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EAACI position paper on the clinical use of the bronchial allergen challenge : Unmet needs and research priorities

Agache, Ioana; Antolin-Amerigo, Dario; de Blay, Frederic; Boccabella, Cristina; Caruso, Cristiano ...

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PROF. IOANA AGACHE (Orcid ID : 0000-0001-7994-364X)

PROF. FRÉDÉRIC DE BLAY (Orcid ID : 0000-0001-5678-2214)

MS. CRISTINA BOCCABELLA (Orcid ID : 0000-0003-1942-8886)

DR. CRISTIANO CARUSO (Orcid ID : 0000-0001-7631-029X)

PROF. MARIANA COUTO (Orcid ID : 0000-0003-4987-9346)

DR. JEAN LUC FAUQUERT (Orcid ID : 0000-0002-6929-9819)

DR. GAIL M GAUVREAU (Orcid ID : 0000-0002-6187-2385)

MS. ALINA GHERASIM (Orcid ID : 0000-0002-3198-1396)

PROF. LUDGER KLIMEK (Orcid ID : 0000-0002-2455-0192)

PROF. CATHERINE LEMIERE (Orcid ID : 0000-0003-3667-9628)

DR. PARAMESWARAN NAIR (Orcid ID : 0000-0002-1041-9492)

DR. LUIS PEREZ DE LLANO (Orcid ID : 0000-0003-2652-6847)

PROF. OLIVER PFAAR (Orcid ID : 0000-0003-4374-9639)

DR. CARMEN RONDON (Orcid ID : 0000-0003-0976-3402)

DR. SANNA TOPPILA-SALMI (Orcid ID : 0000-0003-0890-6686)

DR. IBON EGUILUZ-GRACIA (Orcid ID : 0000-0002-3774-931X)

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Authors: Ioana Agache, Dario Antolin-Amerigo, Frederic de Blay, Cristina Boccabella, Cristiano Caruso, Pascal Chanez, Mariana Couto, Ronina Covar, Serge Doan, Jean-Luc Fauquert, Gail Gauvreau, Alina Gherasim, Ludger Klimek, Catherine Lemiere, Parameswaran Nair, Iñigo Ojanguren, David Peden, Luis Perez-de-Llano, Oliver Pfaar, Carmen Rondon, Maia Rukhazde, Joaquin Sastre, Johannes Schulze, Diana Silva, Susan Tarlo, Sanna Toppila-Salmi, Jolanta Walusiak-Skorupa, Stefan Zielen, Ibon Eguiluz-Gracia.

Affiliations:

Ioana Agache, Faculty of Medicine, Transylvania University, Brasov, Romania

Dario Antolin-Amerigo, Servicio de Alergia, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain.

Frederic de Blay, ALYATEC Environmental Exposure Chamber. Chest Diseases Department Strasbourg University Hospital. University of Strasbourg Strasbourg France.

Cristina Boccabella, Department of Cardiovascular and Thoracic Sciences, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario A. Gemelli - IRCCS, Rome, Italy.

Cristiano Caruso, Allergy Unit, Fondazione Policlinico A. Gemelli, IRCCS, Rome, Italy.

Pascal Chanez, Department of Respiratory CIC Nord INSERMINRAE C2VN, Aix Marseille University, Marseille, France.

Mariana Couto, Centro de Alergia, Hospital CUF Descobertas, Lisboa, Portugal.

Ronina Covar, National Jewish Health, 2930, Pediatrics, Denver, Colorado, United States.

Serge Doan, Fondation A de Rothschild, Paris, France.

Jean-Luc Fauquert, University Hospital, Clermont-Ferrand France.

Gail Gauvreau, Division of Respirology, Department of Medicine, McMaster University, Hamilton, ON, Canada.

Alina Gherasim, ALYATEC Environmental Exposure Chamber, Strasbourg, France.

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Ludger Klimek, Center for Rhinology and Allergology, Wiesbaden, Germany.

Catherine Lemiere, Research Centre, Centre Intégré Universitaire de santé et de services sociaux du Nord-de-l'île-de-Montréal, Montréal, QC, Canada; Faculty of Medicine, Université de Montreal, Montreal, QC, Canada.

Parameswaran Nair, Firestone Institute of Respiratory Health at St. Joseph's Healthcare, Department of Medicine, McMaster University, Hamilton, ON, Canada.

Iñigo Ojanguren, Servei de Pneumología, Hospital Universitari Valld´Hebron, Departament de Medicina, Universitat Autònoma de Barcelona (UAB). Institut de Recerca (VHIR). CIBER de Enfermedades Respiratorias (CIBERES), Barcelona, Spain.

