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Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

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TITLE: Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

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- Ethics: the protocol has been reviewed by the Oxford Tropical Research Ethics Committee and found to comprise fully anonymised data not requiring further ethical approbation.

Patient and public involvement: No patient involved.

Author contributions: SC drafted the protocol and participated in the systematic review literature search, contacted relevant data providers and will participate in data extraction and analysis. ES will provide statistical expertise and support. IDP is the guarantor of this publication, contributed to the writing of the protocol manuscript, participated in the systematic review literature search, and approved the final manuscript. All authors reviewed and approved the final manuscript.

Author declaration of conflicts of interest

SC: received a non-restricted research grant from Sanofi-Genzyme for investigator-initiated type 2 innovation research and received speaker honoraria from GlaxoSmithKline, Sanofi-Regeneron and AstraZeneca; outside the submitted work. **ES:** receives royalties from Springer for the textbook entitled Clinical Prediction Models and received speaker honoraria from GlaxoSmithKline; outside the submitted work. **RB** has received honoraria for presentations or consulting in Adboards from AstraZeneca, Asthma and Respiratory Foundation of New Zealand, Avillion, Cipla and Theravance; and research grants from AstraZeneca, CureKids (NZ), Genentech, and the Health Research Council of New Zealand. **IDP**: In the last 5 years, IP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim, Aerocrine AB, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini, and GSK, and payments for organising educational events from AstraZeneca, GSK, Sanofi/Regeneron, and Teva. He has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AstraZeneca, Boehringer Ingelheim, GSK, Novartis, Teva, Merck, Circassia, Chiesi, and Knopp, and payments to support FDA approval meetings from GSK. He has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, AstraZeneca, Teva, and Chiesi. He has received a grant from Chiesi to support a phase 2 clinical trial in Oxford. He is co-patent holder of the rights to the Leicester Cough Questionnaire and has received payments for its use in clinical trials from Merck, Bayer, and Insmed. In 2014-2015 he was an expert witness for a patent dispute involving AstraZeneca and Teva.

ABSTRACT:

Introduction: The reduction of the risk of asthma attacks is a major goal of guidelines. The fact that type-2 inflammatory biomarkers identify a higher risk, anti-inflammatory responsive phenotype is potentially relevant to this goal. We aim to quantify the relation between blood eosinophils, exhaled nitric oxide (FeNO) and the risk of severe asthma attacks.

Methods and Analysis: A systematic review of randomised controlled trials (RCTs) will be conducted by searching MEDLINE from January 1993 to April 2021. We will include RCTs that investigated the effect of fixed treatment(s) regimen(s) on severe asthma exacerbation rates over at least 6 months and reported a baseline value for blood eosinophils and FeNO. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, and the methodological appraisal of the studies will be assessed by the Cochrane Risk-of-Bias Tool for RCTs. Study authors will be contacted to request anonymised individual participant data for patients randomised to the trial's control arm (*i.e.* no inhaled corticosteroid (ICS), lowest dose ICS, or placebo). An individual participant data meta-analysis will be performed for multivariable prognostic modelling with performance assessment (calibration plots and the c-statistic) in a cross-validation by study procedure. The outcome to predict is the absolute number of severe asthma attacks to occur in the following 12 months if anti-inflammatory therapy is not changed (*i.e.:* if patient were randomised to the control arm of an RCT). A summary prognostic equation and risk stratification chart will be reported as a basis for further analyses of individualized treatment benefit. .

Ethics and Dissemination: The protocol has been reviewed by the relevant Oxford academic ethics committee and found to comprise fully anonymised data not requiring further ethical

approbation. Results will be communicated in an international meeting and submitted to a peer-reviewed journal.

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ARTICLE SUMMARY

Strengths and limitations of this study

- The prognostic (*i.e.* predicting adverse outcomes) and theragnostic (*i.e.* predicting treatment responsiveness) values of type-2 inflammatory biomarkers are established; we thus speculate that a clinical prediction model centred on blood eosinophils and exhaled nitric oxide will provide a useful framework for a preventive, treatable trait-based management.
- This systematic review and individual patient data (IPD) level meta-analysis of randomised controlled trials (RCTs) across the spectrum of asthma severities will offer support for clinical decision-making based on type-2 inflammatory biomarkers and other clinical prognostic factors.
- We aim to include data from a substantial number of RCTs (N>10) for a large number of patients in total (n>5000), which allows for reliable statistical modelling (internal validity) and assessment of transportability across settings (external validity).
- The participating studies' authors and sponsors will form an international, collaborative, and not-for-profit consortium to allow efficient use of high-quality IPD.

- Potential weaknesses are the low number of events reported in RCTs enrolling mild asthmatics and the absence of active arm IPD.

Word count: 5 / 5 one-sentence bullet points

ABBREVIATIONS

CI: confidence intervals

FeNO: fractional exhaled nitric oxide

GINA: Global Initiative for Asthma

ICS: inhaled corticosteroid

IL: interleukin

IPD: individual participant data

MA: meta-analysis

RCT: randomised controlled trial

INTRODUCTION

Reduction of the risk of severe asthma attacks is a major goal of asthma management [1]. The current recommendation is to perform risk assessment based on a history of a previous asthma attack and a list of clinical risk factors (Table 1) [1]. However, many of these prognostic factors are unmodifiable or difficult to modify and a key risk factor (treatment adherence) is difficult to identify and quantify before starting treatment. In contrast, some risk factors are modifiable, such as symptoms and lung function, while they are not necessarily on the causal pathway of asthma attacks. As a result of these deficiencies, risk quantification in asthma is an inexact art and the impact of treatment is difficult to predict [2-13].

One approach to targeted risk reduction is to use a scale centred on readily available prognostic factors that quantify the risk of the adverse outcome of interest in a manner which also predicts the benefits of preventative treatment. This approach has been successful in cardiovascular disease risk reduction where charts [14,15] focus on modifiable factors such as blood pressure and cholesterol with age and gender as key prognostic demographic factors. We speculate that a similar framework can be applied to predict asthma attacks in patients with asthma.

Type-2 airway inflammation is important in the pathogenesis of many asthma attacks [16] where this immune response characterised by interleukin (IL)-4, IL-5, IL-13, and eosinophilic airway infiltration forms a distinct clinical phenotype [16]. In clinic, the actions of type-2 immunity are readily identified by two independent, complementary, and accessible biomarkers: the peripheral blood eosinophil count and fractional exhaled nitric oxide (FeNO) [17–23]. Importantly, the excess risk conferred by raised type-2 biomarkers can be removed with appropriate treatment, be it low-dose inhaled corticosteroids (ICS) in mild asthma [19,24], a higher dose of ICS in moderate asthma

[21], or biological agents targeting type 2 cytokines in moderate and severe asthma [18,25–27]. In effect, blood eosinophils and FeNO have emerged as 'treatable traits' [28].

We have previously established a proof-of-concept biomarker-stratified asthma attack scale using publication-level data which is promising and potentially useful to support clinical decision-making [22]. The prototype lacked detailed and statistically robust assessment of multivariable prognostic relations and systematic assessment of external validity, which is possible with an individual participant data (IPD) meta-analysis (MA).

Review question

In people ≥ 12 years old diagnosed with asthma of any severity randomised to the control arm of a clinical trial, what is the absolute number of severe asthma attacks (defined as acute asthma requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation)[29] to occur in the following 12 months in relation to their peripheral blood eosinophil count, FeNO, and other prognostic factors at baseline?

Objectives

Specific aims of this systematic review are

1. To systematically identify randomised controlled trials (RCTs) in people ≥ 12 years old diagnosed with asthma of any severity which measured i) the peripheral blood eosinophil count and FeNO at baseline and ii) assessed the incident severe asthma attacks over ≥ 6 months of follow-up.
2. To perform an IPD MA for the participants randomised to the control arms (defined as no inhaled corticosteroid (ICS), lowest dose ICS, or placebo) of the RCTs identified in aim 1.

3. To assess the multivariable prognostic relations of the peripheral blood eosinophil count, FeNO, and other risk factors assessed at baseline.
4. To develop and validate a clinical prediction model for the absolute number of severe asthma attacks to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other risk factors at baseline.

METHODS AND ANALYSIS

Eligibility

Types of studies

In keeping with the objectives of the systematic review, we will include RCTs completed between 1 January 1993 and 1 April 2021 that investigated the effect of fixed treatment(s) regimen(s) on severe asthma attack rates over at least 6 months, also reporting a baseline value for blood eosinophils and FeNO.

Types of participants

We will include studies on participants ages 12 and over diagnosed with asthma of any severity according to objective criteria. We will exclude patients if both the baseline blood eosinophil count and FeNO are missing. We will also exclude patients with missing follow-up duration whilst on the allocated therapy, or missing number of severe asthma attacks during follow-up.

Types of interventions

We will request IPD for the control arm(s) of each trial. We define the 'control arm' as. patients with the lowest anti-inflammatory therapy intensity after randomisation (*i.e.* group with no ICS, lowest dose ICS, or placebo).

Types of comparison conditions

Not applicable, as this is a prognostic IPD MA.

Types of outcome measures

The outcome is the occurrence of severe asthma attacks, defined as the number of acute asthma episodes requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation. This was the primary outcome in many RCTs. Severe asthma attacks are important for patients, physicians, and health insurance providers due to the high morbidity and financial burden [29]. The severe asthma attack rate is known to be modifiable following appropriate anti-inflammatory therapy in patients with high type-2 biomarkers [18,19,21]. The minimal clinically important difference for the annualised severe asthma attack rates in RCTs has not been determined, although it has been estimated to be 20-40% in a recent expert consensus document [30].

Search strategy

We will search MEDLINE (PubMed interface) for RCTs from 1 January 1993 to 1 April 2021 that fit the eligibility criteria.

Our search will use the term 'asthma exacerbations' (("asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma s"[All Fields]) AND ("exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields])), filtered for 'randomised controlled trials' 'humans' 'ages 12 and over' and languages English and French. The details of the PubMed query are listed in the Supplementary Material. Literature search results will be uploaded to Microsoft EndNote. Titles and abstracts of all records returned by the literature search will be screened to identify potentially relevant publications which include the word ‘eosinophil’ OR ‘FeNO’ OR ‘nitric oxide’ OR ‘exhaled NO’. Manual reference searching will be performed for completed clinical trials that are in press at the time of the systematic review. Two reviewers (SC and IDP) will independently review the retained publications to select trials for inclusion. We will resolve disagreement through discussion. We will record the reasons for excluding trials. Neither of the authors will be blind to the journal titles or to the study authors or institutions.

Data collection

Request for individual participant data

The authors of the retained studies will be contacted to obtain IPD. The corresponding author of each publication, and the representative(s) of the trial sponsor when applicable, will be sent an invitation letter and a skeleton Microsoft Excel spreadsheet containing the relevant fields for data extraction.

Data items

Anonymised individual patient data to be requested includes demographics (age, body mass index); baseline lung function with post-bronchodilator reversibility; treatment step according to anti-inflammatory components (Table 2); inhaled corticosteroid daily dosage; other asthma controller or reliever medications; presence of any Global Initiative for Asthma (GINA) defined risk factors (Table 1) at baseline, when available; severe asthma attack history in the year prior to trial enrolment; the intervention the patient was randomised to; the peripheral blood eosinophil count, total immunoglobulin E, specific airborne sensitisation, and FeNO at baseline; duration of follow-up under controlled therapy; and the outcome of interest, *i.e.* the number of severe asthma attacks during follow-up.

Risk of bias in individual studies

To facilitate the assessment of possible bias for each study, we will collect information using the Cochrane Collaboration tool for assessing the risk of bias [31], which covers: sequence generation, allocation concealment, blinding, incomplete outcome data (*e.g.* dropouts and withdrawals) and selective outcome reporting. For each domain in the tool, we will detail the procedures undertaken for each study, including verbatim quotes. A judgement as to the risk of bias on each of the six domains will be made from the extracted information, rated as 'high risk' or 'low risk'. If there is insufficient detail reported in the study, we will judge the risk of bias as 'unclear' and the original study investigators will be contacted for more information. These judgements will be made independently by two authors based on the criteria for judging the risk of bias [31]. Disagreements will be resolved first by discussion and then by consulting a third author for arbitration. We will compute graphic representations of potential bias within and across studies. We will consider each item in the risk of bias assessment independently without an attempt to collate and assign an overall score.

Data extraction

Data providers contacted following the systematic review will be provided sufficient time and support to confirm their consent for data extraction through data sharing contracts. Data sharing will be free of charge, financial contributions, and/or barriers to the dissemination of the results.

