eosinophil count higher than 150 cells/ μ L.^{8,19} Blood eosinophil count is the most commonly used biomarker for detecting airway eosinophilic inflammation in adult patients with persistent uncontrolled asthma.⁴³ When eosinophils are activated by proinflammatory cytokines such as interleukin 5 (IL-5), they migrate into the inflammation site and provoke sustained inflammation and tissue damage.⁴⁴ This inflammation mediated by IL-5 is part of a broader concept, that of the so-called “type-2 inflammation” which involves other cytokines, including IL-4 and IL-13. Eosinophil counts, as well as fraction exhaled nitric oxide (FeNO), are now used as biomarkers for type 2 inflammation.⁴⁵ Therapies that disrupt IL-5 signaling such as mepolizumab and reslizumab are used to mitigate blood eosinophil count.⁴⁶ The baseline of blood eosinophils to predict response to treatment remains an unresolved issue due to lack of consensus on the threshold value. However, the cut-off value of 400 cells/ μ l represents the most generally accepted threshold.⁴⁷ Cut-off limits of 300 cells/ μ l and 400 cells/ μ l are the most frequently used values in the studies included in this meta-analysis. Moreover, previous studies showed that blood eosinophil count < 150 cells/ μ l is not associated with any clinical or physiological responses to anti-IL-5 therapies and that the response to treatment is more pronounced at higher levels of eosinophils.⁴⁸ This is in line with our findings that blood eosinophils are associated with asthma exacerbation, starting at a cut-off value of 200 cells/ μ l.

We reported increased odds of emergency department or outpatient visits during asthma exacerbation from elevated blood eosinophil count. However, we could not observe any relation between high eosinophil count and hospitalization. Reports indicate that emergency department visits are more economic than patients' hospitalization and at the same time offer effective treatment.⁴⁹ Accordingly, we hypothesize that asthma attacks, independently of their severity and eosinophil count, are mostly resolved in the clinics or in the emergency departments, which therefore decreases the number of observations in the patient hospitalization chain. It should be emphasized that patients' hospitalization and access to emergency departments vary between healthcare systems.⁶

Furthermore, we observed an increased likelihood of asthma exacerbations in North American and Asian patients with elevated levels of blood eosinophils. The absence of association in European patients is probably due to the inclusion of three studies that reported an opposite effect of increased odds of asthma severity in patients with lower eosinophil counts.^{20,21,40} The authors of two of these studies hypothesized that the exacerbation could have been due to a neutrophilic inflammation caused by rhinovirus.^{20,21} Respiratory tract viruses, especially rhinovirus, are known to be important triggers of asthma exacerbation.^{50,51} On the other side, genetic differences between populations were suggested to influence patients' susceptibility to asthma, response to treatment and the risk of asthma exacerbation.⁵²⁻⁵⁵ Other factors, mainly environmental and socioeconomic predictors, were attributed to differences of asthma occurrence and severity between populations.^{55,56}

Studies within primary care settings have also reported that the risk of asthma exacerbation varies across age-groups, with pediatrics and elderly patients being the most vulnerable populations.⁵⁷ Restricting our analysis to studies with subjects of pediatric age showed that the association of high eosinophil count and asthma exacerbation is stronger than that in the general population. Only one of the 23 studies included in the present meta-analysis, evaluated the specific association in elderly patients, and reported a non-statistically significant increased risk of asthma exacerbation from elevated blood eosinophil count.²⁸

The pooled OR of asthma exacerbation was higher in studies that adjusted for ICS therapy than in studies that failed to adjust for this treatment. The seven studies that did not adjust for ICS

administration were undertaken in Asia, North-America and Europe. The difference in the pooled estimates between regions is then unlikely to be due to a differential use of ICS

Our results are unlikely to be due to publication bias that could have affected the direction and/or the magnitude of the association as indicated by the negative results of Egger's test. Furthermore, although the Trim-and-Fill method imputed two additional studies, the corrected OR was very similar to the one we obtained.

Additional analyses of our data have shown that misclassification of the outcome due to self-ascertainment of asthma exacerbation and misclassification of blood eosinophil count are unlikely to explain our results (data not shown).

