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Blood eosinophils as a predictor of treatment response in adults with difficult-to-treat chronic cough

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Conflict of interests

ARF has received fee from Polpharma for attendance at ERS International Congress (2018), outside the submitted work; MD has received fees from Merck for lectures on chronic cough, outside the submitted work; EMG has received fee for lectures on chronic cough from Merck and Polpharma, outside the submitted work; RK has received fee for lectures from Chiesi, AstraZeneca, Polpharma, outside the submitted work; Boehringer Ingelheim, Chiesi and AstraZeneca have covered his fee and travel expenses for ERS International Congresses (2018, 2019) and ATS Conferences (2018, 2019), outside the submitted work; the authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the study apart from those disclosed.

ABSTRACT

There is lack of evidence on the role of blood eosinophil count (BEC) as a predictor of treatment response in patients with chronic cough (CC). The study aimed to evaluate BEC as a predictor of treatment response in all non-smoking adults with CC and normal chest X-ray referred to cough clinic and in subgroup of patients with CC due to asthma or NAEB (non-asthmatic eosinophilic bronchitis).

This prospective cohort study included 142 consecutive, non-smoking patients referred to our cough centre due to CC. The management of CC was performed according to the current recommendations. At least a 30 mm decrease of 100-mm visual analogue scale in cough severity and a 1.3 points improvement in Leicester Cough Questionnaire were classified as a good therapeutic response.

There was a predominance of females (72.5%), median age 57.5 years with long-lasting, severe cough (median cough duration 60 months, severity 55/100 mm). Asthma, NAEB were diagnosed in 47.2% and 4.9% of patients, respectively. After 12-16 weeks of therapy, a good response to CC treatment was found in 31.0% of all patients. A weak positive correlation was demonstrated between reduction in cough severity and BEC ($r=0.28$, $P<0.001$). AUC for all patients with CC was 0.62 with the optimal BEC cut-off for prediction of treatment response set at 237 cells/μL and for patients with CC due to asthma/ NAEB was 0.68 (95% CI 0.55-0.81) with the cut-off at 150 cells/μL.

BEC is a poor predictor of treatment response in adults with CC treated in the cough centre.

Keywords: asthma; biomarkers; bronchial hyperresponsiveness; chronic cough; eosinophils

INTRODUCTION

Cough is not only a defensive physiological reflex of the airways but also a very common symptom of various pulmonary and extra-pulmonary diseases. When acute, it usually points to a transient underlying disease, such as acute airway infection or asthma exacerbation and suggests a good prognosis in terms of its resolution. In contrast, chronic cough (CC), defined in adults as the cough lasting more than 8 weeks, is considered to be much more cumbersome, at least in some patients. CC has been reported in as many as 5-10% of the adult population and is one of the most common reasons for seeking medical advice from a respiratory specialist.[1-3] The significance and the prognosis in CC is largely related to the underlying disease. Chronic cough frequently results from smoking-related conditions, i.e. chronic bronchitis, chronic obstructive pulmonary disease (COPD) or lung cancer. Cause oriented management, including smoking cessation, inhalation therapy with bronchodilators in combination with inhaled corticosteroids (ICS) is relatively effective in the alleviation of cough.[4-6]

On the other hand, the most frequent causes (or rather triggers) of CC in non-smoking patients with a normal chest radiograph are four common conditions: asthma, non-asthmatic eosinophilic bronchitis (NAEB), rhinitis and rhinosinusitis [which, along with CC, constitute an entity described as upper airway cough syndrome (UACS)] and gastroesophageal reflux (GER).[1,7] Diagnosis and treatment of CC may be challenging, in particular the latter has been shown to have limited efficacy. Despite different therapeutic attempts, the reduction in cough severity is incomplete and regarded unsatisfactory in even up to 46% of patients with CC with persistent impairment of quality of life (QoL).[8,9] If causal treatment is ineffective, refractory chronic cough (RCC) should be diagnosed. While thorough diagnostics do not allow to identify any CC reason, unexplained CC (UCC) is diagnosed.[8] Both RCC and UCC are commonly associated with hypersensitivity of cough reflex, which is a key component in the

pathomechanism of CC regardless of the underlying condition.[1,10] It is characterized by troublesome coughing in response to low level stimuli, loss of inhibitory cough control and abnormal laryngeal sensation. At the same time it is responsible for limited efficacy of CC treatment.[11,12]

The limited effectiveness of cause oriented treatment of CC encourages the application of other approaches including a search for treatable CC traits. Identifying different CC endotypes and biomarkers capable of predicting treatment response may facilitate a more specific therapeutic approach resulting in a higher success rate.[1] It has been shown that up to 25-50% of patients with CC are characterized by eosinophilic airway inflammation, which can be diagnosed by analysis of induced sputum (IS).[13-15] The role of eosinophils in the pathomechanism of CC is complex and still not well recognized. In recent trials, the relationship between eosinophils and increased airway sensory nerve density was documented.[16,17] Moreover, Satia et al. demonstrated heightened cough response to capsaicin when eosinophilic inflammation, caused by allergen exposure, is present.[18] A higher efficacy of CC treatment has been demonstrated in patients with an eosinophilic pattern of airway inflammation (usually in asthma or NAEB).[19] As availability of sputum induction is limited, it has been suggested that peripheral blood eosinophil count (BEC) could be a good indirect tool to assess eosinophilic airway inflammation.[13,14] However, previous studies showed a moderate diagnostic accuracy of BEC as a marker for eosinophilic airway inflammation in asthma[20] and only a weak correlation between BEC and sputum eosinophilia was found in patients with COPD.[21]

As there is the lack of high-quality data on the role of BEC in the prediction of treatment response in patients with CC, the objective of this study was to find out whether BEC may play this role in predicting treatment response in non-smoking adults with CC and a

normal chest X-ray referred to the tertiary cough centre and whether BEC could be useful marker in patients with CC due to asthma or NAEB.

MATERIALS AND METHODS

General study design

This prospective, single-centre cohort study included all consecutive patients with CC referred to the cough centre in the Department of Internal Medicine, Pulmonary Diseases and Allergy of the Medical University of Warsaw between 2016 and 2019. It was a part of a larger project designed to evaluate the causes of CC and the efficacy of CC treatment which has been running in our institution since 2009. The study protocol was approved by the Institutional Review Board of the Medical University of Warsaw (KB/101/2009) and all enrolled patients signed written informed consent.

