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

Couillard S, *et al. BMJ Open* 2022; 12:e058215. doi: 10.1136/bmjopen-2021-058215

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]

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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

STATISTICAL ANALYSIS PLAN

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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 $\geq 0.3 \times 10^9$/L compared to those with a FeNO <25 ppb and blood eosinophils $< 0.15 \times 10^9$/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.

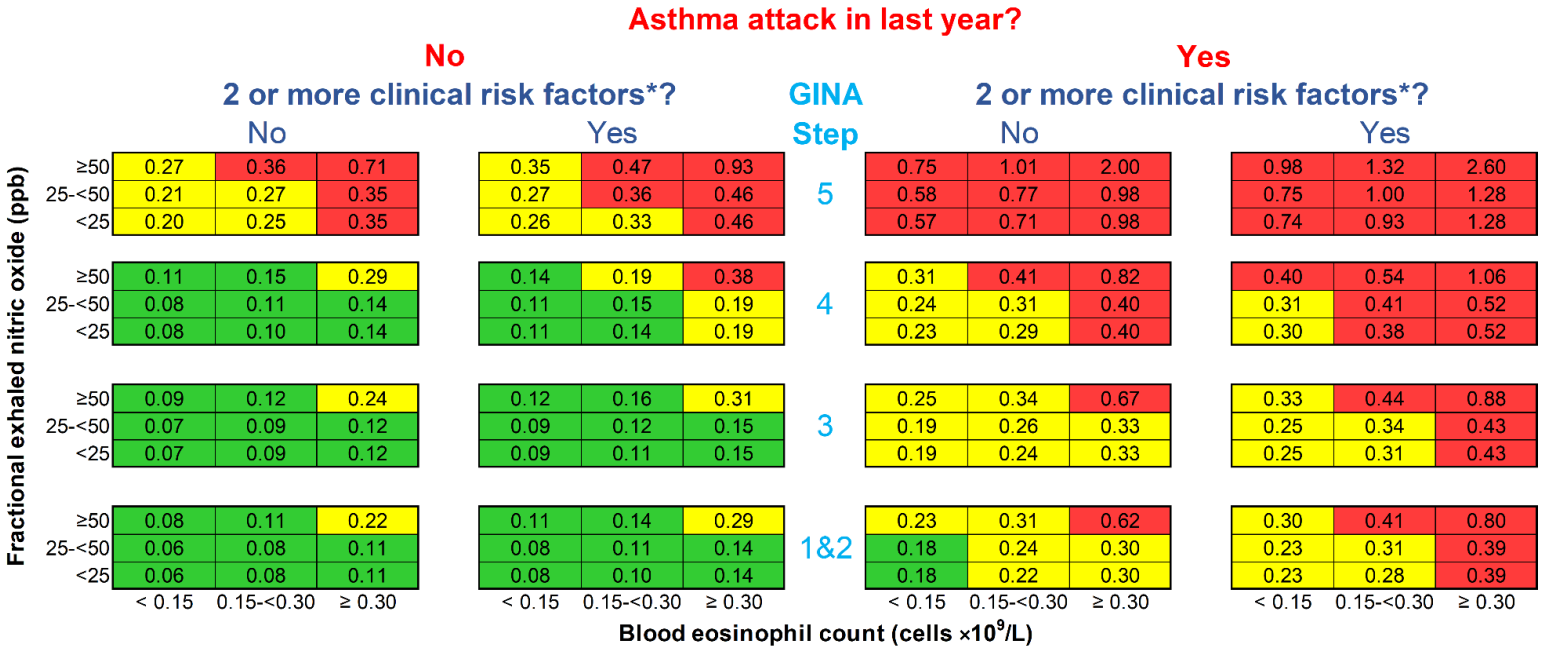

FIGURE 1. Prototype Oxford Asthma Attack Risk ScaLE (ORACLE).

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].

Couillard S, *et al. BMJ Open* 2022; 12:e058215. doi: 10.1136/bmjopen-2021-058215

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].

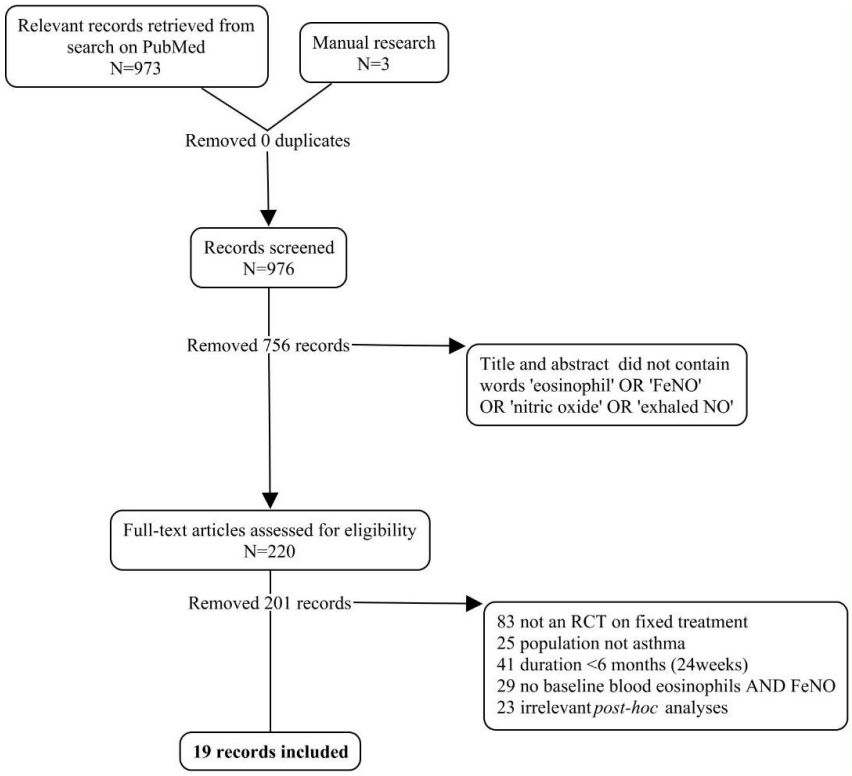

FIGURE 2. PRISMA flowchart of the preliminary results from the systematic review pre-specified in [13][12]

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. Inclusion criteria

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. Definition of baseline

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. Duration of the controlled treatment period

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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