We also found a large amount of heterogeneity between studies that remained present in many subgroup analyses. This heterogeneity was to a certain point expected, due to the wide range of cut-off limits and the differences in the definitions of asthma worsening used in the studies. We, therefore, based our interpretation on random effects estimates as recommended.^{58,59} Meta-analysis experts emphasize that no degree of heterogeneity can be deemed unacceptable, provided the eligibility criteria are clear and the data are correct,⁵⁸ and that heterogeneity in meta-analysis, due to the differences in methods and populations, should be viewed as the "expectation, rather than the exception".⁶⁰

Finally, due to the lack of data, it was not possible to assess whether the increase in the odds of asthma exacerbation was associated in a dose-response fashion to the increase in the count of blood eosinophils.

Asthma is a frequent life-long inflammatory disorder that causes a global substantial burden of disease. Eosinophilic inflammation in asthma patients is a well-characterized feature of the disease. We showed that the occurrence of asthma exacerbation is related to increased levels of blood eosinophils, a modifiable exposure factor. Future research is needed to investigate this association in understudied vulnerable populations including elderly patients. The findings of this meta-analysis could prove useful in the management of asthma.

DATA AVAILABILITY

The data that support the findings of this study are openly available in “FigShare” at: <https://figshare.com/s/e7c1358ae298d033fa91>

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Table 1. Main characteristics of included studies about increased blood eosinophil count and asthma exacerbation

Author, Year	Country	Setting	Main participants' Characteristics	Blood eosinophils cut- off point (cells/ μ l) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
Cohort Studies							
Sohn et al, 2020 ²⁸	Korea	Hospital/Emergency department and outpatient visits	Group 1: Elderly Age: mean (SD): 78.24 (7.18) Female: 44.1%	400	1200	1.69 (1.29, 2.20)	Unadjusted
Shah et al, 2019 ³⁶	Georgia	Hospital/Emergency department and outpatient visits	Group 1: Non-Elderly Age: mean (SD): 36.89 (10.28) Female: 61.8% Group 1: Children Age: mean (SD): 9.1 (1.8) Female: 40.5% Group 2: Adolescents Age: mean (SD): 16.2 (3.2) Female: 43.2%	300	589	1.52 (1.30, 1.77)	Sex, smoking habits, asthma treatment, ethnicity, race, household education level

Nagasaki et al, 2019 ³⁹	Japan	Hospital/Emergency department	Age: mean (SD): 63 (15) Female: 62%0	300	160	1.25 (0.58, 2.67)	Age, sex, asthma treatment, baseline exacerbation, FEV1, FeNO, atopic status
Hakansson et al, 2019 ⁴⁰	Denmark	Hospital/Emergency department	Age: median (IQR): 45.3 (30.1 – 63.1) Female: 65.9%	300	1341	0.64 (0.5, 0.9)	Age, sex, CCI, Soluble Urokinase Plasminogen Activator Receptor, C-RP
Yii et al, 2019 ¹⁸	Singapore	Hospital/Emergency department	Age: mean (SD): 55 (18) Female: 59%	1200	376	1.80 (1.10, 2.90)	Smoking habits, asthma treatment

Table 1. Main characteristics of included studies about increased blood eosinophil count and asthma exacerbation (continued)

Author, Year	Country	Setting	Main participants' Characteristics	Blood eosinophils cut- off point (cells/ μ l) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
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Kerkhof et al, 2018 ¹⁰	United Kingdom	Hospital/Emergency department	Group 1: Eosinophil count: <350 cells/ μ l Age: median (IQR): 54.0 (39.0 - 70.3) Female: 75.7%	350	2613	1.49 (1.04, 2.13)	Age, sex, weight, smoking, CCI, timing of blood eosinophil count relative to the first hospitalization, comorbidities, asthma treatment
			Group 2: Eosinophil count: >350 cells/ μ l Age: median (IQR): 45.0 (30.0 - 65.0) Female: 70.1%				
Turner et al, 2018 ³⁸	United Kingdom	Primary care	Age: mean (SD): 9.0 (2.3) Female: 44%	400	3776	1.46 (1.20, 1.78)	Age, PEF, asthma treatment, hay fever, history of asthma exacerbations, comorbidities, eczema
Pola-Bibian et al, 2017 ²¹	Spain	Hospital/Emergency department	Age: mean (range): 57.3 (14-102) Female: 67.7%	>260	831	0.46 (0.33, 0.64)	Unspecified