Patients

The major inclusion criteria were as follows: 1) cough lasting more than 8 weeks, 2) age over 18 years, 3) without any antitussive treatment for at least 4 weeks prior to enrolment to the study, 4) non-smoking (for at least 1 year) status, 5) normal or near-normal chest radiograph. The exclusion criteria included: 1) some well-defined pulmonary and extrapulmonary conditions which require specific therapeutic intervention (e.g. interstitial lung diseases, COPD, lung cancer or other malignant diseases, tuberculosis or nontuberculous *mycobacteria* infections, treatment with angiotensin-converting enzyme inhibitors), 2) discontinuation of the recommended therapy.

Diagnostic work-up

The causes of CC were diagnosed according to the European Respiratory Society, American College of Chest Physicians (ACCP) and British Thoracic Society (BTS) recommendations.[22-24] The routine stepwise diagnostic work-up included: a detailed medical

history, physical examination and additional investigations, including basic laboratory tests (total and differential blood cell count, total serum IgE level), spirometry, fractional exhaled nitric oxide (FeNO), methacholine challenge, IS differential cell count, skin prick tests and multiple aeroallergens IgE screening assay, imaging studies (chest X-ray, paranasal sinus and chest computed tomography), ENT specialist consultation as well as 24-hour oesophageal impedance and pH-monitoring (see supplementary Figure 1). In the first step the exclusion of other causes of CC was made (smoking, angiotensin-converting enzyme inhibitor therapy, COPD, lung cancer or interstitial lung disease). The next step included diagnostics of asthma, and in the case of high clinical probability, introduction of anti-asthmatic treatment. In case of no improvement or in the absence of evidence for the diagnosis of asthma, the further diagnostics (GER, UACS and other reasons for cough) and causal treatment were introduced (Supplementary Figures 1, 2). BEC was measured twice in independent blood samples (Sysmex XN-2000, Kobe, Japan), before the onset of CC treatment and the higher of the two measurements was considered in further analysis.

Definitions

Chronic cough was defined as cough lasting at least 8 weeks.[23] Blood eosinophilia was defined as BEC ≥300 cells/μL, while the cut-off value for sputum eosinophilia was set at 3%.[25,26] The atopic status of the patient was established based on at least one positive skin prick test (a mean wheal diameter ≥3 mm) or presence of serum specific IgE antibody for at least one allergen.[27]

Asthma was defined as: 1) the presence of typical symptoms (dyspnea or cough or wheezing) together with 2) variable airway obstruction (diurnal variation of peak expiratory flow >10% or spirometry with positive reversibility test or provocative concentration [PC_{20}] in methacholine challenge test <16 mg/mL).[26,28] NAEB was diagnosed in CC patients with elevated sputum eosinophilia and absence of airway hyperresponsiveness.[25] UACS was

diagnosed in patients presenting with CC and signs and symptoms of rhinitis or chronic rhinosinusitis confirmed by medical history and ENT examination.[29] Diagnostic criteria for GER were as follows: 1) esophagitis revealed in gastroscopy, 2) elevated number (> 73/24 h) reflux episodes registered in 24-hour pH-impedance monitoring, 3) typical symptoms of GER disease (e.g. heartburn, regurgitation) and improvement after proton pump inhibitors (PPI) therapy.[30-32] At least one of the above criteria had to be met to diagnose GER. Refractory chronic cough (RCC) was diagnosed when 6-8 weeks of recommended treatment with good patient adherence did not reduce cough severity. Unexplained CC (UCC) was defined if no reason for cough was found despite thorough diagnostics.[8]

Management, monitoring and outcome assessment

Once any CC trigger was diagnosed, patient received specific treatment. Asthma was treated in accordance to Global Initiative for Asthma (GINA), including the three-step therapy, starting with moderate doses of inhaled corticosteroids (ICS, daily 400 μg of beclometasone dipropionate pMDI, HFA, extrafine particle or 800 μg of budesonide pMDI) with long-acting β_2-agonist (LABA, 12-24 μg of formoterol daily) and, if not effective, an add-on therapy with leukotriene receptor antagonist (LTRA, montelukast 10 mg daily) and then short-term add-on oral corticosteroids (OCS prednisone 0.5 mg/kg for 10 days).[23,26,33] NAEB was treated with moderate doses of ICS (320 μg of ciclesonide or 800 μg of budesonide daily).[34] UACS therapy consisted of intranasal corticosteroids (INCS) and add-on oral or intranasal antihistamines (in case of allergic rhinitis).[35,36] GER therapy included lifestyle and diet modification and PPI, if not effective – an add-on therapy with prokinetic drugs.[30] RCC was treated with speech therapy or neuromodulators and continuation of treatment of diagnosed cough associated conditions.[8] UCC was treated with speech therapy or neuromodulators.[9] Patients with more than one cause of CC were treated with therapies for all

identified cough reasons.[8] Decisions on the initiation of the treatment were not dependent on BEC.

Cough severity was measured with the use of the visual analogue scale (VAS), with a score range from 0 – no cough to 100 mm – the worst possible cough.[37] Cough-related QoL was measured with Leicester Cough Questionnaire (LCQ) which is a 19-item questionnaire, assessing cough-related QoL with a total score ranging from 3 to 21 points, with the lower total LCQ score indicating higher impairment in QoL.[23,38] Reassessment of cough severity and cough-related QoL was performed between 12-16 weeks after the onset of the treatment. A treatment response was defined as a decrease in cough severity by ≥30 mm on VAS and improvement ≥1.3 points in LCQ.[39] The major steps of the study protocol are presented in Figure 1.

Statistical analysis

As there has been no previous studies on accuracy of BEC in predicting decrease of CC after therapy, we assumed that accuracy of BEC in predicting response to therapy of CC measured as receiver operating characteristic (ROC) area under the curve (AUC) is 0.6. Thus, the size of the group was calculated for 120 patients based on study by Hajian-Tilaki assuming 10% marginal error and 80% power of the study.[40] The number of enrolled subjects was planned to be increased by 12 subjects to allow for a 10% drop-out rate. Thus, a total number of 132 subjects was the minimum participants required to perform this study.