Data management and sharing

Secure digital transfer and storage solutions are provided by the University of Oxford. Under the terms of the data sharing agreements, access to the complete dataset is restricted to the named authors on the current study protocol who are bound by contract to the University of Oxford. Future third-party data sharing requests will need to be submitted to the original study authors.

Data analysis and synthesis

In relation with the objectives of this study, the data will be analysed and presented according to the following formats:

1. Results of the systematic review will be reported as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [32]. All identified studies will be enumerated and detailed, irrespective of the provision of individual participant data.
2. Results of the multivariable prognostic analysis will report on univariate and multivariable coefficients from binomial negative regression on the annualised severe asthma attack rates. Important predictors to be assessed are the baseline blood eosinophil count and baseline FeNO values. Reporting will be in categories according to commonly accepted cut-offs (blood eosinophils, 0.15-<0.30, ≥0.30×10^9 cells/L; FeNO, <25, 25-<50, ≥50 ppb), with more detailed modelling as continuous variables. Non-linearity will be explored with `rcs` functions, with the number of knots guided by AIC. Relations will be plotted with 95% confidence intervals (CI). Other important prognostic factors include treatment steps (as per Table 2), asthma attack history, postbronchodilator forced expiratory volume in 1 second percentage predicted, mean score on the 5-item Asthma Control Questionnaire, and body mass index. Interactions between blood eosinophil and FeNO values will be assessed according to AIC. If relevant, combined effects will be summarised in a 3×3 matrix stratified by the blood eosinophil count (<0.15, 0.15-<0.30, ≥0.30×10^9 cells/L) and FeNO

(<25, 25-<50, ≥50 ppb), and plotted in interaction plots with 95% CI. Heterogeneity in estimates between studies will be quantified by I^2 statistics.

3. Clinical prediction modelling will be based on the statistical analysis plan (version 1) presented in the Supplementary Material. Briefly, we will use the study population as a derivation cohort, with stratification by study. Validation will be according to an internal – external cross-validation procedure, where each study is left out once [33]. The selection of predictors will be based on the results of the multivariable prognostic analyses. A summary prognostic equation will be produced, assessed by the principal investigators, and adapted to GINA treatment step reference attack rates (*e.g.*: [34]) to allow for a user-friendly prediction summary table similar to the reported prototype (figure). Performance of the predictive equation and table will be assessed separately with calibration plots, c-statistic, and decision-analytic measures as outlined in the statistical analysis plan (see Supplementary Material).

Study power

Considering a mean annualised severe asthma attack of 0.6 in the entire study population and a conservative estimate that the derivation cohort will comprise 50% of the individual patient data reported in our prototype scale (0.5*3051 = 1525) [22], there should be approximately 915 events to derive a clinical prediction model. This provides for a solid basis for statistical modelling considering the limited number of potential predictors (around 10), leading a favourable event per variable (EPV) ratio (EPV=92) [35]. However, we concede that the EPV will be considerably lower for mild asthma populations, where trials identified less than 100 severe asthma attack events in their control arms [24,36]. Conversely, the study will be more than adequately powered for moderate-to-severe asthma.

Statistical software and confidence intervals

Data analysis will be conducted in collaboration with the study statistician (ES) using R software and the `rms` package. Reported outputs will present estimates and accompanying two-sided 95% CI. Bootstrap resampling will be applied to assess internal validity. Cross-validation by study will be performed to assess external validity.

Ethics and dissemination

The protocol has been reviewed by the academic ethics committee (Oxford Tropical Research Ethics Committee (OxTREC)) and found to comprise fully anonymised data not requiring further ethical approbation. The results of the systematic review, patient-level multivariable prognostic MA, and clinical prediction models will be presented in an international scientific meeting and submitted for publication.

DISCUSSION

This protocol for a systematic review and IPD MA of RCTs across the spectrum of asthma severities coincides with a clinical prediction modelling effort centred on the peripheral blood eosinophil count and FeNO. Indeed, we speculate that these two biomarkers are the airway equivalent of high blood pressure and serum cholesterol, insofar as they identify a pathological process which relates to the risk key adverse outcomes (asthma attacks) that is modifiable by treatment (anti-inflammatory medication).

The focus on two biomarkers to predict the modifiable risk of asthma attacks is novel compared to existing clinical prediction models [2-13], where prognostic variables do not include nor adjust for blood eosinophils and FeNO. The established prognostic (*i.e.* predicting adverse outcomes) and theragnostic (*i.e.* predicting treatment responsiveness) values of these type-2 inflammatory biomarkers [17–23] provide a strong basis for a clinical prediction model centred on these independent, additive, and, most importantly, modifiable risk factors. The current protocol extends our previous proof-of-concept [22] work suggesting that traditional clinical risk factors can and should be adjusted for type-2 inflammatory biomarkers. Another novel aspect of our project is our intention to collaborate with a wide variety of authors and sponsors to form an international, data-driven, and not-for-profit consortium to support the development and validation of a robust clinical prediction model.

Despite the rigorous PRISMA [32] and Cochrane [31] methodologies which will be used to identify high-quality RCTs, there are areas of potential weaknesses in our study design which warrant discussion. First, we will limit our search strategy to MEDLINE. This approach was decided after a preliminary search in MEDLINE alone showed potential for >5000 control arm patients eligible to the IPD MA component; more than required to power our multivariable prognostic assessment and sufficient to claim that the included studies will be identified systematically rather than subjectively. Second, RCTs enrolling mild asthmatics have reported low absolute severe asthma attack rates [24,36], which may limit the model's reliability for low-risk patients. Third, a RCT-based clinical prediction model will be difficult to subsequently validate in real-world settings where treatment intensity fluctuates in response to the perceived risk of asthma attacks. Such real-world fluctuation in treatment regimens may weaken the relation between static biomarker measurements and 12-month observed asthma attack rates. Nevertheless, we speculate that physician-patient discussions can be assisted by a clinical prediction model which estimates the risk of asthma attacks if anti-inflammatory treatment is not changed, *i.e.:* if the patient were randomised to the control arm of an RCT. Fourth, controlled trials in asthma are notorious for a strong placebo effect. This caveat may be due to improved adherence to ICS, the Hawthorne effect, regression to the mean, or a combination of factors [37]. It is potentially surmountable by adapting the resultant clinical prediction model using reference asthma attack rates according to treatment intensity, as previously reported in a claims-based study [34] and proposed in our statistical analysis plan. Last, we have not planned to request active arm individual participant data, thus limiting our ability to assess the individual treatment benefit [38] or model heterogeneity of treatment effects [39]. We will not pursue the active arms' data for political reasons but envision a de-centralised computation of individual treatment benefit and aggregate performance measures, such as the c-for-benefit statistic, at a later stage.

To conclude, we propose a systematic review and IPD MA to predict severe asthma attacks based on the inflammatory and clinical risk profile. Our emphasis on the risk conferred by raised type-2 inflammatory biomarkers and the consortium approach central to our endeavour and may distinguishes it from existing prediction models [2-13]. We speculate that a clinical prediction model centred on blood eosinophils and FeNO will provide a useful basis for a preventive, treatable trait-based management.

TABLE 1

Clinical risk factors for asthma exacerbations with their traditional categorisations

Risk factors	Value (if pertinent)
Poor control of asthma symptoms	mean ACQ score ≥ 1.5
Limited lung function:	
low FEV1	< 60-80% predicted
high postbronchodilator reversibility	>12% change in FEV1
Adherence poor (inadequate technique or inhaler use)	
Reliever use excessive	> one 200-dose canister/month
Intubation or ICU admission for asthma on history	
Comorbidities:	
chronic rhinosinusitis	
obesity	body mass index ≥ 35 kg/m²
psychiatric disease	psychosis, substance abuse
Environmental exposure:	
smoking	
allergen exposure in sensitised patient	
air pollution	especially high O_3 and/or NO_3

ACQ = asthma control questionnaire; FEV1 = forced expiratory volume in 1 second; ICU = intensive care unit; PoLAR ICE = mnemonic (see bold characters in table). Adapted from Global Initiative for Asthma Guidelines [1]. Where possible, risk factors will also be analysed in continuous versions with restricted cubic splines to allow for non-linear associations.

TABLE 2
Treatment step definitions

Treatment step	Definition
Step 1	As-needed short-acting beta2-agonist
Step 2	Daily low dose ICS or As-needed low dose ICS-formoterol Daily leukotriene receptor agonist
Step 3	Daily low dose ICS + an additional controller therapy
Step 4	Any medium dose ICS-containing regimen
Step 5	Any high dose ICS-containing regimen or Any maintenance systemic corticosteroid use (defined as use of systemic corticosteroids for ≥50% of the previous year)

ICS, inhaled corticosteroid. Modified from Global Initiative for Asthma 2017 and 2021 [1] guidelines.

FIGURE LEGEND

The prototype OxfoRd Asthma attaCk risk scaLE. Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days and/or hospitalisation. The blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level (FeNO) is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [22] with permission under the original CC BY public copyright license.

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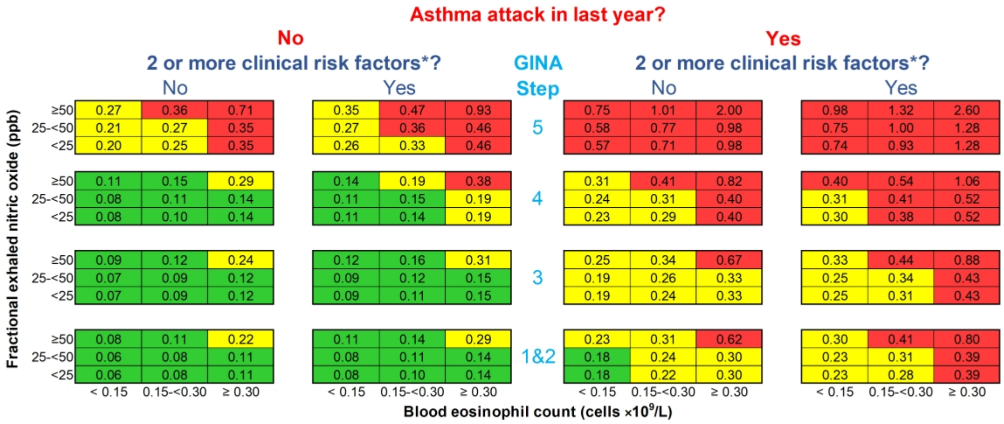

The prototype OxfoRd Asthma attaCk risk scaLE. Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days and/or hospitalisation. The blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level (FeNO) is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [22] with permission under the original CC BY public copyright license.

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Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

Simon Couillard, Ewout W Steyerberg, Richard Beasley, Ian D Pavord

Supplementary Material

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Appendix 1 – Medline search details

1.1. PubMed Search URL

https://pubmed.ncbi.nlm.nih.gov/?term=asthma+exacerbations&filter=pubt.randomizedcontrolledtrial&filter=dates.1993%2F1%2F1-2021%2F4%2F1&filter=hum_ani.humans&filter=lang.english&filter=lang.french&filter=age.adolescent&filter=age.alladult&filter=age.youngadult&filter=age.adult&filter=age.middleagedaged&filter=age.middleaged&filter=age.aged&filter=age.80andover&sort=date

1.2. PubMed Search details:

Search: asthma exacerbations Filters: Randomized Controlled Trial, Humans, English, French, Adolescent: 13-18 years, Adult: 19+ years, Young Adult: 19-24 years, Adult: 19-44 years, Middle Aged + Aged: 45+ years, Middle Aged: 45-64 years, Aged: 65+ years, 80 and over: 80+ years, from 1993/1/1 - 2021/4/1 Sort by: Most Recent

(("asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma s"[All Fields]) AND ("exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields])) AND ((randomizedcontrolledtrial[Filter]) AND (humans[Filter]) AND (1993/1/31:2021/4/1[pdat]) AND (english[Filter] OR french[Filter]) AND (adolescent[Filter] OR alladult[Filter] OR youngadult[Filter] OR adult[Filter] OR middleagedaged[Filter] OR middleaged[Filter] OR aged[Filter] OR 80andover[Filter]))

1.3. Translations

asthma: "asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma's"[All Fields]

exacerbations: "exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields]

Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

STATISTICAL ANALYSIS PLAN

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E5

1. Changes from previous version of SAP

Version number Issue date	Author of this issue	Significant changes from previous version together with reasons
V0.1_2021-06-02	Couillard	Not applicable as this is the 1st issue
V0.2_2021-06-07	Couillard and Steyerberg	Preliminary input by study statistician
V0.3_2021-08-25	Couillard	Minor changes
V0.4_2021-09-15	Couillard	Minor changes to harmonise protocol manuscript draft.
V1.0_2021-10-09	Couillard and Steyerberg	Adjustments to harmonise with final protocol manuscript

2. Background and Objectives

2.1. Background and rationale

Assessment and reduction of the risk of attacks is a major goal of asthma management [1]. However, our ability to do this is limited because the independent risk associated with clinical risk factors has not been defined, some are difficult to identify and/or modify, and others can be modified independent of an effect on asthma attacks. These limitations mean that a precise estimation of the risk of asthma attacks and the likely benefit of treatment is not possible.