David Peden, Division of Pediatric Allergy and Immunology, Center for Environmental Medicine, Asthma and Lung Biology, The School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC, US.

Luis Perez de Llano, Department of Respiratory Medicine, University Hospital Lucus Augusti, Spain.

Oliver Pfaar, Department of Otorhinolaryngology, Head and Neck Surgery, Section of Rhinology and Allergy, University Hospital Marburg, Philipps-Universität Marburg, Marburg, Germany.

Carmen Rondon, Allergy Unit, Hospital Regional Universitario de Malaga, and Instituto de Investigacion Biomedica de Malaga (IBIMA). Malaga, Spain.

Maia Rukhazde, Center of Allergy & Immunology, Teaching University Geomedi LLC, Tbilisi, Georgia.

Joaquin Sastre, Allergy Unit, Hospital Universitario Fundación Jiménez Díaz. Center for Biomedical Network of Respiratory Diseases (CIBERES), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

Johannes Schulze, Department for Children and Adolescents, Division of Allergology, Pulmonology and Cystic Fibrosis, Goethe-University Hospital, Frankfurt am Main, Germany.

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Diana Silva, Basic and Clinical Immunology Unit, Department of Pathology, Faculty of Medicine, University of Porto and Serviço de Imunoalergologia, Centro Hospitalar São João, EPE - Porto, Portugal..

Susan Tarlo, Respiratory Division, Department of Medicine, University Health Network, Toronto Western Hospital, University of Toronto Department of Medicine, and Dalla Lana Department of Public Health, Toronto, Ontario, Canada.

SannaToppila-Salmi, Haartman Institute, Medicum. Skin and Allergy Hospital, Hospital District of Helsinki and Uusimaa, Helsinki University Hospital and University of Helsinki. Helsinki, Finland.

Jolanta Walusiak-Skorupa, Department of Occupational Diseases and Environmental Health, Nofer Institute of Occupational Medicine, Łódź, Poland.

Stefan Zielen, Department for Children and Adolescents Division of Allergology, Pulmonology and Cystic fibrosis, Goethe-University Hospital, Frankfurt am Main, Germany.

Ibon Eguiluz-Gracia, Allergy Unit, Hospital Regional Universitario de Malaga, and Instituto de Investigacion Biomedica de Malaga (IBIMA). Malaga, Spain.

Corresponding author:

Ibon Eguiluz-Gracia, Allergy Unit, Hospital Regional Universitario de Malaga, Allergy Research Group, Instituto de Investigacion Biomedica de Malaga (IBIMA) and ARADyAL, Hospital Civil. Plaza del Hospital Civil, s/n. 29009 Malaga, Spain. E-mail: iboneguiluz@gmail.com

ORCID

Ioana Agache: 0000-0001-7994-364X

DarioAntolin-Amerigo: 0000-0001-5699-4022

Frederic de Blay: 0000-0001-5678-2214

Cristina Boccabella: 0000-0003-1942-8886.

Cristiano Caruso: 0000-0001-7631-029X

Pascal Chanez: 0000-0003-4059-0917

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Mariana Couto: 0000-0003-4987-9346

Gail Gauvreau: 0000-0002-6187-2385

Alina Gherasim: 0000-0002-3198-1396

LudgerKlimek: 0000-0002-2455-0192

Catherine Lemiere: 0000-0003-3667-9628

ParameswaranNair: 0000-0002-1041-9492

Iñigo Ojanguren: 0000-0003-1270-5772

David Peden: 0000-0003-4526-4627

Luis Perez de Llano: 0000-0003-2652-6847

Oliver Pfaar: 0000-0003-4374-9639

Carmen Rondon: 0000-0003-0976-3402

MaiaRukhazde: 0000-0002-1413-8161

Joaquin Sastre: 0000-0003-4689-6837

Diana Silva: 0000-0002-0656-7206

Susan M Tarlo: 0000-0002-4746-5310

SannaToppila-Salmi: 0000-0003-0890-6686

JolantaWalusiak-Skorupa: 0000-0003-0836-6800

Ibon Eguiluz-Gracia: 0000-0002-3774-931X

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Abbreviations

AA = allergic asthma

AIT = allergen immunotherapy

BAC = bronchial allergen challenge

CAC = conjunctival allergen challenge

EAACI = European Academy of Allergy and Clinical Immunology

EAR = early asthmatic reaction

HDM = house dust mites

LAR = late asthmatic reaction

NAC = nasal allergen challenge

Mab = monoclonal antibody

SLIT = sublingual immunotherapy

SPT = skin prick test

T2 = type 2

Box 1: Glossary of terms
Atopy = genetic predisposition to develop IgE-driven immune responses towards non-pathogenic environmental antigens (allergens). Atopyrepresents a risk factor for

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allergic diseases like allergic rhinitis, allergic asthma, atopic dermatitis or IgE-mediated food allergy.