Data were presented as a median and interquartile range or numbers and percentages. The statistical analysis was performed using Statistica 13.1 software package (StatSoft, Tulsa, USA). The correlation analysis between BEC and the reduction in cough severity and LCQ score, FeNO and IS eosinophils was made using the Pearson correlation coefficient. Due to non-normal distribution of BEC, this variable was analyzed in Pearson correlation after logarithmic transformation. ROC curve was constructed to evaluate the value of BEC as the

predictor of treatment response. AUC was calculated to find an optimal BEC cut-off for the prediction of a good response. For further analyses, the patients were classified with low and high BEC, based on the cut-off point estimated by the ROC curve. The differences between these two groups, due to differences in group size, were analysed with non-parametric tests using the Mann-Whitney U test for continuous and χ^2 test for categorical variables. The final evaluation included the subgroup analysis – asthma/NAEB, asthma/NAEB/UACS, UACS, GER, and BHR (BHR+) patients with an attempt to determine a cut-off value of BEC for a prediction of treatment response. A *P* value lower than 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of patients

One hundred sixty-three non-smoking patients with CC were initially enrolled; ultimately, 21 patients were excluded from the final analysis (Figure 2). Thus, the proper study group included 142 patients with CC; median age 57.5 years (46-67), predominantly female (103, 72.5%). Patients' characteristic was presented in the Table 1. The median BEC was 148.8 cells/μL (99.1-237.8) and 18 (12.7%) patients presented with BEC ≥ 300 cells/μL (see Figure 3). There was a moderate positive correlation between BEC and IS eosinophils and between BEC and FeNO (r=0.30, *P*<0.001 and r=0.25, *P*=0.008, respectively).

BEC and response to therapy in all CC patients

A significant reduction in cough severity following cause oriented therapy was observed in 44 (31.0%) patients. Higher BEC and FeNO were noted in patients who reported improvement (184.8 cells/μL vs. 141.8 cells/μL, *P*=0.026 and 18.2 ppb vs. 14.7 ppb, *P*=0.040, respectively). Although no difference was found between the level of BHR (PC_{20}) in patients who responded and in those who did not respond to treatment, the prevalence of BHR was significantly higher in those with observed clinical improvement (55.3% vs. 32.1%, P=0.015, Table 2). Next, in patients who did not respond to causal treatment, the prevalence of GER was significantly higher (57.1% vs. 38.6%, *P*=0.041, Table 2). A weak positive correlation between the decrease of cough severity (dVAS) and BEC was shown (r=0.28, *P*<0.001, see Figure 4 a).

The area under ROC curve for the performance of BEC in all patients was 0.62 (95% CI 0.51–0.72). The optimal BEC cut-off for the prediction of good treatment response was 237 cells/μL (Table 3, Figure 4 b). Comparison of groups with lower (< 237 cells/μL) vs. higher (≥ 237 cells/μL) BEC showed longer cough duration [69 months (36-138) vs. 48 months (24-84), *P*=0.018] and less severe cough [53 mm (35.5-74) vs. 70 mm (44-80),

P=0.033] in the low-BEC group but did not reveal any differences in demographic (age, gender, BMI), clinical data (cough related QoL, atopy, IS eosinophil percentage, FeNO, BHR severity). Moreover, in the low BEC group the prevalence of GER was significantly higher [59 (56.7%) vs. 14 (36.8%), *P*=0.036].

BEC and treatment effects in patients with asthma/NAEB

Among patients with asthma or NAEB 28 of 74 (37.8%) individuals showed improvement after treatment. There was a moderate correlation between BEC and dVAS (r=0.49 *P*<0.001, Figure 4 c). The analysis of BEC as a surrogate biomarker of eosinophilic airway inflammation in patients with asthma/NAEB showed slightly stronger correlations than in the general CC group (BEC and FeNO r=0.29, *P*=0.02 and BEC and IS eosinophil percentage r=0.36, *P*=0.003).

The AUC ROC for prediction of favourable response in asthma/NAEB was 0.68 (95% CI 0.55-0.81) with the cut-off for BEC at 150 cells/μL (Table 3, Figure 4 d). The subanalysis of individuals who responded to ICS or ICS+LABA revealed higher accuracy, with AUC ROC 0.69 (95% CI 0.56-0.82) with the cut-off for BEC at 150 cells/μL (Table 3). Diagnostic accuracy of BEC in predicting response to anti-cough therapy was similar for all patients, patients with asthma/NAEB and for patients with asthma/NAEB/UACS (Table 3).

BEC and treatment effects in patients with other causes of CC

Positive correlations were found for BEC and FeNO and BEC and IS eosinophil percentage in the subgroup with UACS (r=0.28, *P*=0.04; r=0.39, *P*=0.003, respectively), however, there was no correlation for patients with GER. Moreover, the improvement after treatment (dVAS) moderately correlated with BEC in UACS patients (r=0.31, *P*=0.01). In contrast, no specific cut-offs were established either for GER, UACS or for BHR+ (Table 3).

DISCUSSION

Our study implies that BEC is a poor predictor of clinical response to antitussive treatment in adults with CC. Surprisingly, an elevated baseline BEC was found in only 12.7% of these patients. Using the optimal cut-off level for the entire study group (237 cells/μL), BEC was found to have a modestly high specificity (0.82) but low sensitivity (0.41). Even though the subanalysis of patients treated with ICS or ICS+LABA showed a lower cut-off level (150 cells/μL) with a higher AUC value, the sensitivity and specificity were still unsatisfactory. Almost the third of our group (31.0%) responded to therapy and responders were characterized with higher BEC, FeNO and more prevalent BHR. Additionally, in non-responders, GER was diagnosed more frequently. Although the results of our study are not encouraging in terms of the application of BEC as a predictor of therapeutic response in unselected patients with CC treated in cough centre, to the best of our knowledge, it is the first study reporting this issue. Thus, we believe this study may fill the gap in knowledge on the treatable traits in the management of CC.

Regardless of the progress in the knowledge on the pathophysiology and management of CC, the therapeutic effects are still disappointing. According to previous studies, cough persists in up to 46% of patients, despite thorough clinical assessment and the application of cause oriented treatment.[8,41] The complexity of the diagnostics and specific therapies is further augmented by the coexistence of different causes of CC in the same patient. Previous studies showed co-occurrence of multiple CC causes in up to 72% patients.[41] Unsatisfactory results of CC treatment may also result from hypersensitivity of cough reflex, which is a common pathomechanism of RCC. In these patients other treatment modalities should be implemented - primarily speech and language intervention or treatment with neuromodulators. The complexity of CC management requires a multidisciplinary approach and a high level of expertise. This also generates a high economic burden for healthcare systems. In the era of

personalized medicine, the need to identify specific biomarkers which could facilitate diagnostics and treatment is emphasized not only in asthma or COPD but also in CC.[42] A therapeutic strategy based on "treatable traits" is currently a recognized paradigm for different respiratory diseases. Treatable traits are phenotypic features or biomarkers that enable the implementation of precision treatment. Eosinophilic airway inflammation is a well-recognized treatable trait in chronic airway diseases.[43] Due to limited access to markers of eosinophilic airway inflammation, blood eosinophilia is used as a surrogate marker for airway eosinophilic inflammation in both asthma and COPD.[21]

The recent ERS and ACCP guidelines on the management of CC highlighted the necessity of the assessment of airway and systemic eosinophilic inflammation.[1,44] However, to our knowledge the significance of BEC as a predictor of treatment effectiveness in patients with CC has not been previously evaluated. We assumed that it might be useful in the management of patients with CC related to airway eosinophilic inflammation. A T2 inflammatory pattern is certainly responsible for the pathogenesis of some causes of CC, such as asthma, NAEB, allergic rhinitis or chronic rhinosinusitis.[15] On the other hand, the role of BEC in patients with CC associated with other underlying diseases seemed to be uncertain. Furthermore, the coexistence of different cough associated conditions is common. Therefore, we decided to analyse BEC as a predictor of response to therapy in unselected patients with CC and normal chest X-ray referred to our cough clinic.