Recently, five analyses of clinical trials in asthma showed that fractional exhaled nitric oxide (FeNO) and the blood eosinophil count provide additive prognostic information on the occurrence of severe asthma attacks [2–6]. The effect is large, with a three-fold greater rate ratio for asthma attacks seen in patients with FeNO ≥50 ppb and blood eosinophils $\geq 0.3\times10^9$/L compared to those with a FeNO <25 ppb and blood eosinophils $<0.15\times10^9$/L [7]. The excess risk of asthma attacks associated with the highest biomarker combination compared to the lowest was effectively removed by low-dose inhaled corticosteroids (ICS) in mild asthma [6], an increased dosage of ICS in moderate asthma [5], and biologics in severe asthma [4,8].

These findings suggest that the blood eosinophil count and FeNO could form the basis of a risk scale analogous to those that have had a large impact in cardiovascular medicine [9,10]. We have previously explored this hypothesis by developing a prototype scale (figure) which showed reasonable agreement between the observed and predicted asthma attack rates in the derivation trial-level data [7]. The prototype scale showed feasibility and potential to predict asthma attacks which can be prevented by treatment.

Asthma attack in last year?

GINA Step	Fractional exhaled nitric oxide (ppb)	No / 2 or more clinical risk factors*? No: < 0.15	No / No: 0.15-<0.30	No / No: ≥ 0.30	No / Yes: < 0.15	No / Yes: 0.15-<0.30	No / Yes: ≥ 0.30	Yes / 2 or more clinical risk factors*? No: < 0.15	Yes / No: 0.15-<0.30	Yes / No: ≥ 0.30	Yes / Yes: < 0.15	Yes / Yes: 0.15-<0.30	Yes / Yes: ≥ 0.30
5	≥50	0.27	0.36	0.71	0.35	0.47	0.93	0.75	1.01	2.00	0.98	1.32	2.60
5	25-<50	0.21	0.27	0.35	0.27	0.36	0.46	0.58	0.77	0.98	0.75	1.00	1.28
5	<25	0.20	0.25	0.35	0.26	0.33	0.46	0.57	0.71	0.98	0.74	0.93	1.28
4	≥50	0.11	0.15	0.29	0.14	0.19	0.38	0.31	0.41	0.82	0.40	0.54	1.06
4	25-<50	0.08	0.11	0.14	0.11	0.15	0.19	0.24	0.31	0.40	0.31	0.41	0.52
4	<25	0.08	0.10	0.14	0.11	0.14	0.19	0.23	0.29	0.40	0.30	0.38	0.52
3	≥50	0.09	0.12	0.24	0.12	0.16	0.31	0.25	0.34	0.67	0.33	0.44	0.88
3	25-<50	0.07	0.09	0.12	0.09	0.12	0.15	0.19	0.26	0.33	0.25	0.34	0.43
3	<25	0.07	0.09	0.12	0.09	0.11	0.15	0.19	0.24	0.33	0.25	0.31	0.43
1&2	≥50	0.08	0.11	0.22	0.11	0.14	0.29	0.23	0.31	0.62	0.30	0.41	0.80
1&2	25-<50	0.06	0.08	0.11	0.08	0.11	0.14	0.18	0.24	0.30	0.23	0.31	0.39
1&2	<25	0.06	0.08	0.11	0.08	0.10	0.14	0.18	0.22	0.30	0.23	0.28	0.39

Blood eosinophil count (cells ×10^9/L)

FIGURE 1. Prototype Oxford Asthma Attack Risk ScaLE (ORACLE).

Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days. Blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [7].

3. Objectives and Outcomes

3.1.1. Hypothesis

We hypothesise that the blood eosinophil count and FeNO could form the basis of a robust and useful prediction model; we speculate that these two biomarkers are the airway equivalent of high blood pressure and serum cholesterol, insofar as they identify a pathological process which relates to the risk of adverse outcome (asthma attacks) that is modifiable by treatment (anti-inflammatory medication).

3.1.2. Objective

To develop and validate a clinical prediction model for the absolute number of severe asthma attacks to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other risk factors assessed at baseline.

3.1.3. Outcome to predict

The outcome to predict was the absolute number of severe asthma attacks to occur in the following 12 months. Severe asthma attacks are defined as acute asthma episodes requiring treatment with systemic steroids for 3 or more days and/or hospitalisation [11].

3. Study Details

This is the statistical analysis plan for the meta-analysis of individual participant data collected following a pre-specified systematic review protocol [12].

3.1. Study population

We will search MEDLINE (PubMed interface) for randomised controlled trials (RCT) from 1 January 1993 to 1 April 2021 that investigated the effect of fixed treatment(s) regimen(s) on severe asthma attack rates over at least 6 months, also reporting a baseline value for blood eosinophils and FeNO [12].

The included RCT control arm data will be analysed to develop a risk scale to predict asthma attacks. We will focus on risk which is known to be modifiable by treatment. This modifiable excess risk relates to two surrogate measures of airway inflammation (biomarkers): the peripheral blood eosinophil count and FeNO. The contribution of other less modifiable and non-modifiable risk factors defined by the current Global Initiative for Asthma guidelines [1] will also be assessed.

3.2. Study population

Following the preliminary systematic review, we identified 19 records comprising 23 independent RCTs [5,8,13–29].

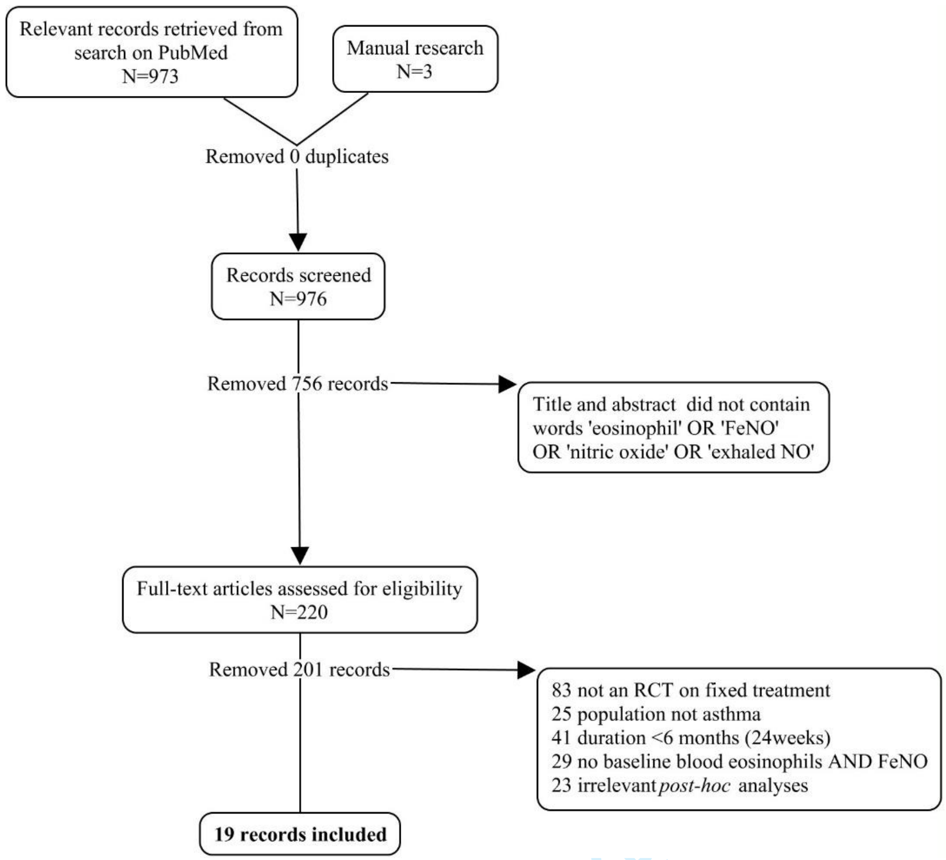

FIGURE 2. PRISMA flowchart of the preliminary results from the systematic review pre-specified in [12]

We will request data from the trial investigators and/or sponsors for patients diagnosed with asthma ages 12 and over that were randomised to the control arm (*i.e.* no ICS, lowest dose ICS, or placebo). The requested dataset will be functionally anonymised by design. The planned analysis pertains to the intention-to-treat population, modified to respect the inclusion criteria defined below.

3.2.1. <u>Inclusion criteria</u>

To be included, patients need to respect the following criteria:

- Asthma diagnosed according to the Global Initiative for Asthma (GINA) guideline-defined criteria (any severity)[1].
- 12 years of age or older
- Randomised to the control arm of the included study (*i.e.* placebo or no change in anti-inflammatory therapy).
- Data available for the following variables:
  - Peripheral blood eosinophil count ($x10^9/L$) at baseline
  - FeNO (ppb) at baseline
  - Sufficient information on the patients' medication to determine the treatment step (*i.e.* disease severity)(see section 3.1.4, table 2)[1].
  - Number of severe asthma attacks in the 12 months previous to the baseline visit. Severe asthma attacks are defined as acute asthma requiring ≥3 days of systemic corticosteroid therapy and/or hospitalisation.
  - Duration of the controlled treatment period (days)
  - Number of severe asthma attacks observed during the study period.

3.2.2. <u>Exclusion criteria</u>

We will exclude patients if both baseline blood eosinophil count and baseline FeNO are missing. We will also exclude patients with missing follow-up duration whilst on the allocated therapy, or missing number of severe asthma attacks during follow-up.

3.3. Cross-validation by study to assess external validity

The study population will be used for derivation and subsequent validation, stratifying by source RCT in cross-validation by study design, where each study serves as a validation set once [30].

3.4. Sources of data for complimentary external validation

The follow sources of data will be used for external validation:

i) cross-validation by study is the initial external validation procedure that will be performed in the meta-analysis population;

ii) observational prospective cohorts envisioned to contribute to later external validation are the Novelty cohort [31]; the outpatient general practice cohort derived from the Optimum Patient Care Research Database [32]; and any other RCTs or cohorts that do not share their data to a central repository.

4. Primary and secondary variables

4.1. General definitions

4.1.1. <u>Definition of baseline</u>

In general, the last non-missing measurement on or prior to the date of randomisation will serve as the baseline measurement for predictors.

4.1.2. <u>Duration of the controlled treatment period</u>

The controlled treatment period for the assessment of severe asthma attacks starts at the date of randomisation and ends at the minimum (date of last dose of placebo + appropriate wash-out period as per source RCT protocol, date of death, date of study withdrawal).

4.1.3. Concomitant medication

Medications taken by the subject at any time during the controlled treatment period will be used to define the treatment step. Concomitant medications during the controlled treatment period which are recorded are defined in section 2.3 (study variables).

4.1.4. Treatment step

A modified version of the 2017 and 2021 GINA guidelines definitions will be used to determine treatment step.

TABLE 2
Modified treatment step definitions for this study

Treatment step	Definition
Step 1	As-needed short-acting beta2-agonist
Step 2	Daily low dose ICS or As-needed low dose inhaled corticosteroid (ICS)-formoterol Daily leukotriene receptor agonist
Step 3	Daily low dose ICS + an additional controller therapy
Step 4	Any medium dose ICS-containing regimen
Step 5	Any high dose ICS-containing regimen or Any maintenance systemic corticosteroid use (defined as use of systemic corticosteroids for ≥50% of the previous year)

Modified from GINA 2017 and 2021 [1] guidelines.

4.1.5. Calculation of inhaled corticosteroid (ICS) dosing

ICS-dose strength will be determined using the following table, retained from the 2021 GINA guidelines:

TABLE 3

Low, medium and high daily metered doses of inhaled corticosteroids in adults and adolescents (12 years and older)

Inhaled corticosteroid	Total daily ICS dose (mcg)		
	Low	Medium	High
Beclomethasone dipropionate CFC-propellent MDI	200-500	>500-1000	>1000
Beclomethasone dipropionate extrafine particle MDI or DPI	100-200	>200-400	>400
Budesonide	200-400	>400-800	>800
Fluticasone dipropionate	100-250	>250-500	>500
Fluticasone furoate	100	100	200
Ciclesonide	80-160	>160-320	>320
Mometasone furoate	200-400	200-400	>400

Adapted from [1]. CFC, chlorofluorocarbon; DPI, dry powder inhaler; MDI, multidose inhaler.