Zeiger et al, 2017 ¹¹	United States	Primary care	Age: mean (SD): 52.1 (16.1) Female: 66.7%	400	261	1.40 (1.02, 1.90)	Age, sex, weight, CCI, education, ethnicity, musculoskeletal disease, asthma medication ratio, perennial allergens, history of asthma exacerbations
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Table 1. Main characteristics of included studies about increased blood eosinophil count and asthma exacerbation (continued)

Author, Year	Country	Setting	Main participants' Characteristics	Blood eosinophils cut- off point (cells/μl) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
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Vedel-Krogh et al, 2017 ¹⁶	Denmark	General population	Group 1: Eosinophil count: 180 cells/ μ l Age: median (IQR): 56 (47–66) Female: 69%	290	4838	1.37 (1.18, 1.60)	Age, sex, weight, smoking habits, asthma treatment, FEV1
			Group 2: Eosinophil count: 180–290 cells/ μ l Age: median (IQR): 56 (47–65) Female: 60%				
			Group 3: Eosinophil count: 290 cells/ μ l Age: median (IQR): 57 (47–68) Female: 51%				
Price et al, 2015 & 2016 ^{14,15}	United Kingdom	Primary care	Group 1: Eosinophil count: $\leq$ 400 cells/ μ l Age: median (IQR): 50 (37-63) Female: 69%	400	130248	1.42 (1.36, 1.47)	Age, sex, weight, smoking habits, CCI, comorbidities
			Group 2: Eosinophil count: >400 cells/ μ l Age: median (IQR): 45 (31-61) Female: 61%				

Table 1. Main characteristics of included studies about increased blood eosinophil count and asthma exacerbation (continued)

Author, Year	Country	Setting	Main participants’ Characteristics	Blood eosinophils cut- off point (cells/μl) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
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Casciano et al, 2016 ¹³	United States	Primary care	Group 1: Eosinophil count: ≥ 400 cells/ μ l Age: groups: 12–17 years: 2 (5.71%) 18–35 years: 8 (22.86%) 36–64 years: 16 (45.71%) 65 years or older: 9 (25.71%) Female: 68.6% Group 2: Eosinophil count: ≤ 400 cells/ μ l Age: groups: 12–17 years: 5 (3.5%) 18–35 years: 18 (12.50%) 36–64 years: 92 (63.89%) 65 years or older: 29 (20.14%) Female: 68.1%	400	179	3.06 (1.14, 8.22)	Patient demographics, asthma severity, CCI
Zeiger et al, 2015 ³⁷	United States	Primary care	Age: mean (SD): 7.7 (1.9) Female: 38.1%	400	333	1.40 (1.02, 1.94)	Age, sex, weight, education, ethnicity, asthma treatment, history of asthma exacerbations

Zeiger et al, 2014 ¹⁹	United States	Primary care	Age: mean (SD): 47.0 (12.43) Female: 70.4%	400	2392	1.31 (1.07, 1.60)	Age, sex, weight, education, ethnicity, asthma treatment, history of asthma exacerbations
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Table 1. Main characteristics of included studies about increased blood eosinophil count and asthma exacerbation (continued)

Author, Year	Country	Setting	Main participants' Characteristics	Blood eosinophils cut- off point (cells/μl) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
Belda et al, 2001 ¹²	Spain	Outpatient visits	Age: mean (SD): 31 (14) Female:61.3%	400	31	4.5 (1.8, 38.0)	Asthma treatment
Case-control studies							
Velthove et al, 2009 ¹⁷	Netherlands	Outpatient visits	Age: ≥ 18 Female: unspecified for asthma category	400	24	15.3 (3.9, 60.0)	Age, sex, asthma treatment