Previous studies showed that airway eosinophilia is a frequent feature of CC, accounting for up to 25-50% of patients, with a mean IS eosinophil percentage of 3%.[1,13,14] The above data are inconsistent with our results demonstrating sputum eosinophilia in only 13.6% of patients. This may be at least in part attributed to the investigated population, i.e. patients with CC referred for management to a tertiary cough centre. Irrespective of the differences between the prevalence of IS eosinophilia in patients with CC, limited access to IS

cytology imposes a search for surrogate biomarkers, including BEC.[45] However, Hastie et al. showed that diagnostic accuracy of BEC in prediction eosinophilic phenotype of asthma is as low as 0.63.[46] The other accessible and non-invasive tool for the measurement of airway eosinophilic inflammation is FeNO. Despite the low prevalence of airway eosinophilic inflammation in this study, significant correlations between BEC and FeNO as well as BEC and IS eosinophil count were found for all patients, as well as the subgroups with asthma, UACS but not GER. These findings are consistent with those reported by Sadeghi et al., who showed a moderate correlation between IS eosinophils, FeNO and BEC in CC patients.[47] Despite numerous previous studies on the utility of FeNO in the prediction of asthma or NAEB in CC patients, no strong evidence for its usefulness as a predictor of response to corticosteroids in CC patients was found.[48]

The relationship between blood eosinophils and the efficacy of asthma treatment was confirmed in previous studies.[49] It has been shown that eosinophil-targeted therapy is effective in the asthmatic population with BEC even as low as 150 cells/μL, which is consistent with our results.[19] Although the previous studies did not confirm the efficacy of biologic therapies targeting eosinophils in reduction of cough in asthmatics,[50] a new randomized trial concerning impact of mepolizumab on CC in asthma is planned (NCT04765722). Besides, the recent cohort trial showed that mepolizumab is effective in reduction of cough in patients with asthma.[51]

Although we found a positive correlation between response to therapy and BEC among asthmatics and NAEB patients, the clinical importance of this finding is questionable as this correlation was weak. This weak association between improvement after treatment and BEC could be caused by a low proportion of patients with blood eosinophilia in our study population. As expected, we demonstrated that BEC is not useful in predicting therapeutic response in CC associated with GER. Furthermore, we found that GER was more common in

patients who failed to respond to CC therapy. As demonstrated previously, GER may be considered as an unfavourable factor in CC treatment.[52] The low efficacy of GER-related cough therapy is thought to be affected by the lack of available causal treatment options and the coexistence of cough hypersensitivity syndrome.[53,54]

Our finding that the significant reduction in cough severity was achieved in approximately 31% of patients suggests low treatment efficacy and it is consistent with previous studies.[41] Recent reports emphasize the role of cough hypersensitivity syndrome as the important reason for this failure.[10] It has been pointed out that the causes of CC, including asthma, NAEB, GER or UACS could be only the triggers for cough and treatment of underlying conditions may be insufficient to cough resolution.[54] Thus, irrespective of the identified CC trigger, the management of patients with refractory CC should include speech and language intervention and neuromodulators or new antitussive drugs.[8,55]

We are aware of several limitations in our study. Firstly, this was a single-centre analysis with a limited number of patients. Certainly, the design of this non-controlled study diminishes the power of the results. However, the study design is due to the fact that this analysis was part of a larger real life study on the causes and efficacy of treatment of CC which has been running in since 2009. Thus confirmation of these results requires the design of the randomized controlled trial. Secondly, blood cell count was evaluated in two blood samples but only the higher value for each patient was included in the analysis. This seems to be an unresolved issue in different studies with no consensus, to date.[56-58] The third limitation could be a potential selection bias associated with the recruitment of patients in the tertiary cough clinic in a university hospital (a reference centre). This may have increased the proportion of patients with difficult-to-treat CC, characterized with severe, long-lasting CC, with a history of therapy failure, which could also explain the low prevalence of BEC in this group. Next, a cough monitoring system was not available in our cough clinic, so the cough

severity assessment was based on VAS and LCQ. Although VAS had not been validated in cough severity assessment and no minimal clinical important difference was established in CC so far, VAS is commonly used in clinical practice and clinical trials. The 30-mm cut-off point in VAS was set arbitrarily, but the same cut off has been proposed recently in COUGH-1 and COUGH-2 trials.[39] Lastly, as the study was a non-experimental, real-life, cohort study, the treatment applied to patients differed in some aspects (i.e. different types of inhalers and different doses of drugs) which certainly could affect the treatment efficacy. Despite all these limitations, we believe that the results of this study indicating a low value of BEC as a prognostic marker could may fill the gap in knowledge on the treatable traits in the management of unselected patients with CC.

In conclusion, we found that due to its low accuracy, BEC was a weak predictor of favourable response to therapy in adults with CC treated in the tertiary cough centre. Moreover, an elevated BEC was rather uncommon in the investigated population. Therefore, despite the availability of BEC, its clinical utility in management of CC appears to be limited.

LIST OF ABBREVIATIONS

ACCP, American College of Chest Physicians

AUC, area under the curve

BEC, blood eosinophil count

BHR, bronchial hyperresponsiveness

BTS, British Thoracic Society

CC, chronic cough

CHS, cough hypersensitivity syndrome

COPD, chronic obstructive pulmonary disease

dVAS, reduction in cough severity

ERS, European Respiratory Society

FeNO, fractional exhaled nitric oxide

$FEV_{1,}$ forced expiratory volume in 1 second

GER, gastroesophageal reflux

GINA, Global Initiative for Asthma

ICS, inhaled corticosteroids

IgE, immunoglobulin E

INAH, intranasal antihistamines

INCS, intranasal corticosteroids

IS, induced sputum

LABA, long-acting β_2-agonist

LCQ, Leicester Cough Questionnaire

LTRA, leukotriene receptor antagonist

MCT, methacholine challenge test

NAEB, non-asthmatic eosinophilic bronchitis

OAH, oral antihistamines

OCS, oral corticosteroids

PC_{20}, provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second

PPI, proton pump inhibitors

QoL, quality of life

RCC, refractory chronic cough

ROC, receiver operating characteristic

UACS, upper airway cough syndrome

UCC, unexplained chronic cough

VAS, visual analogue scale

VC, vital capacity

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ARF and MD had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses; ARF, MD, EMG, KBG, KK, OT, RK contributed to the study concept and design; ARF, MD, EMG, KBG, KK, OT, PNG, MPG collected, analyzed or interpreted the data; ARF, MD, RK wrote the draft manuscript and did the statistical analyses; all authors revised the final manuscript for important intellectual content.