The following ICS dose equivalence table will be used to characterise patients' concomitant ICS use:

TABLE 4
Equivalent doses between inhaled corticosteroids

Inhaled corticosteroid type	**Equivalent dose**
Beclomethasone dipropionate CFC-propellent MDI	1 mcg
Beclomethasone dipropionate HFA or DPI	2.5 mcg
Budesonide	1.25 mcg
Fluticasone dipropionate	2.5 mcg
Fluticasone furoate	5 mcg
Ciclesonide	3.125 mcg
Mometasone furoate	2.27 mcg
Triamcinolone acetonide	0.5 mcg

Adapted from [1].

4.2. Primary variable and study endpoint

The effect to measure and predict is number of severe asthma attacks (defined as acute asthma requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation)[11] to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other prognostic actors assessed at baseline.

The start of an exacerbation is defined as the start date of systemic corticosteroids, emergency room (ER), urgent care (UC) visits, or hospital admissions due to asthma, whichever occurs earlier. The end date is defined as the last day of systemic corticosteroids or ER/UC/hospital discharge, whichever occurs later.

Two or more exacerbations with the same start date and end date will be counted as one exacerbation for the purposes of calculating the number and duration of exacerbations for a subject. In the case that one or more exacerbations are recorded as starting or ending during another exacerbation, these will be counted as one exacerbation, using the earliest exacerbation start date and the latest exacerbation stop date to calculate duration.

Additional systemic corticosteroid treatments, ER visit or UC visit requiring use of systemic corticosteroids, or hospital admission will not be regarded as a new exacerbation. To be counted as a new exacerbation it must be preceded by at least 7 days in which neither criterion is fulfilled. If the end date of the first exacerbation and the start date of the second exacerbation are less than 7 days apart, then these will be counted as one exacerbation.

The number of days the subject experiences a protocol defined exacerbation, including the subsequent 7 days (when a further exacerbation would not be considered as a second exacerbation), will be subtracted from the time at risk defined above for the primary analysis. For example, if a subject has a single exacerbation which lasts 4 days then 7 + 4 =11 days will be subtracted from the time at risk.

4.3. Subgrouping for biomarker-stratified clinical prediction modelling

3.1.1. Biomarker-stratified subgroups

The main multivariable prognostic modelling analysis will use continuous values of the blood eosinophil count, FeNO, and other clinical risk factors (table 1). If relevant, combined effects will be summarised in a 3×3 matrix stratified by the blood eosinophil count (<0.15, 0.15-<0.30, ≥0.30×109 cells/L) and FeNO (<25, 25-<50, ≥50 ppb), and plotted in interaction plots with 95% confidence intervals (CI). Heterogeneity in estimates between studies will be quantified by I^2 statistics. Additional analyses will consider continuous versions of predictors with restricted cubic splines [33].

3.1.2. Comparative subgroup rate ratio analysis

If relevant following analyses on continuous data, crude and adjusted rate ratios of the annualised severe exacerbation rate for each of the 9 categories (3×3 matrix according to the blood eosinophil count (<0.15, 0.15-<0.30, ≥0.30×10^9 cells/L) and FeNO (<25, 25-<50, ≥50 ppb) will be determined. Rate ratios for each subgroup are calculated as the weighted annualised exacerbation rate for the selected subgroup divided by the mean for the remainder of the matrix, weighted by patient-years of data. The adjusted rate ratios will account for asthma severity (treatment step), history of asthma attacks (≤1 or >1 in previous 12 months); as well as age, sex, and source RCT to control for unsuspected confounding factors relating to the three latter variables.

The potentially relevant clinical risk factors for asthma attacks listed in section 3.4 will be assessed using a bootstrapped backward stepwise selection procedure during regression analysis in a random effects model. Key predictors are: blood eosinophils, FeNO, treatment step and the past history of exacerbations (0 or ≥1 in previous 12 months).

4.4. Potential clinical predictors

The following variables will be assessed as potential clinical predictors, in addition to the forced variables (treatment step, past history of exacerbations (<1 or ≥1 in previous 12 months), age, sex, and source RCT).

- Ethnicity: categorical
- Comorbidities: categorical (list of comorbidities following the Charlson comorbidity index [34])
- Socioeconomic status (anonymised and operationalised depending dataset)

- Body mass index: continuous
- Postbronchodilator (BD) FEV1, as % predicted (or preBD if no postBD): continuous
- % change in FEV1 post-bronchodilator (calculated as (FEV1 post BD minus FEV1 preBD in litres) divided by FEV1 preBD in litres: continuous
- FEV1/FVC ratio, calculated as FEV1 postBD in litres divided by FVC postBD in litres (or using preBD values if no postBD)
- Smoking status (current, ex-, passive, never-smokers): categorical
- Airborne allergies reported (yes/no): categorical
- Allergy testing positive (yes/no): categorical
- Chronic rhinosinusitis (yes/no): categorical
- Nasal polyposis (yes/no): categorical
- Adherent to medications (operationalised definition depending on the dataset): continuous (or categorical if not feasible to operationalise in a continuous variable)
- Inhalers prescribed:
  - ICS: categorical (yes/no)
  - ICS daily equivalent dose (continuous)
  - Short-acting beta2-agonist (yes/no) and number of actuations used per month (continuous)
  - Long-acting beta2-agonist (yes/no)
  - Long-acting muscarinic antagonist (yes/no)
  - Leukotriene receptor antagonist (yes/no)
  - Theophylline or aminophylline (yes/no)
- On maintenance oral corticosteroids (OCS) (yes/no): categorical

- Severe exacerbation in the preceding 12 months (defined as an acute event requiring ≥3 days of systemic corticosteroids and/or hospitalisation): yes/no category and continuously by number of episodes in preceding 12 months.
- Previous intensive care or intubation for airways disease (yes/no): categorical
- Asthma control questionnaire (ACQ) score (or asthma control test (ACT) or any other standardised symptom score test if ACQ not available): continuous (ACQ or ACT) and categorical (according to established cut points for uncontrolled disease: ACQ ≥1.5 or ACT <20)

4.5. Missing values

Missing values will be assessed for their mechanism (missing completely at random, missing at random or missing not at random) by the main investigators in conjunction with the study statistician. When data is missing at random, 10 complete datasets will be generated by multiple imputation.

4.6. Heterogeneity assessment

The variability between studies will be quantified in a random effect analysis and quantified with I^2 statistics.

4.7. Optimism correction

The adjusted biomarker-stratified and clinical predictors' incidence rate ratios will be corrected for overoptimistic predictions. Penalty terms will be used and/or linear shrinkage factors, as estimated from cross-validation and/or bootstrap resampling procedures as implemented in `rms` and `glmnet` libraries for R.

4.8. Statistical software and confidence intervals

Data analysis will be conducted in collaboration with the study statistician (ES) using R software. Estimates will be accompanied by two-sided 95% CI.

4. Clinical prediction model presentation formats

A summary prognostic equation will be produced, assessed by the principal investigators, and adapted to previously reported GINA treatment step reference attack rates [35] to allow for a user-friendly prediction summary chart similar to the reported prototype (figure 1).

5. Performance evaluation

5.1. General performance measures

The resultant prognostic equation and chart will be assessed in the validation cohorts defined in section 2.4. Discrimination will be evaluated. Calibration plots will be created with focus on centiles of risk (10^{th}, 50^{th} and 90^{th} of the distribution of predicted attack rates), and summary measures of the plot will be computed. Sensitivity, specificity and receiving operating characteristic (ROC) analyses of the model will be assessed. Reliability will be evaluated using the intraclass correlation coefficient (two-ways mixed model for absolute agreement, single measures, with 95% CI). Calibration will be assessed graphically, with characterization of calibration in the large by a calibration intercept, and overall prognostic strength by the calibration slope. Discrimination will be assessed by the c-statistic, and clinical utility by Net Benefit plotted in decision curves.

5.2. Subgroup performance measures

The performance of the resultant chart will be evaluated across the selected clinical predictors (composite biomarker category; treatment step; asthma attack history; retained clinical risk factors) as stated in section 4.1 for each subsection of the chart in each of the validation cohorts. In effect, assuming the final chart resembles the prototype (figure 1), this will result in performance assessment for each of the 16 subsections and/or each of the 144 squares, depending on the validation cohort size.

6. Study power

Considering a mean annualised severe asthma attack of 0.6 in the entire study population and a conservative estimate that the derivation cohort will comprise 50% of the individual patient data reported in our prototype scale (0.5*3051 = 1525) [7], there should be approximately 915 events to derive a clinical prediction model. With a target maximum of 10 prediction variables, the event per variable (EPV) number is 92; well over the recommended 10-20 EPV [36]. However, we concede that the EPV will be considerably lower for mild asthma populations, where trials identified less than 100 severe asthma attack events in their control arms [21,23]. Conversely, the study will be more than adequately powered for moderate-to-severe asthma.

Strengths and limitations of our approach

6.1. Strengths

- The study design and its objective – to derive and validate a clinical prediction tool based on biomarkers of type-2 inflammation – fulfils an unmet clinical need. We speculate that a risk stratification strategy centred on modifiable type-2 airway inflammation rather than difficult-to-modify clinical characteristics would facilitate better treatment decisions by providing a framework for a preventive, treatable trait-based management.

- A proof-of-concept evaluation of this project has already been completed and shows feasibility and potential to predict asthma attacks which can be prevented by treatment [7] (Figure 1).
- Study selection bias is reduced via the pre-specified systematic review approach.
- Adequate study power. As stated above, with an estimated overall attack rate equal to that reported in the prototype scale (0.6 attacks per year) and a conservative estimate of individual participant data provided (50% of the prototype study population), there should ample events observed for model derivation validation.
- Detection bias of the outcome of interest (severe asthma attacks) is minimised by its rigorous monitoring and documentation in the context of RCTs.
- In addition to the cross-validation by study [30], we plan to validate the resultant chart in different validation cohorts: a part of the base RCT population, Novelty [31] and the Optimum Patient Care Research Database [32].

6.2. Limitations

- Many of the included RCT study populations were positively selected to be at high risk of asthma attacks, and trials enrolling mild asthmatics have reported low asthma attack rates ; this may result in the model overestimating the risk of events and underperforming in mild asthma.
- The assumption at the basis of our approach is that the type-2 biomarkers blood eosinophils and FeNO carry additive and independent predictive value for the risk of asthma attacks at all disease severities. It is unclear if FeNO exerts a similar predictive value in mild asthma [6]. This modification of risk will be addressed by statistical interaction terms.
- There is no clear reference for treatment step asthma attack rates adapted for the most recent GINA 2021 guidelines; it is possible we will need to model around the previously reported GINA 2017 classification reference asthma attack rates [35].
- We suspect that some of the important clinical risk factors emphasised by current management guidelines [1] will not be present in the RCT population (*e.g.* nonadherence is usually an exclusion criteria; salbutamol over-use is not always reported).
- Controlled trial populations in asthma are notorious for a strong placebo effect and do not necessarily reflect clinical practice, where treatment fluctuates according to the perceived or observed risk of asthma attacks; this may impact external validation in observational cohorts.

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Manuscript page (of 29 page Word document)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	p.1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	p.1
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	p.1 + **p.5**
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	p.1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	p.2
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	p.2
Sponsor	5b	Provide name for the review funder and/or sponsor	p.2
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	p.2
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	p.7
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	p.8
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	p.10-12
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	p.11-12
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	p.11-12 + Appendix 1

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	p.11-12
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	p.11-12
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	p.12
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	p.12
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	p.11 + p.12
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	p.13
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	p.13-15 + Appendix 2
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	p.13-15 + Appendix 2
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	p.13-15 + Appendix 2
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	N/A
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	N/A

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Immunology (including allergy), Epidemiology, Diagnostics, General practice / Family practice
Keywords:	Asthma < THORACIC MEDICINE, Immunology < THORACIC MEDICINE, Epidemiology < THORACIC MEDICINE, Thoracic medicine < INTERNAL MEDICINE, PREVENTIVE MEDICINE

TITLE: Blood Eosinophils, Fractional Exhaled Nitric Oxide, and the Risk of Asthma Attacks in Randomised Controlled Trials: Protocol for a Systemic Review and Control Arm Patient-Level Meta-Analysis for Clinical Prediction Modelling

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Registration: In accordance with reporting guidelines, the systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 23 March 2021 and was last updated on 23 June 2021 (registration number CRD42021245337)

Word count: 2781/4000

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- For the purpose of Open Access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.
- Ethics: the protocol has been reviewed by the Oxford Tropical Research Ethics Committee and found to comprise fully anonymised data not requiring further ethical approbation.

Patient and public involvement: None.

Author contributions: SC drafted the protocol and participated in the systematic review literature search, contacted relevant data providers and will participate in data extraction and analysis. ES will provide statistical expertise and support. RB provided insight on the study design and contributed to manuscript writing. IDP is the guarantor of this publication, contributed to the writing of the protocol manuscript, participated in the systematic review literature search, and approved the final manuscript. All authors reviewed and approved the final manuscript.