Cross-sectional studies

Price et al, 2019 ²⁵	United Kingdom	Primary care	Group 1: FENO normal; non-high blood eosinophils Age: mean (SD): 51.9±13.1 Female: 58.6%	300	372	1.53 (1.09, 2.15)	Unadjusted
			Group 2: FENO normal; high blood eosinophils Age: mean (SD): 51.8±13.7 Female: 58.6%				
Gonzalez-Barcala et al, 2018 ²⁰	Spain	Hospital/Emergency department	Age: median (SD): 60.02 (20.2) Female: 70%	400	1316	0.59 (0.37, 0.92)	Age, sex, asthma treatment
Mogensen et al, 2018 ⁴²	United States		Age (percentage of total population): <18: 27% 18-44: 42% >44: 31% Female (percentage of total population): 58%	400	1419	1.56 (1.15, 2.01)	Age, sex, smoking, weight, ethnicity, age of asthma onset, hay fever, S-cotinine levels, asthma treatment, study period, inflammatory medications

Table 1. Main characteristics of studies included in the meta-analysis about increase blood eosinophil count and asthma exacerbation (continued)

Author, Year	Country	Setting	Main participants' Characteristics	Blood eosinophils cut- off point (cells/ μ l) used in the meta-analysis	Study Size	General OR (95%CI)	Adjustment Variables
Malinovschi et al, 2016 ⁷	Sweden	Primary care and outpatient visits	Group 1: FENO normal; blood eosinophil count increased Age: mean (SD): 20.6 $\pm$ 7.8 Female: 40.9%	300	410	1.25 (0.76, 2.07)	Age, smoking habits, weight, atopy, asthma treatment
Westerhof et al, 2016 ⁴¹	Netherlands	Unspecified	Group 2: FENO increased; blood eosinophil count increased Age: mean (SD): 17.3 $\pm$ 5.8 Female: 46.3%	250	153	2.94 (1.1, 8.1)	Asthma treatment, asthma duration, FEV1, blood neutrophils, atopy, ACQ-6 score
			Group 1: Ex-smokers Age: mean (SD): 53 (10) Female: 60%				
			Group 2: Never smokers Age: mean (SD): 50 (13) Female: 77%				

Tran et al, 2014 ⁸	United States	General population	Group1: Children Age: mean (SD): 12 (0.1) Female: 46%	400	2910	1.23 (0.92, 1.64)	Age, sex, weight, smoking habits, education, ethnicity, survey year, blood neutrophil level, age at asthma onset, treatment
			Group2: Adults Age: mean (SD): 40 (0.4) Female: 63%				

FEV1: Forced Expiratory Volume in 1 second; FeNO: Fractional Exhaled Nitric Oxide; CCI: Charlson Comorbidity Index; CRP: C-Reactive Protein; PEF: Peak Expiratory Flow

Table 2. Pooled OR and 95% CI of increased blood eosinophil count and asthma exacerbation

	Number of studies	OR (95% CI) Fixed effects	OR (95% CI) Random effects	R_t^2	Q test p-value
All studies	22†	1.39 (1.35, 1.44)	1.31 (1.16, 1.49)	0.90	<0.0001
Study design					
Cohort	15	1.40 (1.36, 1.45)	1.30 (1.13, 1.49)	0.92	<0.0001
Cross-Sectional	6	1.31 (1.13, 1.51)	1.27 (0.94, 1.72)	0.73	0.003
Cohort study type					
Prospective	7	1.42 (1.37, 1.46)	1.35 (1.17, 1.56)	0.92	<0.0001
Retrospective	8	1.26 (1.13, 1.41)	1.29 (0.95, 1.76)	0.86	<0.0001
Age category					
Only children	4	1.38 (1.22, 1.56)	1.38 (1.22, 1.56)	0.00	0.64
Exacerbation ascertainment					
Medical Record	18	1.40 (1.36, 1.44)	1.30 (1.13, 1.50)	0.93	<0.0001
Self-Reporting	4	1.40 (1.17, 1.67)	1.40 (1.17, 1.67)	0.00	0.42
Adjustment for ICS treatment					
Not adjusted for ICS or unspecified	7	1.08 (0.94, 1.24)	1.13 (0.73, 1.74)	0.90	<0.0001
Adjusted for ICS	15	1.42 (1.38, 1.46)	1.42 (1.28, 1.56)	0.79	0.001
Quality Score					
< 4 point	11	1.39 (1.35, 1.44)	1.21 (0.93, 1.59)	0.98	<0.0001
≥ 4 points	11	1.44 (1.33, 1.55)	1.44 (1.33, 1.55)	0.00	0.86
Geographical location					
Europe	12	1.39 (1.35, 1.44)	1.19 (0.95, 1.50)	0.97	<0.0001
North America	7	1.43 (1.31, 1.57)	1.43 (1.31, 1.57)	0.00	0.54
Asia	3	1.67 (1.34, 2.08)	1.67 (1.34, 2.08)	0.00	0.70
Blood eosinophils cut-off limit					
150	4	1.21 (1.08, 1.34)	1.16 (0.77, 1.74)	0.93	<0.0001