Atopic sensitisation: positivity of skin prick test and/or detectable serum specific IgE (sIgE) against allergens.

Allergic asthma = asthma phenotype driven by sIgE-mediated inflammation, and where the allergen is the main trigger of asthma symptoms.

Bronchial challenge(provocation) = A procedure to identify and characterise subjects with hyperresponsive airways consisting on the inhalation of an agent known to cause (or suspected of causing) a drop in lung function.

Local respiratory allergy = rhinitis or asthma symptoms driven by the allergen in a subject without systemic atopic sensitization

T2 asthma = asthma endotype with evidence of a type-2 immune response driving asthma pathogenetic features. Three major pathways account for T2 asthma: the IgE pathway, the IL-5 pathway and the IL-4/IL-13 pathway together with the contribution of resident cells (epithelium, mucus producing cells and airway smooth muscle cells), neural inflammation and epigenetic mechanisms.

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Abstract

Allergic asthma (AA) is a common asthma phenotype and its diagnosis requires both the demonstration of IgE-sensitization to aeroallergens, and the causative role of this sensitization as a major driver of asthma symptoms. Therefore, a bronchial allergen challenge (BAC) would be occasionally required to identify AA patients among atopic asthmatics. Nevertheless, BAC is usually considered a research tool only, with existing protocols being tailored to mild asthmatics and research needs (e.g. long washout period for inhaled corticosteroids). Consequently, existing BAC protocols are not designed to be performed in moderate-to-severe asthmatics or in clinical practice.

The correct diagnosis of AA might help select patients for immunomodulatory therapies. Allergen sublingual immunotherapy is now registered and recommended for controlled or partially controlled patients with house dust mite-driven AA and with FEV1≥70%. Allergen avoidance is costly and difficult to implement for the management of AA, so the proper selection of patients is also beneficial.

In this Position Paper, the EAACI Task Force proposes a methodology for clinical BAC that would need to be validated in future studies. The clinical implementation of BAC could ultimately translate into a better phenotyping of asthmatics in real life, and into a more accurate selection of patients for long-term and costly management pathways.

1. Introduction

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Allergic asthma (AA) is defined by the presence of atopic sensitisations in an asthma patient in association with the allergen being a driver of asthma symptoms. AA is a common asthma phenotype (1, 2) which is encountered across the lifespan, and across all asthma severity steps. Moreover, atopic sensitisation is related to asthma severity and control (3-5). Nevertheless, not every patient with asthma with sensitisation to an aeroallergen experiences asthma symptoms upon exposure to the specific allergen. In patients sensitised to seasonal allergens or suffering from intermittent symptoms, the clinical history often suffices to establish the relevance of atopic sensitisations. Conversely, in patients with persistent asthma, with sensitisation to perennial allergens or polysensitised, demonstrating the clinical relevance of atopic sensitisation poses more difficulties. In some cases, the demonstration of the involvement of the allergen as a major driver of asthma symptoms could be only achieved through in vivo provocations, such as the nasal or the bronchial allergen challenge (NAC and BAC, respectively) (6). The BAC aims to evaluate the response of the bronchi to a controlled inhaled allergen exposure.

Interest in clarifying the allergic triggers of asthma symptoms is mainly due to the possibility of establishing personalized management strategies based on the pattern of allergen-specific reactivity. Measures to control environmental exposure to indoor allergens (e.g. epithelia from furry animals or house-dust mites) can control AA symptoms (7). Moreover, the identification of allergic triggers of asthma might be paramount to decide on life choices (e.g. hobbies, place of residence, pet keeping, etc.). On the other hand, new modalities of allergen immunotherapy (AIT), such as sublingual tablets of house dust mite extract (HDM) (HDM-SLIT) registered as pharmaceutical products, have demonstrated a robust capacity to improve critical asthma outcomes (dose of inhaled corticosteroids (ICS) needed to maintain control, exacerbations, etc.) (8, 9). Therefore, HDM-SLIT is now recommended by international guidelines for the treatment of HDM-driven AA (10, 11). Unlike other AIT modalities requiring prior good asthma control to be initiated, HDM-SLIT is recommended for patients with partially controlled asthma and with forced expiratory volume in the 1st second (FEV_1)≥70%,with the aim to facilitate asthma control. Nevertheless, HDM-SLIT requires distinguishing between AA and asthma with atopic sensitisation, as only patients with AA should receive AIT (10). The ability of the clinical history to interpret atopic sensitizations in severe asthmatics is particularly limited, due to the severity and

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persistence of their symptoms. Moreover,>70% of severe asthmatics have positive skin prick tests (SPT) to aeroallergens (2).