Author declaration of conflicts of interest

SC: received a non-restricted research grant from Sanofi-Genzyme for investigator-initiated type 2 innovation research and received speaker honoraria from GlaxoSmithKline, Sanofi-Regeneron and AstraZeneca; outside the submitted work. **ES:** receives royalties from Springer for the textbook entitled Clinical Prediction Models and received speaker honoraria from GlaxoSmithKline; outside the submitted work. **RB** has received honoraria for presentations or consulting in Adboards from AstraZeneca, Asthma and Respiratory Foundation of New Zealand, Avillion, Cipla and Theravance; and research grants from AstraZeneca, CureKids (NZ), Genentech, and the Health Research Council of New Zealand. **IDP**: In the last 5 years, IP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim, Aerocrine AB, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini, and GSK, and payments for organising educational events from AstraZeneca, GSK, Sanofi/Regeneron, and Teva. He has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AstraZeneca, Boehringer Ingelheim, GSK, Novartis, Teva, Merck, Circassia, Chiesi, and Knopp, and payments to support FDA approval meetings from GSK. He has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, AstraZeneca, Teva, and Chiesi. He has received a grant from Chiesi to support a phase 2 clinical trial in Oxford. He is co-patent holder of the rights to the Leicester Cough Questionnaire and has received payments for its use in clinical trials from Merck, Bayer, and Insmed. In 2014-2015 he was an expert witness for a patent dispute involving AstraZeneca and Teva.

ABSTRACT:

Introduction: The reduction of the risk of asthma attacks is a major goal of guidelines. The fact that type-2 inflammatory biomarkers identify a higher risk, anti-inflammatory responsive phenotype is potentially relevant to this goal. We aim to quantify the relation between blood eosinophils, exhaled nitric oxide (FeNO) and the risk of severe asthma attacks.

Methods and Analysis: A systematic review of randomised controlled trials (RCTs) will be conducted by searching MEDLINE from January 1993 to April 2021. We will include RCTs that investigated the effect of fixed treatment(s) regimen(s) on severe asthma exacerbation rates over at least 24 weeks and reported a baseline value for blood eosinophils and FeNO. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, and the methodological appraisal of the studies will be assessed by the Cochrane Risk-of-Bias Tool for RCTs. Study authors will be contacted to request anonymised individual participant data for patients randomised to the trial's control arm. An individual participant data meta-analysis will be performed for multivariable prognostic modelling with performance assessment (calibration plots and the c-statistic) in a cross-validation by study procedure. The outcome to predict is the absolute number of severe asthma attacks to occur in the following 12 months if anti-inflammatory therapy is not changed (*i.e.:* annualised number of attacks requiring ≥3 days of systemic corticosteroids and/or hospitalisation if the patient was randomised to the control arm of an RCT). A summary prognostic equation and risk stratification chart will be reported as a basis for further analyses of individualized treatment benefit.

Ethics and Dissemination: The protocol has been reviewed by the relevant Oxford academic ethics committee and found to comprise fully anonymised data not requiring further ethical

approbation. Results will be communicated in an international meeting and submitted to a peer-reviewed journal.

Registration: PROSPERO CRD42021245337.

Word count: 300/300

ARTICLE SUMMARY

Strengths and limitations of this study

- The prognostic (*i.e.* predicting adverse outcomes) and theragnostic (*i.e.* predicting treatment responsiveness) values of type-2 inflammatory biomarkers are established; we thus speculate that a clinical prediction model centred on blood eosinophils and exhaled nitric oxide will provide a useful framework for a preventive, treatable trait-based management.
- This systematic review and individual patient data (IPD) level meta-analysis of randomised controlled trials (RCTs) across the spectrum of asthma severities will support clinical decision-making based on type-2 inflammatory biomarkers and other clinical prognostic factors.
- We aim to include data from a substantial number of RCTs (N>10) for a large number of patients in total (n>5000), which allows for reliable statistical modelling (internal validity) and assessment of transportability across settings (external validity).
- The participating studies' authors and sponsors will form an international, collaborative, and not-for-profit consortium to allow efficient use of high-quality IPD.

- Potential weaknesses are the low number of events reported in RCTs enrolling mild asthmatics and the absence of active arm IPD.

Word count: 5 / 5 one-sentence bullet points

ABBREVIATIONS

CI: confidence intervals

FeNO: fractional exhaled nitric oxide

GINA: Global Initiative for Asthma

ICS: inhaled corticosteroid

IL: interleukin

IPD: individual participant data

MA: meta-analysis

RCT: randomised controlled trial

INTRODUCTION

Reduction of the risk of severe asthma attacks is a major goal of asthma management [1]. The current recommendation is to perform risk assessment based on a history of a previous asthma attack and a list of clinical risk factors (Table 1) [1]. However, many of these prognostic factors are unmodifiable or difficult to modify and a key risk factor (treatment adherence) is difficult to identify and quantify before starting treatment. In contrast, some risk factors are modifiable, such as symptoms and lung function, while they are not necessarily on the causal pathway of asthma attacks. As a result of these deficiencies, risk quantification in asthma is an inexact art and the impact of treatment is difficult to predict [2–13].

One approach to targeted risk reduction is to use a scale centred on readily available prognostic factors that quantify the risk of the adverse outcome of interest in a manner which also predicts the benefits of preventative treatment. This approach has been successful in cardiovascular disease risk reduction where charts [14,15] focus on modifiable factors such as blood pressure and cholesterol with age and gender as key prognostic demographic factors. We speculate that a similar framework can be applied to predict asthma attacks in patients with asthma.

Type-2 airway inflammation is important in the pathogenesis of many asthma attacks [16] where this immune response characterised by interleukin (IL)-4, IL-5, IL-13, and eosinophilic airway infiltration forms a distinct clinical phenotype [16]. In clinic, the actions of type-2 immunity are readily identified by two independent, complementary, and accessible biomarkers: the peripheral blood eosinophil count and fractional exhaled nitric oxide (FeNO) [17–24]. Importantly, the excess risk conferred by raised type-2 biomarkers can be removed with appropriate treatment [24], be it low-dose inhaled corticosteroids (ICS) in mild asthma [19,25], a higher dose of ICS in moderate asthma [21,26], or biological agents targeting type 2 cytokines in moderate and severe asthma [18,27–29]. In effect, blood eosinophils and FeNO have emerged as 'treatable traits' [30].

We have previously established a proof-of-concept biomarker-stratified asthma attack scale using publication-level data which is promising and potentially useful to support clinical decision-making [23]. The prototype lacked detailed and statistically robust assessment of multivariable prognostic relations and systematic assessment of external validity, which is possible with an individual participant data (IPD) meta-analysis (MA).

Review question

In people ≥ 12 years old diagnosed with asthma of any severity randomised to the control arm of a clinical trial, what is the annualised rate of severe asthma attacks (defined as acute asthma requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation)[31] to occur in relation to their peripheral blood eosinophil count, FeNO, and other prognostic factors at baseline?

Objectives

Specific aims of this systematic review are

1. To systematically identify randomised controlled trials (RCTs) in people ≥ 12 years old diagnosed with asthma of any severity which measured i) the peripheral blood eosinophil count and FeNO at baseline and ii) assessed the incident severe asthma attacks over ≥ 24 weeks of follow-up.
2. To perform an IPD MA for the participants randomised to the control arms (defined as no inhaled corticosteroid (ICS), lowest dose ICS, or placebo) of the RCTs identified in aim 1.

3. To assess the multivariable prognostic relations of the peripheral blood eosinophil count, FeNO, and other risk factors assessed at baseline.
4. To develop and validate a clinical prediction model for the absolute number of severe asthma attacks to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other risk factors at baseline.

METHODS AND ANALYSIS

Eligibility

Types of studies

In keeping with the objectives of the systematic review, we will include RCTs completed between 1 January 1993 and 1 April 2021 that investigated the effect of fixed treatment(s) regimen(s) on severe asthma attack rates over at least 6 months, also reporting a baseline value for blood eosinophils and FeNO.

Types of participants

We will include studies on participants ages 12 and over diagnosed with asthma of any severity according to objective criteria. We will exclude patients if both the baseline blood eosinophil count and FeNO are missing. We will also exclude patients with missing follow-up duration whilst on the allocated therapy, or missing number of severe asthma attacks during follow-up.

Types of interventions

We will request IPD for the control arm(s) of each trial. We define the 'control arm' as. patients with the lowest anti-inflammatory therapy intensity after randomisation (*i.e.* group with no ICS, lowest dose ICS, or placebo).

Types of comparison conditions

Not applicable, as this is a prognostic IPD MA.

Types of outcome measures

The outcome is the occurrence of severe asthma attacks, defined as the number of acute asthma episodes requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation. This was the primary outcome in many RCTs. Severe asthma attacks are important for patients, physicians, and health insurance providers due to the high morbidity and financial burden [31]. The severe asthma attack rate is known to be modifiable following appropriate anti-inflammatory therapy in patients with high type-2 biomarkers [18,19,21]. The minimal clinically important difference for the annualised severe asthma attack rates in RCTs has not been determined, although it has been estimated to be 20-40% in a recent expert consensus document [32].

Search strategy

We will search MEDLINE (PubMed interface) for RCTs from 1 January 1993 to 1 April 2021 that fit the eligibility criteria.

Our search will use the term 'asthma exacerbations' (("asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma s"[All Fields]) AND ("exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields])), filtered for 'randomised controlled trials' 'humans' 'ages 12 and over' and languages English and French. The details of the PubMed query are listed in the Supplementary Material. Literature search results will be uploaded to Microsoft EndNote. Titles and abstracts of all records returned by the literature search will be screened to identify potentially relevant publications which include the word 'eosinophil' OR 'FeNO' OR 'nitric oxide' OR 'exhaled NO'. Manual reference searching will be performed for completed clinical trials that are in press at the time of the systematic review. Two reviewers (SC and IDP) will independently review the retained publications to select trials for inclusion. We will resolve disagreement through discussion. We will record the reasons for excluding trials. Neither of the authors will be blind to the journal titles or to the study authors or institutions.

Data collection

Request for individual participant data

The authors of the retained studies will be contacted to obtain IPD. The corresponding author of each publication, and the representative(s) of the trial sponsor when applicable, will be sent an invitation letter and a skeleton Microsoft Excel spreadsheet containing the relevant fields for data extraction.

Data items

Anonymised individual patient data to be requested includes demographics (age, body mass index); baseline lung function with post-bronchodilator reversibility; treatment step according to anti-inflammatory components (Table 2); inhaled corticosteroid daily dosage; other asthma controller or reliever medications; presence of any Global Initiative for Asthma (GINA) defined risk factors (Table 1) at baseline, when available; severe asthma attack history in the year prior to trial enrolment; the intervention the patient was randomised to; the peripheral blood eosinophil count, total immunoglobulin E, specific airborne sensitisation, and FeNO at baseline; duration of follow-up under controlled therapy; and the outcome of interest, *i.e.* the number of severe asthma attacks during follow-up.

Risk of bias in individual studies

To facilitate the assessment of possible bias for each study, we will collect information using the Cochrane Collaboration tool for assessing the risk of bias [33], which covers: sequence generation, allocation concealment, blinding, incomplete outcome data (*e.g.* dropouts and withdrawals) and selective outcome reporting. For each domain in the tool, we will detail the procedures undertaken for each study, including verbatim quotes. A judgement as to the risk of bias on each of the six domains will be made from the extracted information, rated as 'high risk' or 'low risk'. If there is insufficient detail reported in the study, we will judge the risk of bias as 'unclear' and the original study investigators will be contacted for more information. These judgements will be made independently by two authors based on the criteria for judging the risk of bias [33]. Disagreements will be resolved first by discussion and then by consulting a third author for arbitration. We will compute graphic representations of potential bias within and across studies. We will consider each item in the risk of bias assessment independently without an attempt to collate and assign an overall score.

Data extraction

Data providers contacted following the systematic review will be provided sufficient time and support to confirm their consent for data extraction through data sharing contracts. Data sharing will be free of charge, financial contributions, and/or barriers to the dissemination of the results.

Data management and sharing

Secure digital transfer and storage solutions are provided by the University of Oxford. Under the terms of the data sharing agreements, access to the complete dataset is restricted to the named authors on the current study protocol who are bound by contract to the University of Oxford. Future third-party data sharing requests will need to be submitted to the original study authors.