200	5	1.16 (1.11, 1.22)	1.22 (1.02, 1.46)	0.92	<0.0001
250	3	1.24 (1.18, 1.29)	1.42 (1.09, 1.83)	0.95	0.005
300	11	1.29 (1.24, 1.35)	1.23 (1.08, 1.41)	0.83	<0.0001
350	2	1.34 (1.28, 1.41)	1.34 (1.28, 1.41)	0.00	0.56
400	12	1.41 (1.34, 1.48)	1.41 (1.23, 1.62)	0.78	<0.0001
>400	3	1.54 (1.46, 1.61)	1.54 (1.46, 1.61)	0.00	0.70

Definition of asthma exacerbation

Asthma related hospitalization	8	0.99 (0.86, 1.15)	1.40 (0.82, 2.40)	0.92	<0.0001
Asthma related emergency room visit	3	1.63 (1.29, 2.07)	1.63 (1.29, 2.07)	0.00	0.58
Asthma related outpatient acute visits	2	1.46 (1.25, 1.70)	1.25 (0.71, 2.20)	0.90	0.14

Frequency of asthma attack

Frequent (≥ 2)	5	1.47 (1.38, 1.56)	1.28 (0.94, 1.74)	0.92	0.05
Less frequent	4	1.04 (0.90, 1.20)	0.95 (0.70, 1.29)	0.71	0.04

†: The total number of studies is 22, as 2 cohort studies were analyzed together (e-Appendix 2).

‡: Proportion of total variance due to between-study variance

Figure Legends

Figure 1. Flow diagram of studies' localization and selection

Figure 2. Study-specific and pooled Odds Ratios of blood eosinophil count and asthma exacerbation

Figure 3. Funnel plot of Odds Ratios of blood eosinophil count and asthma exacerbation

Appendices

e-Appendix 1: Quality Assessment of included studies

We assessed the following aspects that were common between the three study designs: 1) if the method used to count blood eosinophils was described (1 point), else (0 point); 2) if asthma exacerbation was determined based on medical records (1 point), else (0 point).

For cohort studies we also determined: 1) if the exposed cohort was representative of the population (1 point), else (0 point); 2) if non-exposed patients were recruited from the same population as the exposed patients (1 point), else (0 point); 3) if included patients were free from asthma exacerbation events when recruited (1 point), else (0 point); 4) if the measure of effect was adjusted for sex, age, weight, asthma treatment and smoking habits (1 point), else (0 point); 5) if patients were followed up for a minimum of 1 year (1 point), else (0 point); 6) if the proportion of patients who left the study or were lost to follow up was $\leq 20\%$ (1 point), else (0 point).

For case-control studies, we determined: 1) if cases were representative of patients with asthma exacerbation in the population (1 point), else (0 point); 2) if controls were selected from the same population as the cases (1 point), else (0 point); 3) if blood eosinophil count in controls was ascertained using the same method as that applied for cases (1 point), else (0 point); 4) if participation rate was $\geq 80\%$ (1 point), else (0 point).