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Tables

Table 1. Characteristics of the study population

	n=142
Age (years)	57.5 (46.0-67.0)
Gender (female n, %)	103 (72.5%)
Smoking history (n ex-smokers, %)	41 (28.9%)
Smoking history (packyears)	7.5 (5.0-15.0)
Cough duration (months)	60.0 (36.0-120.0)
Serum total IgE concentration (IU/mL)	19.0 (8.0-49.0)
BMI (kg/m^2)	26.6 (22.4-30.8)
FeNO (ppb)	15.8 (11.6-25.1)
FEV_1 (L)	2.5 (2.1-3.0)
FEV_1 (% predicted)	94.0 (81.0-100.0)
VC (L)	3.4 (2.7-4.2)
VC (% predicted)	105.0 (95.0-113.0)
FEV_1/FVC (%)	75.3 (69.7-79.0)
FEV_1/FVC (percentile)	28.0 (11.0-52.0)
Bronchial hyperresponsiveness (n, %)[&]	48 (39.3%)
PC_{20} (mg/mL)	1.4 (0.3-4.0)
IS eosinophil percentage (%)[*]	1.0 (0.0-2.0)
IS eosinophil count > 3% (n, %)[*]	17 (13.6%)
Blood eosinophil count (cells/µL)	148.8 (99.1-237.8)
Blood eosinophils ≥ 300 cells/µL (n, %)	18 (12.7%)
Baseline LCQ score (points)	11.1 (8.9-14.0)
Baseline cough severity score in VAS (mm)	55.0 (37.0-76.0)
Atopy (n, %)	43 (30.3%)
NAEB (n, %)	7 (4.9%)
Asthma (n, %)	67 (47.2%)
UACS (n, %)	65 (45.8%)
GER (n, %)	73 (51.4%)
Baseline RCC/UCC (n, %)	6 (4.2%)
Only asthma (n, %)	35 (24.6%)
Only NAEB (n, %)	1 (0.7%)
Only UACS (n, %)	11 (7.7%)
Only GER (n, %)	24 (16.9%)

Data are presented as median and interquartile range or numbers and percentages

n, number of patients; IgE, immunoglobulin E; BMI, body mass index (kg/m^2); FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; PC_{20}, provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second; IS, induced sputum; LCQ, Leicester Cough Questionnaire; VAS, visual analogue scale; NAEB,

non-asthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome; GER, gastroesophageal reflux; RCC, refractory chronic cough; UCC, unexplained chronic cough [&] methacholine provocation challenge was performed in 122 (85.9%) of patients; [*] sputum was collected in 125 (88.0%) patients

Table 2. Comparison of patients with good and unsatisfactory therapeutic response

	patients without improvement in cough severity (dVAS <30 mm and LCQ <1.3 points) n=98, 69.0%	**patients with decrease in cough severity (dVAS ≥30 mm and LCQ ≥1.3 points) n=44, 31.0%**	***P* value**
Age (years)	56.0 (45.0-67.0)	61.0 (47.0-67.0)	0.526
Gender (female n, %)	69 (70.4%)	34 (77.3%)	0.398
Cough duration (months)	60.0 (34.0-120.0)	60.0 (36.0-120.0)	0.949
Serum total IgE concentration (IU/mL)	16.0 (7.0-45.0)	25.0 (10.0-52.0)	0.265
BMI (mg/m^2)	26.9 (23.0-30.8)	25.7 (22.2-31.0)	0.241
FeNO (ppb)	14.7 (10.9-24.6)	18.2 (15.2-26.7)	0.040
FEV_1 (L)	2.5 (2.2-3.2)	2.4 (1.8-2.7)	0.099
FEV_1 (% predicted)	94.0 (81.0-101.0)	93.0 (82.0-99.0)	0.647
VC (L)	3.5 (2.8-4.5)	3.2 (2.5-4.0)	0.234
VC (% predicted)	106.0 (96.0-114.0)	101.5 (93.0-111.0)	0.211
FEV_1/FVC (%)	74.9 (70.0-78.6)	76.5 (68.5-79.0)	0.956
FEV_1/FVC (percentile)	25.0 (11.0-52.0)	38.5 (11.0-52.0)	0.891
Presence of bronchial hyperresponsiveness (n, %)[&]	27 (32.1%)	21 (55.3%)	0.015
PC_{20} (mg/mL) [&]	1.4 (0.3-3.7)	1.0 (0.4-4.3)	0.958
IS eosinophil percentage (%)[*]	1.0 (0.0-2.0)	1.0 (0.0-2.0)	0.630
IS eosinophil count >3% (n, %)[*]	73 (85.9%)	35 (87.5%)	0.806
Blood eosinophil count (cells/μL)	141.8 (97.9-216.5)	184.8 (118.1-278.8)	0.026
Blood eosinophils ≥300 cells/μL (n, %)	90 (91.8%)	34 (77.3%)	0.016
Baseline LCQ score (points)	11.7 (9.6-14.9)	10.1 (8.1-12.9)	0.009
Baseline cough severity in VAS (mm)	45.5 (30.0-70.0)	75.0 (57.5-84.5)	<0.001
Post treatment LCQ score (points)	12.5 (10.0-15.7)	16.9 (14.0-19.0)	<0.001
Post treatment VAS (mm)	40.5 (20.0-68.0)	15.0 (5.5-27.5)	<0.001
Change in LCQ score (points)	1.0 (-0.5-3.3)	5.6 (3.2-7.4)	<0.001
Change in cough severity VAS (mm)	4.5 (-10.0-18.0)	50.0 (40.0-68.0)	<0.001
Atopy (n, %)	29 (29.6%)	14 (31.8%)	0.789
Asthma or NAEB (n, %)	46 (46.9%)	28 (63.6%)	0.065
UACS (n, %)	44 (44.9%)	21 (47.7%)	0.754
GER (n, %)	56 (57.1%)	17 (38.6%)	0.041
Baseline RCC/UCC (n, %)	5 (5.1%)	1 (1.0%)	0.899