Despite these clinical needs, BAC is not currently recommended for daily practice, beyond the diagnosis of occupational AA (14-16).This fact contrasts with the wider implementation of other bronchial challenges (both direct and indirect provocations, see Table 1), which are routinely performed to assess for airway hyperresponsiveness (AHR) as an essential step in asthma diagnosis (17). Several factors can explain the differences in the clinical implementation of the tests. Although bronchial challenges are always laborious procedures requiring appropriate facilities and trained personnel (17), BAC needs a longer observation period due to the higher possibility of a late asthmatic response (LAR) (14). Moreover,allergens can induce a more profound bronchoconstriction than other stimuli (18). This aspect is particularly difficult because asthma medication should be discontinued before bronchial challenges (17). Therefore, according to current protocols, most atopic patients with moderate-to-severe asthma cannot undergo BAC.

BAC is currently a standard tool for translational studies. Research BAC aims to alter lung function to study the mechanisms of airway allergy (18), or analyse the effect of asthma medication (19). Published studies mostly include mild asthmatics with a clinical history indicative of AA who maintain asthma control without ICS for a period long enough to achieve the required washout. Upon BAC, these subjects experience an early asthmatic response (EAR, see section 5 for definition). In some patients, the EAR is followed by a LAR (see section 5 for definition) (20). Interestingly, the frequency of LAR is allergen-specific, ranging from 15% for pollens to 75-90% for HDM (21). Research BAC has been paramount to demonstrate that therapies blocking the inflammatory pathways of AA increase the allergen dose eliciting an EAR and inhibit LAR (22-43). In this regard, LAR is considered the most useful parameter to evaluate the effect of most anti-inflammatory asthma drugs (Figure 1). Research studies show that ICS greatly affect the occurrence and extent of LAR, whereas their influence over EAR is less pronounced. Therefore, there is little flexibility in the washout period for ICS in research BAC protocols.

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For clinical BAC, shorter washout periods could be considered because the occurrence of EAR suffices to establish the positivity of the test. Moreover, shorter washout periods would favour the maintenance of control in moderate-to-severe asthmatics, and they would also decrease the frequency and extent of LAR (44), thus facilitating BAC implementation in the clinic.

In this position paper, a group of experts on bronchial and allergen challenges and AA analyse the available literature on the topic, and identify the methodological aspects of BAC that should be re-examined to facilitate its clinical implementation beyond occupational asthma. These methodological modifications should be validated in future studies in order to progress with the implementation of BAC in daily practice.

2. Indications

The indication of clinical BAC would be to investigate the capacity of an allergen to induce an asthmatic response in a patient sensitised to that allergen when the history is not conclusive regarding the clinical relevance of the sensitization. This clarification would be particularly useful in asthma patients on ICS/long-acting β2 agonists (LABA) who continue having symptoms suggestive of AA, as it can guide a different therapeutic approach (e.g. AIT, allergen avoidance measures like alpine altitude climate therapy, etc.) to achieve and maintain asthma control.

Bronchial allergen-specific reactivity has also been described in patients with local allergic rhinitis (negative for SPT and serum allergen-specific (s)IgE but with symptoms driven by the allergen) (45-47). Although local allergic rhinitis and asthma are not yet well-validated phenotypes, NAC and BAC would be required to diagnose patients with these conditions.

3. Contraindications

The absolute and relative contraindications proposed for clinical BAC are presented in table 2

4. Proposed methodology for clinical BAC

4.1. Preliminary considerations

Clinical BAC should always be performed in a setting fully equipped for pulmonary lung testing and with capability to quickly manage a possible adverse reaction.

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Previous spirometry conducted during a period of controlled disease would be required to demonstrate that the patient can perform correct manoeuvres (6, 14).

Hour of the day

BAC should start early in the morning (14).