For cross-sectional studies we checked: 1) if the sample was representative of the whole population (1 point), else (0 point); 2) if the response rate was reported (1 point), else (0 point); 3) if the statistical analysis was well described and appropriate (1 point), else (0 point); 4) if the sample size was justified (1 point), else (0 point).

The comparability in case-control and cross-sectional studies was rated as follows: 1) if the measurement of effect was adjusted for asthma treatment (1 point), else (0 point); 2) if the measurement of effect was adjusted for sex, age, weight, and smoking habits (1 point), else (0 point).

When details on a specific item were not provided, we graded this item with 0 point.

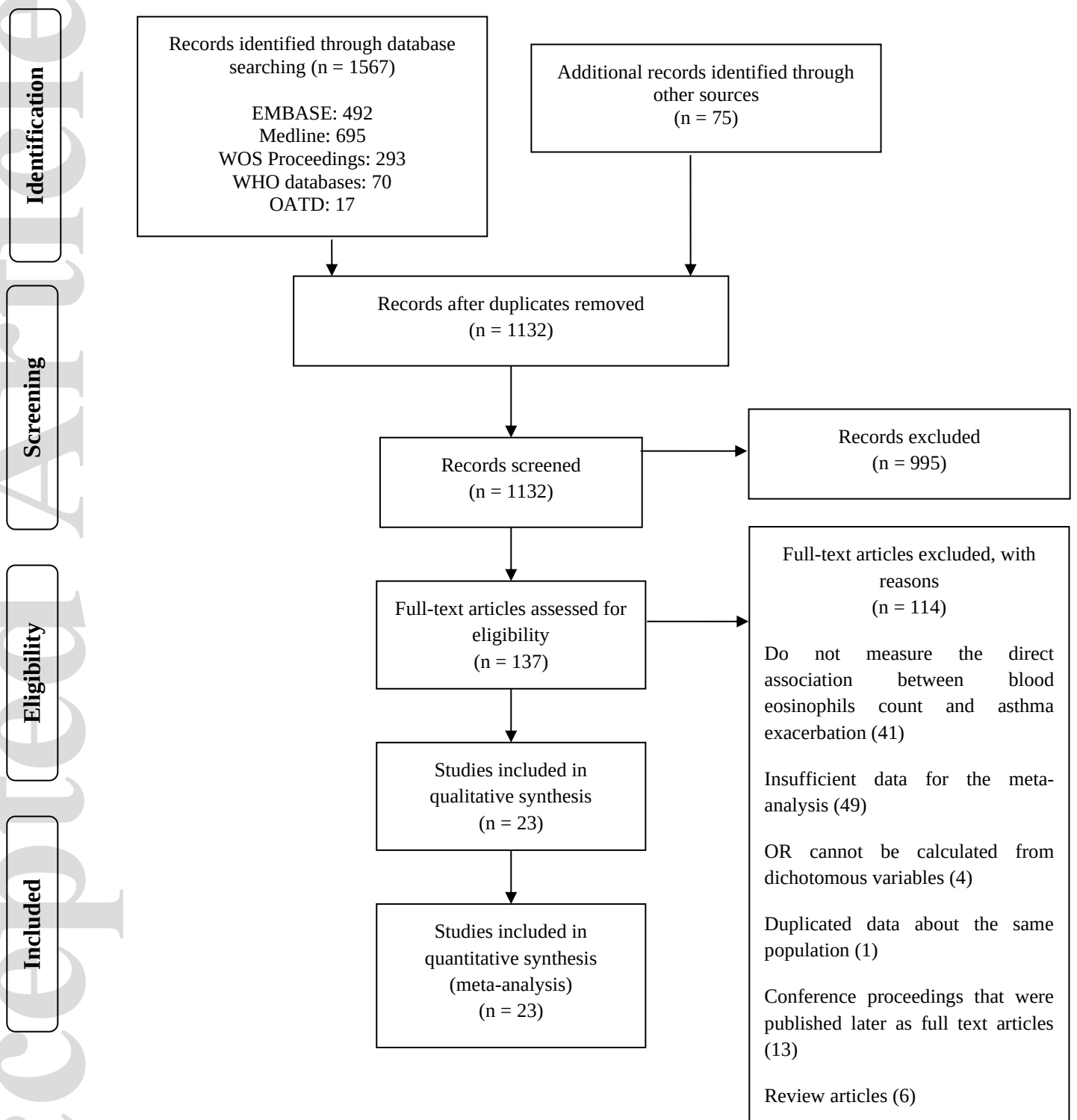
Finally, we summed the points across the items in order to obtain a global quality score of a maximum 8 points.