Data analysis and synthesis

In relation with the objectives of this study, the data will be analysed and presented according to the following formats:

1. Results of the systematic review will be reported as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [34]. All identified studies will be enumerated and detailed, irrespective of the provision of individual participant data.
2. Results of the multivariable prognostic analysis will report on univariate and multivariable coefficients from negative binomial regression on the annualised severe asthma attack rates. Important predictors to be assessed are the baseline blood eosinophil count and baseline FeNO values. Reporting will be in categories according to commonly accepted cut-offs (blood eosinophils, 0.15-<0.30, ≥0.30×10^9 cells/L; FeNO, <25, 25-<50, ≥50 ppb), with more detailed modelling as continuous variables. Non-linearity will be explored with `rcs` functions, with the number of knots guided by AIC. Relations will be plotted with 95% confidence intervals (CI). Other important prognostic factors include treatment steps (as per Table 2), asthma attack history, postbronchodilator forced expiratory volume in 1 second percentage predicted, mean score on the 5-item Asthma Control Questionnaire, and body mass index; potential predictors are listed fully in the statistical analysis plan version 1.1, section 4.4 (Online Supplementary Material). Interactions between blood eosinophil and FeNO values will be assessed according to AIC. If relevant, combined effects will be summarised in a 3×3 matrix stratified by the blood eosinophil count (<0.15, 0.15-<0.30,

≥$0.30×10^9$ cells/L) and FeNO (<25, 25-<50, ≥50 ppb), and plotted in interaction plots with 95% CI. Heterogeneity in estimates between studies will be quantified by I^2 statistics.

3. Clinical prediction modelling will be based on the statistical analysis plan (version 1.1) presented in the Supplementary Material. Briefly, we will use the study population as a derivation cohort, with stratification by study. Validation will be according to an internal – external cross-validation procedure, where each study is left out once [35]. The selection of predictors will be based on the results of the multivariable prognostic analyses. A summary prognostic equation will be produced, assessed by the principal investigators, and adapted to GINA treatment step reference attack rates (*e.g.*: [36]) to allow for a user-friendly prediction summary table similar to the reported prototype (figure). Performance of the predictive equation and table will be assessed separately with calibration plots, c-statistic, and decision-analytic measures as outlined in the statistical analysis plan (see Supplementary Material).

Study power

Considering a mean annualised severe asthma attack of 0.6 in the entire study population and a conservative estimate that the derivation cohort will comprise 50% of the individual patient data reported in our prototype scale (0.5*3051 = 1525) [23], there should be approximately 915 events to derive a clinical prediction model. This provides for a solid basis for statistical modelling considering the limited number of potential predictors (around 10), leading a favourable event per variable (EPV) ratio (EPV=92) [37]. However, we concede that the EPV will be considerably lower for mild asthma populations, where trials identified less than 100 severe asthma attack events in their control arms [25,38]. Conversely, the study will be more than adequately powered for moderate-to-severe asthma.

Statistical software and confidence intervals

Data analysis will be conducted in collaboration with the study statistician (ES) using R software and the `rms` package. Reported outputs will present estimates and accompanying two-sided 95% CI. Bootstrap resampling will be applied to assess internal validity. Cross-validation by study will be performed to assess external validity.

Ethics and dissemination

The protocol has been reviewed by the academic ethics committee (Oxford Tropical Research Ethics Committee (OxTREC)) and found to comprise fully anonymised data not requiring further ethical approbation. The results of the systematic review, patient-level multivariable prognostic MA, and clinical prediction models will be presented in an international scientific meeting and submitted for publication.

DISCUSSION

This protocol for a systematic review and IPD MA of RCTs across the spectrum of asthma severities coincides with a clinical prediction modelling effort centred on the peripheral blood eosinophil count and FeNO. Indeed, we speculate that these two biomarkers are the airway equivalent of high blood pressure or serum cholesterol, insofar as they identify a pathological process which relates to the risk key adverse outcomes (asthma attacks) that is modifiable by treatment (anti-inflammatory medication).

The focus on two biomarkers to predict the modifiable risk of asthma attacks is novel compared to existing clinical prediction models [2–13], where prognostic variables do not include nor adjust for blood eosinophils and FeNO. The established prognostic (*i.e.* predicting adverse outcomes) and theragnostic (*i.e.* predicting treatment responsiveness) values of these type-2 inflammatory biomarkers [17–24] provide a strong basis for a clinical prediction model centred on these independent, additive, and, most importantly, modifiable risk factors. The current protocol extends our previous proof-of-concept [23] work suggesting that traditional clinical risk factors can and should be adjusted for type-2 inflammatory biomarkers. Another novel aspect of our project is our intention to collaborate with a wide variety of authors and sponsors to form an international, data-driven, and not-for-profit consortium to support the development and validation of a robust clinical prediction model.

Despite the rigorous PRISMA [34] and Cochrane [33] methodologies which will be used to identify high-quality RCTs, there are areas of potential weaknesses in our study design which warrant discussion. First, we will limit our search strategy to MEDLINE. This approach was decided after a preliminary search in MEDLINE alone showed potential for >5000 control arm patients eligible to the IPD MA component; more than required to power our multivariable prognostic assessment and sufficient to claim that the included studies will be identified systematically rather than subjectively. Second, RCTs enrolling mild asthmatics have reported low absolute severe asthma attack rates [25,38], which may limit the model's reliability for low-risk patients. Third, a RCT-based clinical prediction model will be difficult to subsequently validate in real-world settings where treatment intensity fluctuates in response to the perceived risk of asthma attacks. Such real-world fluctuation in treatment regimens may weaken the relation between static biomarker measurements and 12-month observed asthma attack rates. Nevertheless, we speculate that physician-patient discussions can be assisted by a clinical prediction model which estimates the risk of asthma attacks if anti-inflammatory treatment is not changed, *i.e.:* if the patient were randomised to the control arm of an RCT. Fourth, controlled trials in asthma are notorious for a strong placebo effect. This caveat may be due to improved adherence to ICS, the Hawthorne effect, regression to the mean, or a combination of factors [39]. It is potentially surmountable by adapting the resultant clinical prediction model using reference asthma attack rates according to treatment intensity, as previously reported in a claims-based study [36] and proposed in our statistical analysis plan. Last, we have not planned to request active arm individual participant data, thus limiting our ability to assess the individual treatment benefit [40] or model heterogeneity of treatment effects [41]. We will not pursue the active arms' data to promote collaboration between competing sponsors but envision a de-centralised computation of individual treatment benefit and aggregate performance measures, such as the c-for-benefit statistic, at a later stage.

To conclude, we propose a systematic review and IPD MA to predict severe asthma attacks based on the inflammatory and clinical risk profile. Our emphasis on the risk conferred by raised type-2 inflammatory biomarkers and the consortium approach central to our endeavour may distinguish it from existing prediction models [2–13]. We speculate that a clinical prediction model centred on blood eosinophils and FeNO will provide a useful basis for a preventive, treatable trait-based asthma management.

TABLE 1
Clinical risk factors for asthma exacerbations with their traditional categorisations

Risk factors	Value (if pertinent)
Poor control of asthma symptoms	mean ACQ score ≥ 1.5
Limited lung function:	
low FEV1	< 60-80% predicted
high postbronchodilator reversibility	>12% change in FEV1
Adherence poor (inadequate technique or inhaler use)	
Reliever use excessive	> one 200-dose canister/month
Intubation or ICU admission for asthma on history	
Comorbidities:	
chronic rhinosinusitis	
obesity	body mass index ≥ 35 kg/m²
psychiatric disease	psychosis, substance abuse
Environmental exposure:	
smoking	
allergen exposure in sensitised patient	
air pollution	especially high O_3 and/or NO_3

ACQ = asthma control questionnaire; FEV1 = forced expiratory volume in 1 second; ICU = intensive care unit; PoLAR ICE = mnemonic (see bold characters in table). Adapted from Global Initiative for Asthma Guidelines [1]. Where possible, risk factors will also be analysed in continuous versions with restricted cubic splines to allow for non-linear associations.

TABLE 2
Treatment step definitions

Treatment step	Definition
Step 1	As-needed short-acting beta2-agonist
Step 2	Daily low dose ICS or As-needed low dose ICS-formoterol Daily leukotriene receptor agonist
Step 3	Daily low dose ICS + an additional controller therapy
Step 4	Any medium dose ICS-containing regimen
Step 5	Any high dose ICS-containing regimen or Any maintenance systemic corticosteroid use (defined as use of systemic corticosteroids for ≥50% of the previous year)

ICS, inhaled corticosteroid. Modified from Global Initiative for Asthma 2017 and 2021 [1] guidelines.

FIGURE LEGEND

The prototype OxfoRd Asthma attaCk risk scaLE. Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days and/or hospitalisation. The blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level (FeNO) is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [23] with permission under the original CC BY public copyright license.

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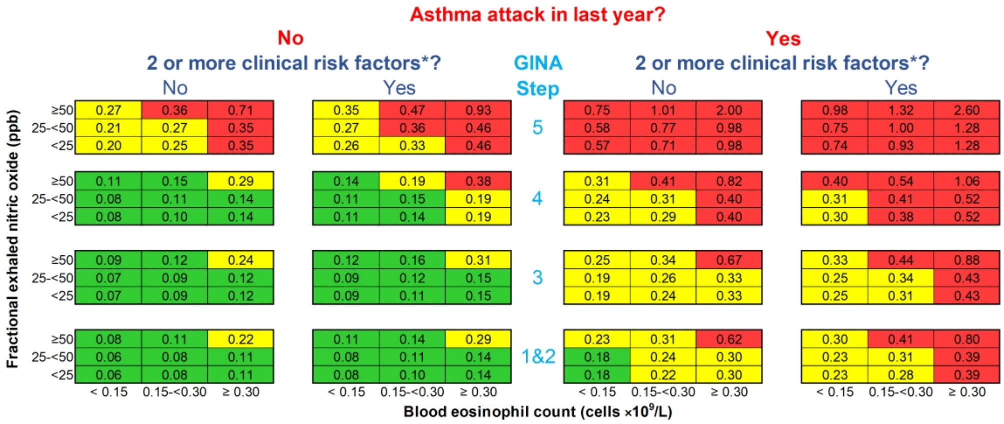

The prototype OxfoRd Asthma attaCk risk scaLE. Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days and/or hospitalisation. The blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level (FeNO) is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [23] with permission under the original CC BY public copyright license.

814x343mm (118 x 118 DPI)

Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

Simon Couillard, Ewout W Steyerberg, Richard Beasley, Ian D Pavord

Supplementary Material

Table of Contents

Appendix 1 – Medline search details

1.1. PubMed Search URL

https://pubmed.ncbi.nlm.nih.gov/?term=asthma+exacerbations&filter=pubt.randomizedcontrolledtrial&filter=dates.1993%2F1%2F1-2021%2F4%2F1&filter=hum_ani.humans&filter=lang.english&filter=lang.french&filter=age.adolescent&filter=age.alladult&filter=age.youngadult&filter=age.adult&filter=age.middleagedaged&filter=age.middleaged&filter=age.aged&filter=age.80andover&sort=date

1.2. PubMed Search details:

Search: asthma exacerbations Filters: Randomized Controlled Trial, Humans, English, French, Adolescent: 13-18 years, Adult: 19+ years, Young Adult: 19-24 years, Adult: 19-44 years, Middle Aged + Aged: 45+ years, Middle Aged: 45-64 years, Aged: 65+ years, 80 and over: 80+ years, from 1993/1/1 - 2021/4/1 Sort by: Most Recent

(("asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma s"[All Fields]) AND ("exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields])) AND ((randomizedcontrolledtrial[Filter]) AND (humans[Filter]) AND (1993/1/31:2021/4/1[pdat]) AND (english[Filter] OR french[Filter]) AND (adolescent[Filter] OR alladult[Filter] OR youngadult[Filter] OR adult[Filter] OR middleagedaged[Filter] OR middleaged[Filter] OR aged[Filter] OR 80andover[Filter]))

1.3. Translations

asthma: "asthma"[MeSH Terms] OR "asthma"[All Fields] OR "asthmas"[All Fields] OR "asthma's"[All Fields]

exacerbations: "exacerbate"[All Fields] OR "exacerbated"[All Fields] OR "exacerbates"[All Fields] OR "exacerbating"[All Fields] OR "exacerbation"[All Fields] OR "exacerbations"[All Fields] OR "exacerbator"[All Fields] OR "exacerbators"[All Fields]

Blood eosinophils, fractional exhaled nitric oxide, and the risk of asthma attacks in randomised controlled trials: protocol for a systemic review and control arm patient-level meta-analysis for clinical prediction modelling

STATISTICAL ANALYSIS PLAN

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E5

1. Changes from previous version of SAP

Version number Issue date	Author of this issue	Significant changes from previous version together with reasons
V0.1_2021-06-02	Couillard	Not applicable as this is the 1st issue
V0.2_2021-06-07	Couillard and Steyerberg	Preliminary input by study statistician
V0.3_2021-08-25	Couillard	Minor changes
V0.4_2021-09-15	Couillard	Minor changes to harmonise protocol manuscript draft.
V1.0_2021-10-09	Couillard and Steyerberg	Adjustments to harmonise with final protocol manuscript
V1.1_2022-01-23	Couillard	Adjustments following BMJ Open peer-review of the protocol

2. Background and Objectives

2.1. Background and rationale

Assessment and reduction of the risk of attacks is a major goal of asthma management [1]. However, our ability to do this is limited because the independent risk associated with clinical risk factors has not been defined, some are difficult to identify and/or modify, and others can be modified independent of an effect on asthma attacks. These limitations mean that a precise estimation of the risk of asthma attacks and the likely benefit of treatment is not possible.