Exposure to environmental allergens

For pollen allergens, BAC should be performed outside the pollination season. For perennial allergens, BAC should be conducted at the moment of minimal allergen burden (6, 14, 18). In polysensitised patients, BAC should be performed when the patient does not report symptoms (conjunctival, nasal or bronchial) that could be attributable to other allergens he/she is allergic to (6, 18).

Acclimation period

The patient should arrive at the clinic 30 minutes before the test is started. No significant efforts (running, cycling, etc.) should be done the morning of the test (17).

Washout period for asthma drugs

All drugs approved for asthma treatment affect allergen-induced bronchial responses and inflammation (28, 51-54) (Figure 1). Moreover, oral antihistamines also attenuate EAR (27). ICS greatly affect the magnitude of LAR, AHR, and sputum eosinophilia in a dose-dependent manner (14, 28, 52-54). Therefore, research BAC requires ICS discontinuation for 2-4 weeks prior to the test. However, the capacity of ICS to dampen EAR nearly disappears 12 hours after treatment administration on average, although significant intra-class variability exists (55). Therefore, a shorter withdrawal period (e.g.24 – 48 hours for ICS) could be considered for clinical BAC. The same rationale applies to all relevant antinflammatory anti-asthmatic drugs.

Therefore, this position paper proposes evaluating the following approach in future studies:

Scenario 1

If patients tolerate the withdrawal of asthma medication (they remain controlled and FEV_1 is ≥70%), the washout periods should be those indicated in the “optimal” column of table 3 (14, 51, 55, 56).

Scenario 2

In patients who lose asthma control and/or experience a drop in FEV_1<70% upon withdrawal of asthma controller medication, the washout periods could be shortened up to the limits indicated

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in the “minimal” column of table 3 (6). The washout periods should be as close to the optimal period as the patient tolerates. To limit false negative results when following this approach we suggest to interpret the results as described in section 5.

4.2 *Allergen extracts and doses*

Wherever available, allergen extracts specifically manufactured for bronchial challenge should be used (57). In any case, they should be standardised. Extracts are usually commercialized as lyophilised powder to be reconstituted with a diluent (e.g. 0.9% saline solution) before administration. Allergen extracts manufactured for SPT are not recommended for BAC, because they are glycerinated and can irritate the respiratory mucosa (58). Some researchers have used dilutions of AIT tablets to perform NAC (59), but this approach has not been tested for BAC.

In mild asthmatics and when a modern dosimeter device is used for inhalation, it is safe and reproducible to start with a dose of 10 standardised biological units (SBU) followed by four doubling concentrations (cumulative dose: 10, 30, 70, 150 and 310 SBU). This 5-dose protocol can be applied safely to patients with baseline FEV_1 82-120% in order to induce an EAR (44). If the allergen content is not provided in SBU, the starting concentration can be determined by endpoint titration of SPT (1/10000, 1/1000, 1/100, 1/10 and 1/1 dilutions). BAC would be initiated with the lowest concentration inducing a ≥3-mm wheal, followed by the inhalation of doubling concentrations or of concentrations following a geometric progression, up to 1/1 (14). This approach also offers additional safety guarantees and should be prioritized in moderate-to-severe asthmatics.

4.3 *Procedure for delivering the inhaled allergen*

An incremental high-dose provocation is recommended for clinical BAC, because a bolus provocation would require a prior incremental screening challenge (60), and repeated low-dose BAC are more time consuming. Before allergen administration, a baseline spirometry should be performed, followed by a diluent inhalation (same solution used to reconstitute the allergen extract). A second spirometry should be conducted 10 minutes after the end of the inhalation (14). If the diluent challenge is negative (post-diluent FEV_1 is >80% of baseline measurement), allergen concentrations are administered at 12-minute intervals with spirometry measured in duplicate 10 minutes after each consecutive allergen dose. The use of dosimeter (e.g. Misty Max© or Wright© disposable nebulizer with an output of 0.13 mL/min) is preferred over jet

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nebulizer (e.g. DeVilbiss 646) because of the lower access of the allergen-containing aerosols fromthe room environment. Nevertheless, both methods are equally reproducible (14, 61). Both tidal breathing (e.g. 2-minute breathing/allergen dose) and counted breath dosimeters can be used to inhale the allergen (61, 62).

4.4 Observation during and after allergen inhalation.

The subject should never be left unattended during the challenge procedure or during observation period (14).