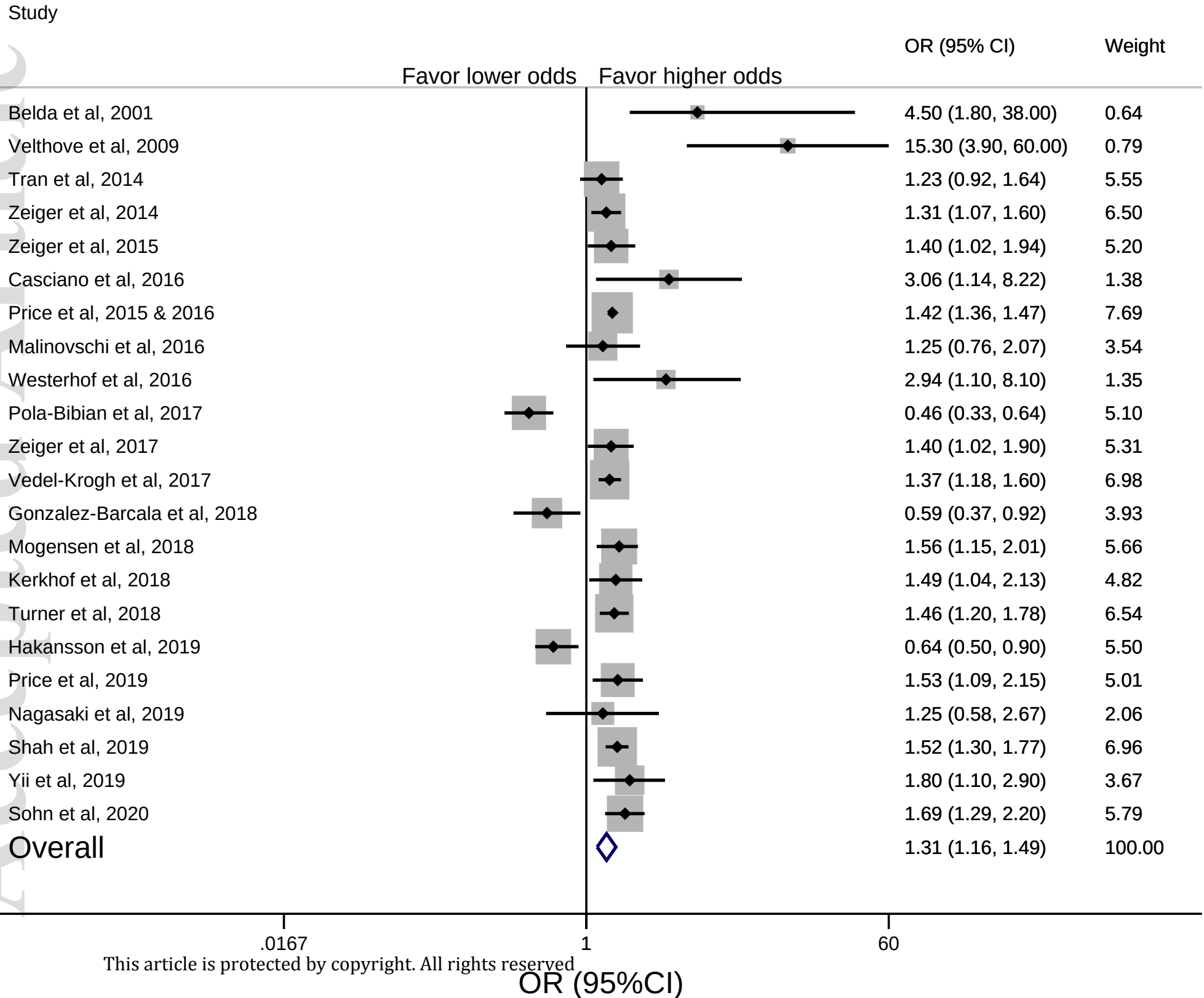
e-Appendix 2: Further details on different publications using the same population