Recently, five analyses of clinical trials in asthma showed that fractional exhaled nitric oxide (FeNO) and the blood eosinophil count provide additive prognostic information on the occurrence of severe asthma attacks [2–6]. The effect is large, with a three-fold greater rate ratio for asthma attacks seen in patients with FeNO ≥50 ppb and blood eosinophils $\geq 0.3\times10^9$/L compared to those with a FeNO <25 ppb and blood eosinophils $<0.15\times10^9$/L [7]. The excess risk of asthma attacks associated with the highest biomarker combination compared to the lowest was effectively removed by low-dose inhaled corticosteroids (ICS) in mild asthma [6], an increased dosage of ICS in moderate asthma [5,8], and biologics in severe asthma [4,9–11].

These findings suggest that the blood eosinophil count and FeNO could form the basis of a risk scale analogous to those that have had a large impact in cardiovascular medicine [12,13]. We have previously explored this hypothesis by developing a prototype scale (figure) which showed reasonable agreement between the observed and predicted asthma attack rates in the derivation trial-level data [7]. The prototype scale showed feasibility and potential to predict asthma attacks which can be prevented by treatment.

Asthma attack in last year?

GINA Step	Fractional exhaled nitric oxide (ppb)	No: 2 or more clinical risk factors*? No			No: 2 or more clinical risk factors*? Yes			Yes: 2 or more clinical risk factors*? No			Yes: 2 or more clinical risk factors*? Yes		
	Blood eosinophil count (cells ×10^9/L)	< 0.15	0.15-<0.30	≥ 0.30	< 0.15	0.15-<0.30	≥ 0.30	< 0.15	0.15-<0.30	≥ 0.30	< 0.15	0.15-<0.30	≥ 0.30
5	≥50	0.27	0.36	0.71	0.35	0.47	0.93	0.75	1.01	2.00	0.98	1.32	2.60
	25-<50	0.21	0.27	0.35	0.27	0.36	0.46	0.58	0.77	0.98	0.75	1.00	1.28
	<25	0.20	0.25	0.35	0.26	0.33	0.46	0.57	0.71	0.98	0.74	0.93	1.28
4	≥50	0.11	0.15	0.29	0.14	0.19	0.38	0.31	0.41	0.82	0.40	0.54	1.06
	25-<50	0.08	0.11	0.14	0.11	0.15	0.19	0.24	0.31	0.40	0.31	0.41	0.52
	<25	0.08	0.10	0.14	0.11	0.14	0.19	0.23	0.29	0.40	0.30	0.38	0.52
3	≥50	0.09	0.12	0.24	0.12	0.16	0.31	0.25	0.34	0.67	0.33	0.44	0.88
	25-<50	0.07	0.09	0.12	0.09	0.12	0.15	0.19	0.26	0.33	0.25	0.34	0.43
	<25	0.07	0.09	0.12	0.09	0.11	0.15	0.19	0.24	0.33	0.25	0.31	0.43
1&2	≥50	0.08	0.11	0.22	0.11	0.14	0.29	0.23	0.31	0.62	0.30	0.41	0.80
	25-<50	0.06	0.08	0.11	0.08	0.11	0.14	0.18	0.24	0.30	0.23	0.31	0.39
	<25	0.06	0.08	0.11	0.08	0.10	0.14	0.18	0.22	0.30	0.23	0.28	0.39

FIGURE 1. Prototype Oxford Asthma Attack Risk ScaLE (ORACLE).

Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma requiring treatment with systemic steroids ≥ 3 days. Blood eosinophil count is contemporaneous or the highest result in last 12 months; fractional exhaled nitric oxide level is contemporaneous. *Risk factors are defined by the Global Initiative for Asthma (GINA) guidelines [1]: poor symptom control (ACQ score ≥1.5), low lung function (FEV1 <80% predicted), adherence issues, reliever over-use (>200-dose salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, comorbidities (one of: chronic rhinosinusitis, obesity, psychiatric disease), environmental exposures (one of: smoking, allergen, pollution). Reproduced from reference [7].

3. Objectives and Outcomes

3.1.1. Hypothesis

We hypothesise that the blood eosinophil count and FeNO could form the basis of a robust and useful prediction model; we speculate that these two biomarkers are the airway equivalent of high blood pressure and serum cholesterol, insofar as they identify a pathological process which relates to the risk of adverse outcome (asthma attacks) that is modifiable by treatment (anti-inflammatory medication).

3.1.2. Objective

To develop and validate a clinical prediction model for the absolute number of severe asthma attacks to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other risk factors assessed at baseline.

3.1.3. Outcome to predict

The outcome to predict was the absolute number of severe asthma attacks to occur in the following 12 months (calculated as the annualised asthma attack rate). Severe asthma attacks are defined as acute asthma episodes requiring treatment with systemic steroids for 3 or more days and/or hospitalisation [14].

3. Study Details

This is the statistical analysis plan for the meta-analysis of individual participant data collected following a pre-specified systematic review protocol [15].

3.2. Study population

We will search MEDLINE (PubMed interface) for randomised controlled trials (RCT) from 1 January 1993 to 1 April 2021 that investigated the effect of fixed treatment(s) regimen(s) on severe asthma attack rates over at least 24 weeks, also reporting a baseline value for blood eosinophils and FeNO [15].

The included RCT control arm data will be analysed to develop a risk scale to predict asthma attacks. We will focus on risk which is known to be modifiable by treatment. This modifiable excess risk relates to two surrogate measures of airway inflammation (biomarkers): the peripheral blood eosinophil count and FeNO. The contribution of other less modifiable and non-modifiable risk factors defined by the current Global Initiative for Asthma guidelines [1] will also be assessed.

3.3. Study population

Following the preliminary systematic review, we identified 19 records comprising 23 independent RCTs [5,9,11,16–31].

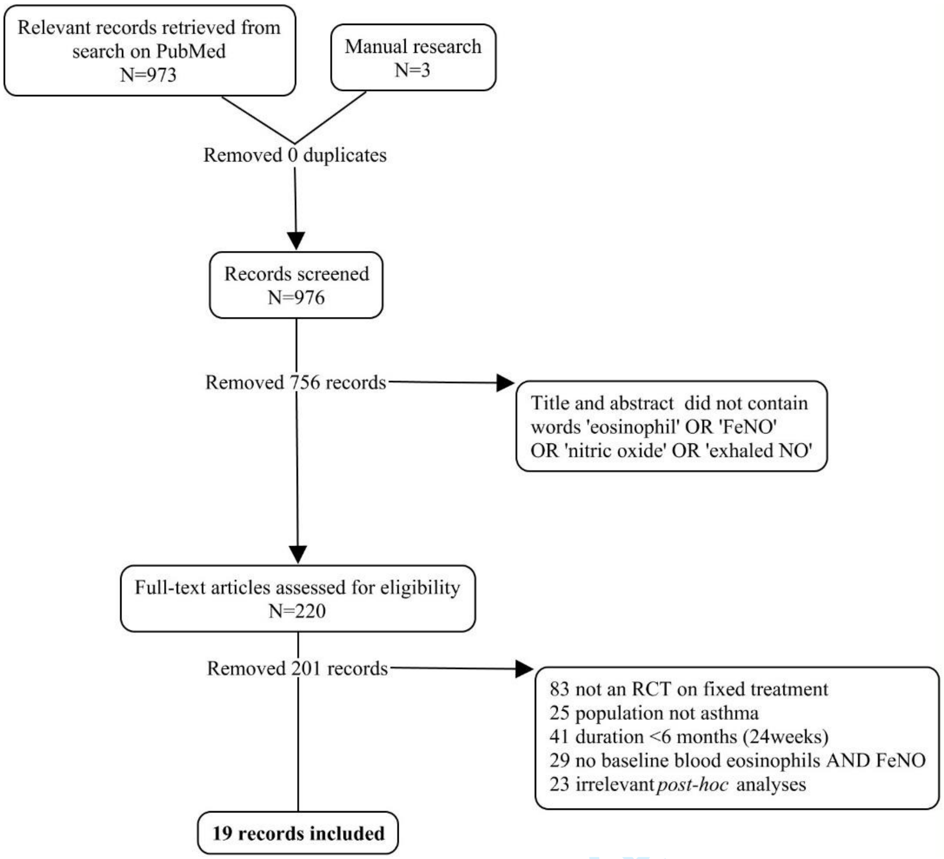

FIGURE 2. PRISMA flowchart of the preliminary results from the systematic review pre-specified in [13][12]

We will request data from the trial investigators and/or sponsors for patients diagnosed with asthma ages 12 and over that were randomised to the control arm (*i.e.* no ICS, lowest dose ICS, or placebo). The requested dataset will be functionally anonymised by design. The planned analysis pertains to the intention-to-treat population, modified to respect the inclusion criteria defined below.

3.3.1. <u>Inclusion criteria</u>

To be included, patients need to respect the following criteria:

- Asthma diagnosed according to the Global Initiative for Asthma (GINA) guideline-defined criteria (any severity)[1].
- 12 years of age or older
- Randomised to the control arm of the included study (*i.e.* placebo or no change in anti-inflammatory therapy).
- Data available for the following variables:
  - Peripheral blood eosinophil count (x10^9/L) at baseline
  - FeNO (ppb) at baseline
  - Sufficient information on the patients' medication to determine the treatment step (*i.e.* disease severity)(see section 3.1.4, table 2)[1].
  - Number of severe asthma attacks in the 12 months previous to the baseline visit. Severe asthma attacks are defined as acute asthma requiring ≥3 days of systemic corticosteroid therapy and/or hospitalisation.
  - Duration of the controlled treatment period (days)
  - Number of severe asthma attacks observed during the study period.

3.3.2. Exclusion criteria

We will exclude patients if both baseline blood eosinophil count and baseline FeNO are missing. We will also exclude patients with missing follow-up duration whilst on the allocated therapy, or missing number of severe asthma attacks during follow-up.

3.4. Cross-validation by study to assess external validity

The study population will be used for derivation and subsequent validation, stratifying by source RCT in cross-validation by study design, where each study serves as a validation set once [32].

3.5. Sources of data for complimentary external validation

The follow sources of data will be used for external validation:

i) cross-validation by study is the initial external validation procedure that will be performed in the meta-analysis population;

ii) observational prospective cohorts envisioned to contribute to later external validation are the Novelty cohort [33]; the outpatient general practice cohort derived from the Optimum Patient Care Research Database [34]; and any other RCTs or cohorts that do not share their data to a central repository.

4. Primary and secondary variables

4.1. General definitions

4.1.1. <u>Definition of baseline</u>

In general, the last non-missing measurement on or prior to the date of randomisation will serve as the baseline measurement for predictors.

4.1.2. <u>Duration of the controlled treatment period</u>

The controlled treatment period for the assessment of severe asthma attacks starts at the date of randomisation and ends at the minimum (date of last dose of placebo + appropriate wash-out period as per source RCT protocol, date of death, date of study withdrawal).

4.1.3. Concomitant medication

Medications taken by the subject at any time during the controlled treatment period will be used to define the treatment step. Concomitant medications during the controlled treatment period which are recorded are defined in section 2.3 (study variables).

4.1.4. Treatment step

A modified version of the 2017 and 2021 GINA guidelines definitions will be used to determine treatment step.

TABLE 2
Modified treatment step definitions for this study

Treatment step	Definition
Step 1	As-needed short-acting beta2-agonist
Step 2	Daily low dose ICS or As-needed low dose inhaled corticosteroid (ICS)-formoterol Daily leukotriene receptor agonist
Step 3	Daily low dose ICS + an additional controller therapy
Step 4	Any medium dose ICS-containing regimen
Step 5	Any high dose ICS-containing regimen or Any maintenance systemic corticosteroid use (defined as use of systemic corticosteroids for ≥50% of the previous year)

Modified from GINA 2017 and 2021 [1] guidelines.