Positive test (EAR occurs, see section 5):

In clinic observation

As soon as EAR is detected the patient should be treated with inhaled salbutamol (2 puffs of metered-dose inhaler (MDI) using a spacer device) every 20 minutes for 1 hour and with 0.5-1 mg/kg of oral prednisone or equivalent. After 1 hour, a full clinical assessment should be performed and spirometry should be repeated. If FEV1 is <90% of post-diluent measurement, 4-10 puffs of salbutamol should be administered (alternatively, a nebulization of 5 mg/2 ml of salbutamol can be considered). If reversibility is not achieved after 20 minutes, intramuscular epinephrine (0.3-0.5 mg) should be given at the same time that the emergency response team (ERT) is contacted for transfer to ICU. The ERT should also be called at any point during BAC protocol if the patient is agitated, confused or drowsy, uses accessory respiratory muscles while having a silent chest, and/or has a heart rate >120 bpm, or a respiratory rate >30/min (Figure 2).

It is important to note that the management of EAR responses proposed in this position paper is meant to be applied in a heterogeneous population of asthmatics and in the clinic. BAC performed for research purposes includes mostly mild asthmatics. Thus, inhaled salbutamol is not always administered after EAR is detected and oral corticosteroids are avoided in order to preserve LAR. The differences between clinical and research protocols are based on safety considerations and on the different goals of each BAC modality.

Patients responsive to salbutamol (FEV_1<10% lower than pre-allergen post-diluent measurement) can be discharged when clinically stable (14). Before the patient leaves the clinic, the physician should check the inhaler technique and all patients should be given a written action plan and a kit containing oral prednisone for a 5-day course, ICS/formoterol inhaler and a peak expiratory flow (PEF) device to monitor their lung function hourly during the 24 hours following allergen

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inhalation (excluding sleeping hours). The physician should also check that the patient has sufficient amount of their regular asthma medication (6, 14).

At home observation

Individuals should be instructed to restart taking their regular controller medication during the evening of BAC day, and to perform extra inhalations of ICS/formoterol (regardless of the drugs used as controller medication) as reliever (11) whenever symptoms occur (maximum 2 puffs every 20 minutes for 1 hour). In case of symptoms, patients should also measure PEF every 30 minutes. If awakenings occur during nighttime due to asthma symptoms, patients should measure PEF immediately before performing ICS/formoterol inhalations. If LAR persists (see section 5) in two consecutive measurements or symptoms do not disappear with the maximum ICS/formoterol dose, the patient should be instructed to call the responsible physician and/or attend the Emergency Room (49).

Negative test: EAR did not occur (see section 5)

In clinic observation

Scenario 1 (symptoms do not occur during the 3 hours following allergen inhalation): the patient can be discharged.

Scenario 2 (symptoms occur during the 3 hours following allergen inhalation): the patient should be treated as described below for positive tests, and is discharged when clinically stable. Nevertheless, the test is not considered positive, and the physician might decide to repeat the BAC in a new appointment.

At home observation

Same recommendations as for patients experiencing EAR apply. In case of symptoms or LAR (see section 5) during home observation, the test is not considered positive, as isolated LAR are unlikely associated with IgE-dependent mechanisms. The physician might decide to repeat BAC in a new appointment.

4.5 Measurement of inflammatory biomarkers

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When the optimal washout period for antiasthma drugs is not achieved, fractional exhaled nitric oxide (FeNO) should be measured prior to the pre-diluent spirometry. In case of inconclusive BAC results (see section 5), the patient is advised not to restart the regular asthma therapy until a second FeNO measurement (24 hours after BAC) is conducted (21, 63). The quantification of blood or sputum eosinophils at baseline and 24 hours after allergen inhalation (52, 60, 64) might also help interpret inconclusive results, yet an increase of blood eosinophils does not always follow positive BAC, while sputum cytology is not readily available in all clinical settings (46). When the optimal washout period for asthma drugs is achieved or BAC results are unequivocal, the measurement of inflammatory biomarkers is optional.

Figure 3 shows a diagram with the proposed methodology for clinical BAC.

5 **Readouts**:

5.1. Change in respiratory symptoms

Although positive BAC frequently induces cough, wheezing or dyspnoea there is no validated score allowing an accurate interpretation of the test without considering changes in airway function.

5.2. Change in airway function

FEV_1:

Optimal washout period of asthma drugs was achieved:

Positivity criteria: occurrence of EAR

EAR would be defined as a drop in FEV_1 ≥20% as compared to pre-allergen post-diluent measurement, and occurring up to three hours following allergen inhalation. EAR usually occurs within ten minutes, peaks between ten and 30 minutes and resolves within two hours after allergen inhalation (65). FEV_1 is the preferred read-out to monitor BAC because it excels in evaluating the obstruction of the large airways, the main event driving EAR.