Data are presented as median and interquartile range or numbers and percentages

n, number of patients; IgE, immunoglobulin E; BMI, body mass index (kg/m^2); FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; VC, vital

capacity; PC_{20}, provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second; IS, induced sputum; LCQ, Leicester Cough Questionnaire; VAS, visual analogue scale; NAEB, non-asthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome; GER, gastroesophageal reflux; RCC, refractory chronic cough; UCC, unexplained chronic cough

[&] methacholine provocation challenge was performed in 122 (85.9%) of patients; [*] sputum was collected in 125 (88.0%) patients

Table 3. Diagnostic accuracy of blood eosinophil count in prediction of a good treatment response in patients with chronic cough

	Youden J index	**AUC**	**95% CI**	**Cut-off BEC threshold (cells/μL)**	***P* value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**dACC**
All patients	0.23	0.62	0.51-0.72	237	0.026	0.41	0.82	0.50	0.75	0.69
Patients with asthma or NAEB										
Asthma/NAEB	0.34	0.68	0.55-0.81	150	0.005	0.75	0.59	0.53	0.79	0.65
Asthma/NAEB/UACS	0.25	0.63	0.52-0.74	171	0.019	0.58	0.68	0.49	0.75	0.64
ICS/ICS+LABA therapy	0.37	0.69	0.56-0.82	150	0.005	0.74	0.63	0.57	0.78	0.67
ICS/ICS+LABA/ ICS+LABA+LTRA therapy	0.34	0.68	0.55-0.81	150	0.005	0.75	0.59	0.53	0.79	0.65
Patients with other causes of chronic cough										
UACS	0.33	0.64	0.49-0.80	319	0.070					
GER	0.17	0.53	0.37-0.69	259	0.680					
BHR+[&]	0.26	0.58	0.41-0.74	150	0.378					

CI, confidence interval; AUC, area under the receiver operating curve; BEC, blood eosinophil count; PPV, positive predictive value; NPV, negative predictive value; dACC, diagnostic accuracy; NAEB, non-asthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome GER, gastroesophageal reflux; BHR+, presence of bronchial hyperresponsiveness; ICS, inhaled corticosteroids; LABA, long-acting β_2-agonist; LTRA, leukotriene receptor antagonist; [&] methacholine provocation challenge was performed in 122 (85.9%) of patients

Figures legends

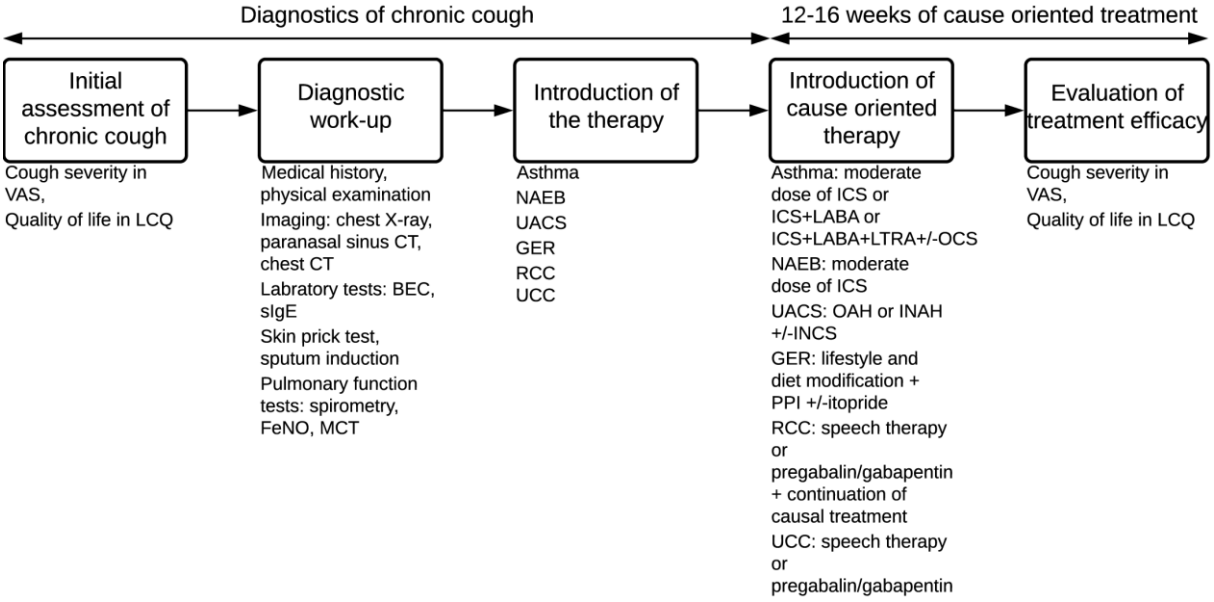


Figure 1. Study design.

VAS, visual analogue scale; CT, computed tomography; BEC, blood eosinophil count; sIgE, specific immunoglobulin E; FeNO, fractional exhaled nitric oxide; MCT, methacholine challenge test; NAEB, non-asthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome; GER, gastroesophageal reflux; RCC, refractory chronic cough; UCC, unexplained chronic cough; ICS, inhaled corticosteroids; LABA, long-acting β_2-agonist; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroid; OAH, oral antihistamines; INAH, intranasal antihistamines; INCS, intranasal corticosteroids

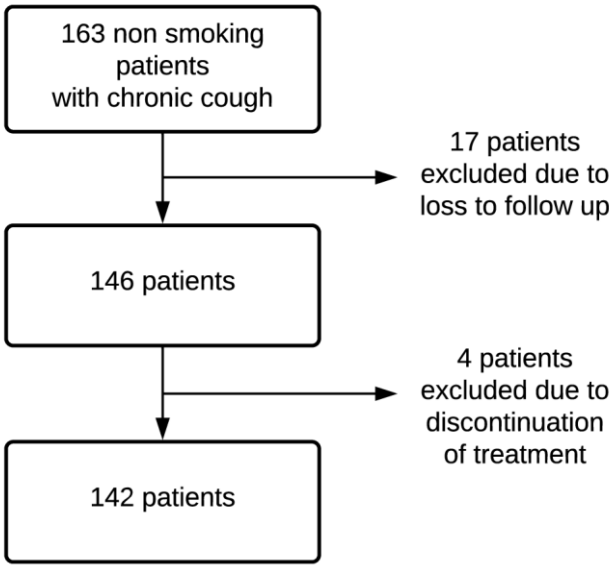


Figure 2. Flowchart of included group.