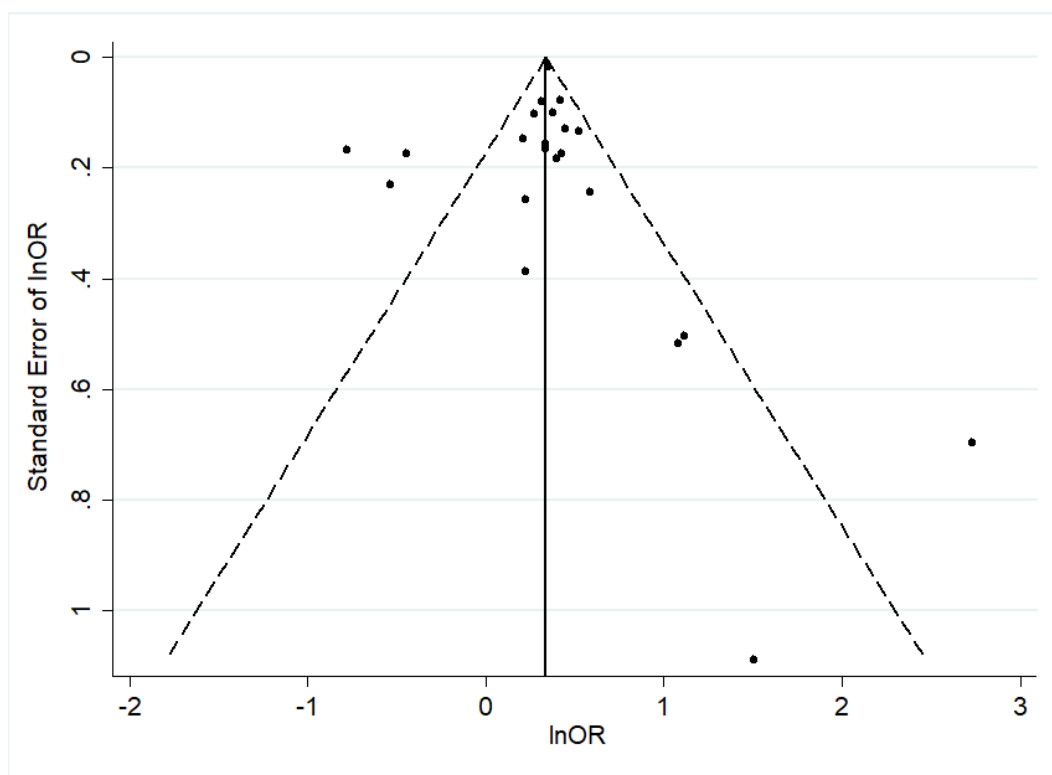
Two different publications using the same population were considered for our meta-analysis as they measured a different outcome. The first publication measured the risk of “any asthma exacerbation” while the second assessed the risk of “frequent asthma exacerbation”.^{1,2} The results of these studies were never pooled in the same group. We used the first one for the overall pooled estimation and all the subgroup analyses,¹ except for the subgroup “Frequency of asthma attacks” in which we used the second publication in the category “frequent” (≥ 2 attacks).² A third publication by the same group was included in this meta-analysis after confirming with the author that there was no overlap between the population of this study,³ and that of the previous two studies.^{1,2} Other 3 studies, published by the same first author (Zeiger RS), were unrelated and encompassed different populations.⁴⁻⁶ One of these studies involved adult patients recruited between 2009 and 2010,⁴ whereas the second publication included patients ≥ 12 years of age after 2012.⁵ The third study included a population of children aged 5 to 11 years old.⁶

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- Accepted Article
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