Of note, the occurrence of LAR does not define BAC positivity (66). LAR is defined as a drop in FEV_1 ≥15% as compared to pre-allergen post-diluent measurement and occurring three-to-seven hours after allergen inhalation (14).

Shorter than optimal washout period for asthma drugs was achieved

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Positivity criteria: occurrence of EAR.

Same cut-off point as in the previous protocol, but the test is considered inconclusive if FEV_1 falls 15-20% within three hours after allergen inhalation. In this case, two mutually non-exclusive approaches could be followed:

1. To consider the test positive if FeNO increases with 17.5 ppb 24 hours after BAC.
2. To repeat the test on a separate day and consider BAC positive if two consecutive borderline results are observed.

Of note, with this protocol, it would not be possible to evaluate the occurrence of LAR (18).

The observation period and managementof inconclusive results would be the same as cases with confirmed EAR.

Other spirometry parameters:

PEF is not as sensitive as FEV_1 for EAR monitoring (60, 67-70). For logistical reasons, the use of PEF is acceptable for LAR monitoring. A drop ≥20% or an absolute reduction of ≥80 L/min as compared to pre-allergen post-diluent PEF measurement and occurring after three hours following allergen inhalation have been proposed as cut-off points for LAR identification (69). These cut-off points have better specificity (92-93%) than sensitivity (61-71%) (60, 67-70).

FEF_{25-75} and FEF_{50} do not bring any significant advantage over FEV_1 (71, 72) and do not have validated cut-off points for EAR or LAR diagnosis.

Parameters measured by impulse oscillometry:

Although FEV_1 is superior for EAR evaluation (71), impulse oscillometry (IOS) indexes (R5-R20) might be an alternative for patients unable to perform reliable spirometry manoeuvres. However, no clear cut-off point has been validated for EAR interpretation (73).

5.3. Change in airway responsiveness

Direct bronchial challenges: a one- to two-fold decrease in the methacholine or histamine concentration inducing ≥20% fall in FEV1 (PC_{20}) in the 24 hours following allergen exposure is usually observed in patients with positive BAC. Thus, comparing the PC_{20} before and after BAC provides another useful tool for BAC interpretation (14, 18). Nevertheless, considering that it would require three consecutive visits to the clinic, this approach is not recommended for routine practice.

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Indirect bronchial challenges: airway responsiveness to mannitol or adenosine monophosphate decreases (74) 3 to 24 hours (75) after positive BAC, probably due to the depletion of mast cells mediators. Therefore, indirect provocations are not useful for BAC interpretation.

5.4. Change in inflammatory biomarkers

A previous study demonstrated that an increase ≥17.5 ppb in FeNO 24 hours after bronchial challenge with high molecular weight sensitizers displays an optimal sensitivity and specificity for the diagnosis of occupational AA (76). Importantly, the washout period for ICS in this study was 72 hours. Although the overall value of FeNO to monitor bronchial challenges with occupational agents is controversial, a FeNO increase is consistently seen in patients with positive bronchial challenges with high molecular weight sensitizers (mostly subjects with occupational AA) (77). Therefore, a ≥17.5 ppb increase in FeNO could be used as positivity cut-off point for patients who do not achieve the optimal washout period of asthma drugs and display borderline results.

The increase of sputum eosinophils after positive BAC has been shown for decades (65, 78). Although a 2.2% increase in sputum eosinophil counts is more sensitive than FeNO for the identification of positive BAC (79), cytologic evaluation of sputum is not yet widely adopted in the clinic. On the other hand, the increase in peripheral blood eosinophils is not consistently observed after positive BAC (80).

6 **Safety**

Despite being well-tolerated in most cases, BAC can occasionally induce life-threatening bronchoconstriction (81). Moreover, BAC is performed in subjects with mild AA following long washout periods for ICS. Therefore, it is currently unknown how BAC would be tolerated in a heterogeneous population of patients with asthma in the clinic. For additional safety guarantees, a peripheral venous access can be secured before BAC initiation. Although no case has been described following respiratory exposure, aeroallergens can rarely trigger anaphylaxis after ingestion (82).

6.1. *Safety requirements to treat adverse events following BAC*

Setting: a hospital is preferred over outpatient clinic, yet the limiting factor is ≤30-minute distance to ERT.

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Personnel: BAC must be conducted by a competent physician supported by a trained nurse/technician. All members of the team should be trained in resuscitation methods and in the management of acute bronchoconstriction and anaphylaxis.