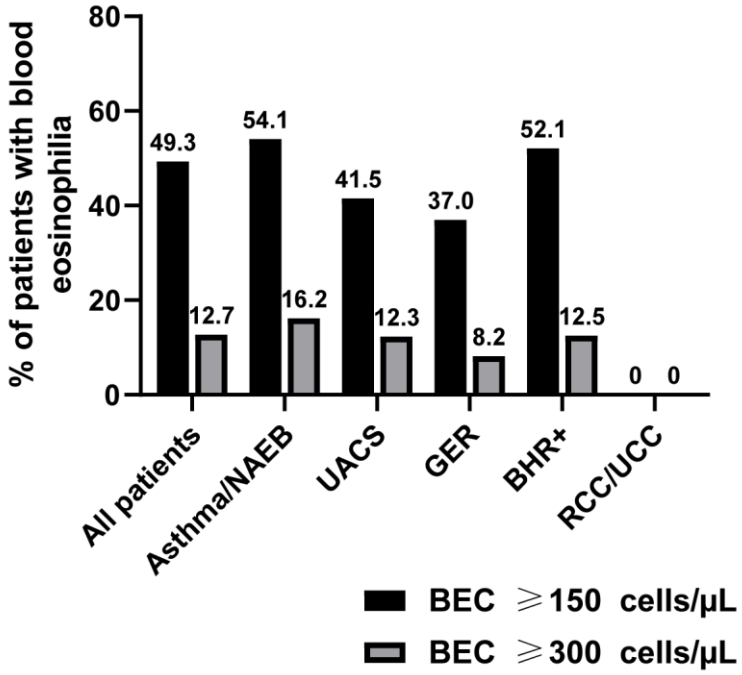


Figure 3. Prevalence of eosinophilia in sub-groups with different causes of chronic cough. BEC, blood eosinophil count; NAEB, non-asthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome; GER, gastroesophageal reflux; BHR+, presence of bronchial hyperresponsiveness; RCC, refractory chronic cough; UCC, unexplained chronic cough

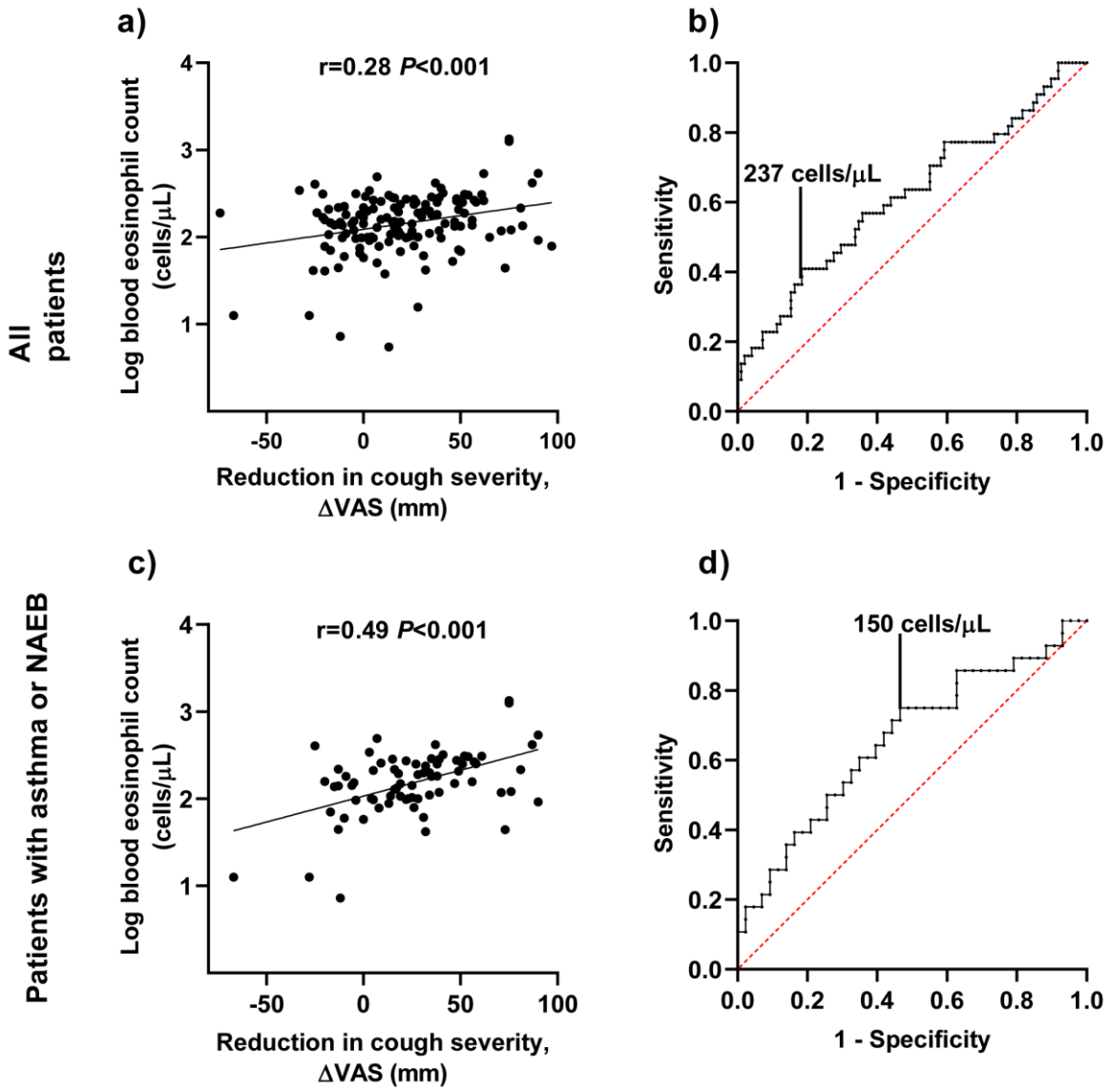
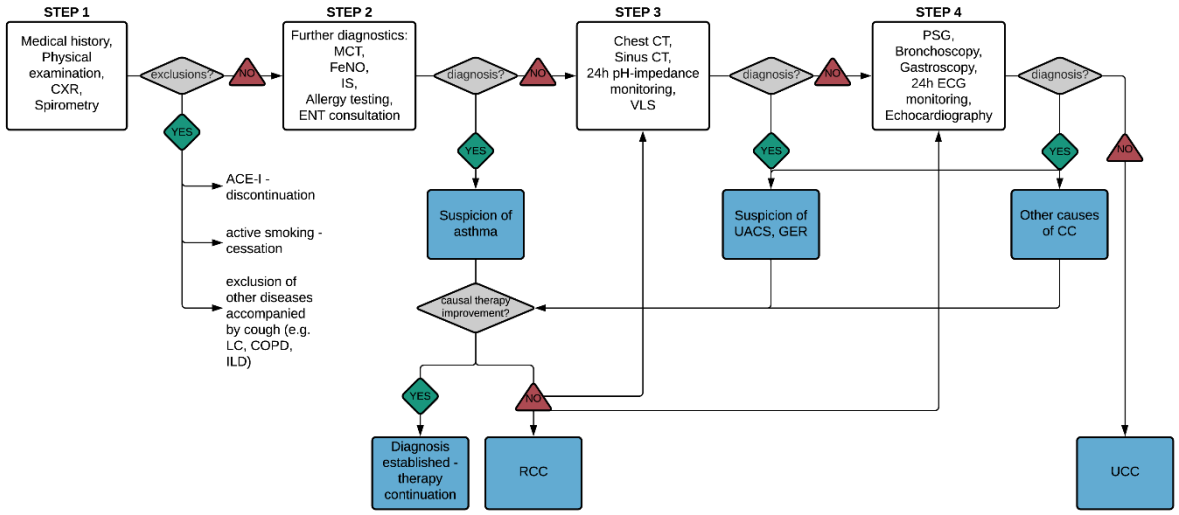