4.1.5. Calculation of inhaled corticosteroid (ICS) dosing

ICS-dose strength will be determined using the following table, retained from the 2021 GINA guidelines:

TABLE 3

Low, medium and high daily metered doses of inhaled corticosteroids in adults and adolescents (12 years and older)

Inhaled corticosteroid	Total daily ICS dose (mcg)		
	Low	Medium	High
Beclomethasone dipropionate CFC-propellent MDI	200-500	>500-1000	>1000
Beclomethasone dipropionate extrafine particle MDI or DPI	100-200	>200-400	>400
Budesonide	200-400	>400-800	>800
Fluticasone dipropionate	100-250	>250-500	>500
Fluticasone furoate	100	100	200
Ciclesonide	80-160	>160-320	>320
Mometasone furoate	200-400	200-400	>400

Adapted from [1]. CFC, chlorofluorocarbon; DPI, dry powder inhaler; MDI, multidose inhaler.

The following ICS dose equivalence table will be used to characterise patients' concomitant ICS use:

TABLE 4
Equivalent doses between inhaled corticosteroids

Inhaled corticosteroid type	Equivalent dose
Beclomethasone dipropionate CFC-propellent MDI	1 mcg
Beclomethasone dipropionate HFA or DPI	2.5 mcg
Budesonide	1.25 mcg
Fluticasone dipropionate	2.5 mcg
Fluticasone furoate	5 mcg
Ciclesonide	3.125 mcg
Mometasone furoate	2.27 mcg
Triamcinolone acetonide	0.5 mcg

Adapted from [1][1].

4.2. Primary variable and study endpoint

The effect to measure and predict is number of severe asthma attacks (defined as acute asthma requiring ≥ 3 days of systemic corticosteroids and/or hospitalisation)[14] to occur in the following 12 months in relation to the peripheral blood eosinophil count, FeNO, and other prognostic actors assessed at baseline.

The start of an exacerbation is defined as the start date of systemic corticosteroids, emergency room (ER), urgent care (UC) visits, or hospital admissions due to asthma, whichever occurs earlier. The end date is defined as the last day of systemic corticosteroids or ER/UC/hospital discharge, whichever occurs later.

Two or more exacerbations with the same start date and end date will be counted as one exacerbation for the purposes of calculating the number and duration of exacerbations for a subject. In the case that one or more exacerbations are recorded as starting or ending during another exacerbation, these will be counted as one exacerbation, using the earliest exacerbation start date and the latest exacerbation stop date to calculate duration.

Additional systemic corticosteroid treatments, ER visit or UC visit requiring use of systemic corticosteroids, or hospital admission will not be regarded as a new exacerbation. To be counted as a new exacerbation it must be preceded by at least 7 days in which neither criterion is fulfilled. If the end date of the first exacerbation and the start date of the second exacerbation are less than 7 days apart, then these will be counted as one exacerbation.

The number of days the subject experiences a protocol defined exacerbation, including the subsequent 7 days (when a further exacerbation would not be considered as a second exacerbation), will be subtracted from the time at risk defined above for the primary analysis. For example, if a subject has a single exacerbation which lasts 4 days then 7 + 4 =11 days will be subtracted from the time at risk.

4.3. Subgrouping for biomarker-stratified clinical prediction modelling

3.1.1. Biomarker-stratified subgroups

The main multivariable prognostic modelling analysis will use continuous values of the blood eosinophil count, FeNO, and other clinical risk factors (table 1). If relevant, combined effects will be summarised in a 3×3 matrix stratified by the blood eosinophil count (<0.15, 0.15-<0.30, ≥0.30×109 cells/L) and FeNO (<25, 25-<50, ≥50 ppb), and plotted in interaction plots with 95% confidence intervals (CI). Heterogeneity in estimates between studies will be quantified by I^2 statistics. Additional analyses will consider continuous versions of predictors with restricted cubic splines [35].

3.1.2. Comparative subgroup rate ratio analysis

If relevant following analyses on continuous data, crude and adjusted rate ratios of the annualised severe exacerbation rate for each of the 9 categories (3×3 matrix according to the blood eosinophil count (<0.15, 0.15-<0.30, ≥0.30×10^9 cells/L) and FeNO (<25, 25-<50, ≥50 ppb) will be determined. Rate ratios for each subgroup are calculated as the weighted annualised exacerbation rate for the selected subgroup divided by the mean for the remainder of the matrix, weighted by patient-years of data. The adjusted rate ratios will account for asthma severity (treatment step), history of asthma attacks (≤1 or >1 in previous 12 months); as well as age, sex, and source RCT to control for unsuspected confounding factors relating to the three latter variables.

The potentially relevant clinical risk factors for asthma attacks listed in section 3.4 will be assessed using a bootstrapped backward stepwise selection procedure during regression analysis in a random effects model. Key predictors are: blood eosinophils, FeNO, treatment step and the past history of exacerbations (0 or ≥1 in previous 12 months).

4.4. Potential clinical predictors

The following variables will be assessed as potential clinical predictors, in addition to the forced variables (treatment step, past history of exacerbations (<1 or ≥1 in previous 12 months), age, sex, and source RCT).

- Ethnicity: categorical
- Comorbidities: categorical (list of comorbidities following the Charlson comorbidity index [35][34])
- Socioeconomic status (anonymised and operationalised depending dataset)

- Body mass index: continuous
- Postbronchodilator (BD) FEV1, as % predicted (or preBD if no postBD): continuous
- % change in FEV1 post-bronchodilator (calculated as (FEV1 post BD minus FEV1 preBD in litres) divided by FEV1 preBD in litres: continuous
- FEV1/FVC ratio, calculated as FEV1 postBD in litres divided by FVC postBD in litres (or using preBD values if no postBD)
- Smoking status (current, ex-, passive, never-smokers): categorical
- Airborne allergies reported (yes/no): categorical
- Allergy testing positive (yes/no): categorical
- Chronic rhinosinusitis (yes/no): categorical
- Nasal polyposis (yes/no): categorical
- Adherent to medications (operationalised definition depending on the dataset): continuous (or categorical if not feasible to operationalise in a continuous variable)
- Inhalers prescribed:
  - ICS: categorical (yes/no)
  - ICS daily equivalent dose (continuous)
  - Short-acting beta2-agonist (yes/no) and number of actuations used per month (continuous)
  - Long-acting beta2-agonist (yes/no)
  - Long-acting muscarinic antagonist (yes/no)
  - Leukotriene receptor antagonist (yes/no)
  - Theophylline or aminophylline (yes/no)
- On maintenance oral corticosteroids (OCS) (yes/no): categorical

- Severe exacerbation in the preceding 12 months (defined as an acute event requiring ≥3 days of systemic corticosteroids and/or hospitalisation): yes/no category and continuously by number of episodes in preceding 12 months.
- Previous intensive care or intubation for airways disease (yes/no): categorical
- Asthma control questionnaire (ACQ) score (or asthma control test (ACT) or any other standardised symptom score test if ACQ not available): continuous (ACQ or ACT) and categorical (according to established cut points for uncontrolled disease: ACQ ≥1.5 or ACT <20)

4.5. Missing values

Missing values will be assessed for their mechanism (missing completely at random, missing at random or missing not at random) by the main investigators in conjunction with the study statistician. When data is missing at random, 10 complete datasets will be generated by multiple imputation.

4.6. Heterogeneity assessment

The variability between studies will be quantified in a random effect analysis and quantified with I^2 statistics.

4.7. Optimism correction

The adjusted biomarker-stratified and clinical predictors' incidence rate ratios will be corrected for overoptimistic predictions. Penalty terms will be used and/or linear shrinkage factors, as estimated from cross-validation and/or bootstrap resampling procedures as implemented in `rms` and `glmnet` libraries for R.

4.8. Statistical software and confidence intervals

Data analysis will be conducted in collaboration with the study statistician (ES) using R software. Estimates will be accompanied by two-sided 95% CI.

4. Clinical prediction model presentation formats

A summary prognostic equation will be produced, assessed by the principal investigators, and adapted to previously reported GINA treatment step reference attack rates [37] to allow for a user-friendly prediction summary chart similar to the reported prototype (figure 1).

5. Performance evaluation

5.1. General performance measures

The resultant prognostic equation and chart will be assessed in the validation cohorts defined in section 2.4. Discrimination will be evaluated. Calibration plots will be created with focus on centiles of risk (10^{th}, 50^{th} and 90^{th} of the distribution of predicted attack rates), and summary measures of the plot will be computed. Sensitivity, specificity and receiving operating characteristic (ROC) analyses of the model will be assessed. Reliability will be evaluated using the intraclass correlation coefficient (two-ways mixed model for absolute agreement, single measures, with 95% CI). Calibration will be assessed graphically, with characterization of calibration in the large by a calibration intercept, and overall prognostic strength by the calibration slope. Discrimination will be assessed by the c-statistic, and clinical utility by Net Benefit plotted in decision curves.

5.2. Subgroup performance measures

The performance of the resultant chart will be evaluated across the selected clinical predictors (composite biomarker category; treatment step; asthma attack history; retained clinical risk factors) as stated in section 4.1 for each subsection of the chart in each of the validation cohorts. In effect, assuming the final chart resembles the prototype (figure 1), this will result in performance assessment for each of the 16 subsections and/or each of the 144 squares, depending on the validation cohort size.

6. Study power

Considering a mean annualised severe asthma attack of 0.6 in the entire study population and a conservative estimate that the derivation cohort will comprise 50% of the individual patient data reported in our prototype scale (0.5*3051 = 1525) [7], there should be approximately 915 events to derive a clinical prediction model. With a target maximum of 10 prediction variables, the event per variable (EPV) number is 92; well over the recommended 10-20 EPV [38]. However, we concede that the EPV will be considerably lower for mild asthma populations, where trials identified less than 100 severe asthma attack events in their control arms [17,31]. Conversely, the study will be more than adequately powered for moderate-to-severe asthma.

Strengths and limitations of our approach

6.1. Strengths

- The study design and its objective – to derive and validate a clinical prediction tool based on biomarkers of type-2 inflammation – fulfils an unmet clinical need. We speculate that a risk stratification strategy centred on modifiable type-2 airway inflammation rather than difficult-to-modify clinical characteristics would facilitate better treatment decisions by providing a framework for a preventive, treatable trait-based management.

- A proof-of-concept evaluation of this project has already been completed and shows feasibility and potential to predict asthma attacks which can be prevented by treatment [7,10] (Figure 1).
- Study selection bias is reduced via the pre-specified systematic review approach.
- Adequate study power. As stated above, with an estimated overall attack rate equal to that reported in the prototype scale (0.6 attacks per year) and a conservative estimate of individual participant data provided (50% of the prototype study population), there should ample events observed for model derivation validation.
- Detection bias of the outcome of interest (severe asthma attacks) is minimised by its rigorous monitoring and documentation in the context of RCTs.
- In addition to the cross-validation by study [32], we plan to validate the resultant chart in different validation cohorts: a part of the base RCT population, Novelty [33] and the Optimum Patient Care Research Database [34].

6.2. Limitations

- Many of the included RCT study populations were positively selected to be at high risk of asthma attacks, and trials enrolling mild asthmatics have reported low asthma attack rates ; this may result in the model overestimating the risk of events and underperforming in mild asthma.
- The assumption at the basis of our approach is that the type-2 biomarkers blood eosinophils and FeNO carry additive and independent predictive value for the risk of asthma attacks at all disease severities. It is unclear if FeNO exerts a similar predictive value in mild asthma [6][6]. This modification of risk will be addressed by statistical interaction terms.
- There is no clear reference for treatment step asthma attack rates adapted for the most recent GINA 2021 guidelines; it is possible we will need to model around the previously reported GINA 2017 classification reference asthma attack rates [37].
- We suspect that some of the important clinical risk factors emphasised by current management guidelines [1] will not be present in the RCT population (*e.g.* nonadherence is usually an exclusion criteria; salbutamol over-use is not always reported).
- Controlled trial populations in asthma are notorious for a strong placebo effect and do not necessarily reflect clinical practice, where treatment fluctuates according to the perceived or observed risk of asthma attacks; this may impact external validation in observational cohorts.

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Manuscript page (of 29 page Word document)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	p.1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	p.1
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	p.1 + **p.5**
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	p.1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	p.2
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	p.2
Sponsor	5b	Provide name for the review funder and/or sponsor	p.2
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	p.2
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	p.7
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	p.8
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	p.10-12
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	p.11-12
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	p.11-12 + Appendix 1

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	p.11-12
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	p.11-12
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	p.12
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	p.12
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	p.11 + p.12
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	p.13
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	p.13-15 + Appendix 2
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	p.13-15 + Appendix 2
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	p.13-15 + Appendix 2
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	N/A
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	N/A

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.