Figure 4. a) Correlation between reduction in cough severity and blood eosinophil count in all patients with chronic cough; b) The receiver operating characteristic curve with analysis of blood eosinophil count cut-off for prediction of a treatment response in all patients with chronic cough; c) Correlation between reduction in cough severity and blood eosinophil count in patients with asthma or NAEB; d) The receiver operating characteristic curve with analysis of blood eosinophil count cut-off for prediction of a treatment response in patients with asthma or NAEB

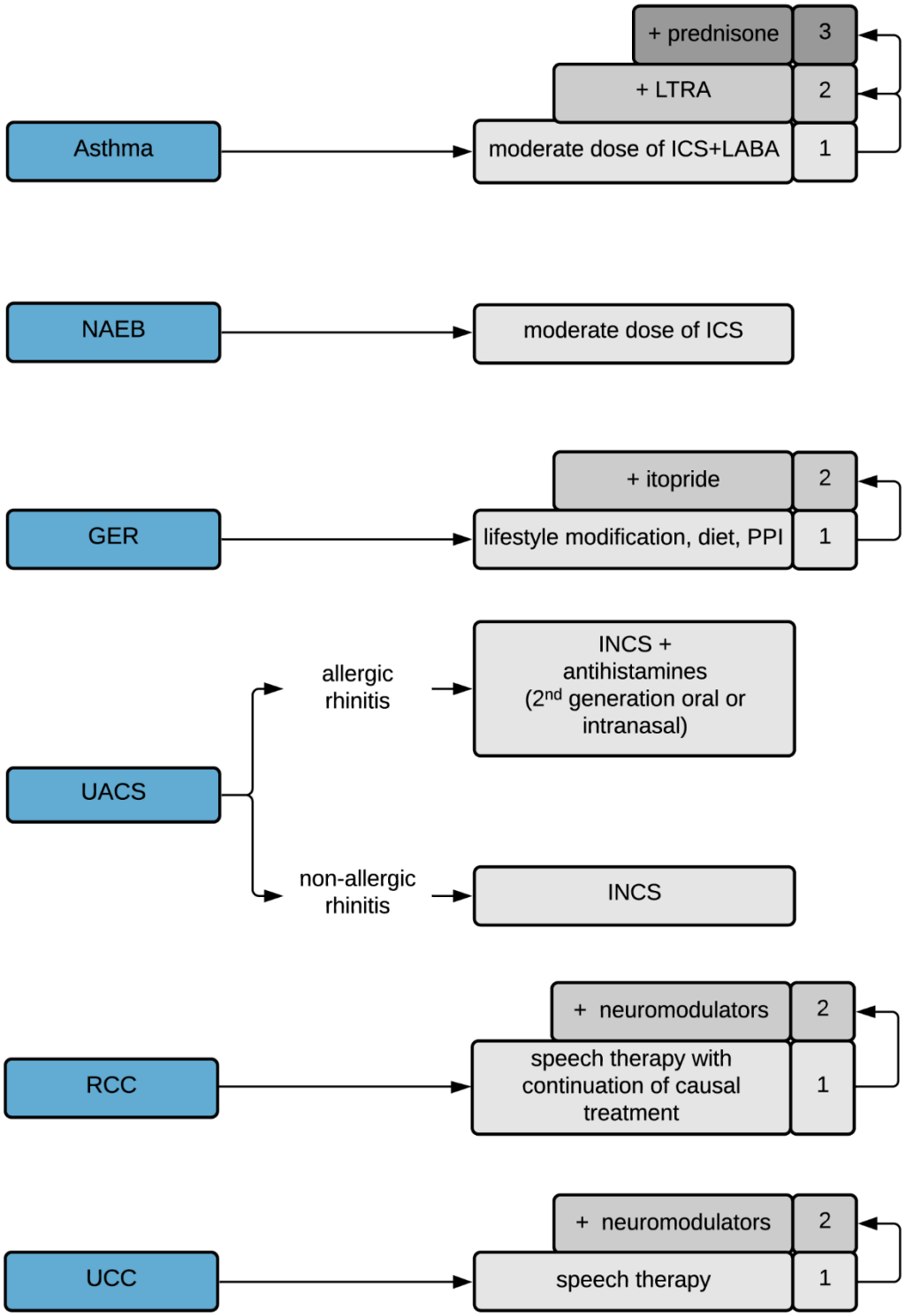
NAEB, non-asthmatic eosinophilic bronchitis; VAS, visual analogue scale



Supplementary Figure 1

Diagnostic stepwise protocol for patients with chronic cough

CXR, chest X-ray; ACE-I, angiotensin-converting enzyme inhibitors; LC, lung cancer; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; MCT, methacholine challenge test; ENT, otorhinolaryngology; RCC, refractory chronic cough; UACS, upper airway cough syndrome; GER, gastroesophageal reflux, PSG, polysomnography, ECG, electrocardiogram; CC, chronic cough; UCC, unexplained chronic cough


Asthma
moderate dose of ICS+LABA
1
+ LTRA
2
+ prednisone
3
NAEB
moderate dose of ICS
GER
lifestyle modification, diet, PPI
1
+ itopride
2
UACS
allergic rhinitis
INCS + antihistamines (2nd generation oral or intranasal)
non-allergic rhinitis
INCS
RCC
speech therapy with continuation of causal treatment
1
+ neuromodulators
2
UCC
speech therapy
1
+ neuromodulators
2

Supplementary Figure 2

General treatment algorithm in patients with chronic cough

ICS, inhaled corticosteroids; LABA, long-acting β_2-agonist; LTRA, leukotriene receptor antagonist; PPI, proton pump inhibitors; INCS, intranasal corticosteroids