Emergency equipment: written protocols for bronchoconstriction, anaphylaxis and resuscitation together with ERT phone number should be close-at-hand. Moreover, emergency equipment must be available on site including: a) a ready-to-use oxygen source and mask and pulse oximeter; b) a nebulizing device; c) inhaled and intravenous bronchodilators (both short-acting β2 agonist and anti-cholinergic); d) oral and parenteral corticosteroids; e) cardiopulmonary resuscitation equipment, intravenous fluids (plasma expander and normal saline), intramuscular and intravenous epinephrine, oral and intravenous antihistamines, needles, and syringes.

6.2 Safety requirements to protect healthcare professionals and other patients from infection, sensitisation or allergen-driven symptoms

Patients: national/regional guidelines should be followed. In some cases, a prior negative PCR or rapid antigen test for SARS-CoV-2 for patients undergoing spirometry is required.

Setting: Simultaneous challenges should not be performed in the same room (even if different devices are used) and a complete air exchange of the room is required between different patients and every 30 minutes (14). Exhaust or fume hoods or high-efficiency particulate air (HEPA) cleaners constitute another option (14, 83). Automated UVC sterilisation might also be used.

Personnel: health care professionals conducting BAC must wear a face filtering piece (FFP)2/FFP3 respiratory mask or a mask equipped with HEPA filter, face shields or goggles, and nitrile gloves.

BAC equipment: a dosimeter equipped with a validated expiratory filter should be used for allergen inhalation, because this method is associated with a lower access of the aerosols to the room environment, as compared to jet nebulizers (14, 61, 84). Moreover, the use of breath-activated devices can further limit environmental pollution.

7 Reproducibility

Many aspects influence BAC outcome including the baseline condition of the patient, environmental exposures in previous days (85), allergen dosing and the type of the aeroallergen. Other important aspect is the time interval between consecutive BACs, or between BAC and

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other bronchial or allergen provocations. The minimal interval recommended between consecutive challenges would be:

- Between consecutive BAC or between BAC and an environmental allergen challenge with the same or different allergen: 4 weeks (86).
- Between BAC and indirect bronchial challenge: 3 weeks (17, 52, 54, 64, 87).
- Between BAC and direct bronchial challenge: 2 weeks (83).
- Between BAC and nasal or conjunctival challenges with the same or different allergen: 1 week (6, 58, 88).

When controlling for all these factors, BAC has a good reproducibility in mild asthmatics (89). The dose eliciting EAR in a patient subjected to consecutive bronchial challenges with the same allergen remains within a two-fold variation (87), with an excellent (0.80) intra-class correlation coefficient (ICC) demonstrated for the percentage fall in FEV_1 during the three hours following allergen inhalation (86). Moreover, a good-to-excellent (0.60-0.77) ICC has been described for LAR, whereas a good (0.60) ICC was reported for sputum eosinophilia seven hours after allergen inhalation (90).

8 Proposal for integrating BAC in the care pathways of asthma patients sensitised to HDM

Several studies investigated serum HDM-sIgE cut-off points for the identification of atopic asthmatics displaying positive BAC results. Nevertheless, neither the sensitivity nor the specificity of these cut-off points was optimal (91, 92). Therefore, further studies are required to evaluate whether a certain level of serum HDM-sIgE can replace BAC for the diagnosis of HDM-driven AA.

The presence of rhinitis is a risk factor for asthma onset, and this association is particularly strong in allergic individuals (93). Interestingly, a nasal endoscopy in asthma patients without rhinitis symptoms often reveals signs of nasal inflammation (94), whereas the performance of NAC in allergic rhinitis individuals without asthma induces an eosinophilic inflammation detectable in the bronchoalveolar lavage (95). In this regard, NAC could be a useful tool to phenotype the inflammatory disease affecting the airways regardless of its organ-specific manifestations (conjunctivitis, rhinitis and asthma). Of note, 83-89% of atopic asthmatics with rhinitis who tested positive to the nasal challenge with HDM have positive BAC results (46, 96). The NAC is a

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safe and reproducible technique (97, 98) with a validated methodology (58) and defined cut-off points for positivity (99). Moreover, challenge protocols are considerably shorter than those of BAC (58, 100). The conjunctival allergen challenge (CAC) was also proposed to phenotype nasal inflammatory disease (101), and validated protocols for its clinical use have been recently published (88, 102). Nevertheless, no study has investigated to date the correlation between BAC and CAC results. Table 4 shows a comparison between the features of CAC, NAC and BAC.

In asthma patients sensitised to HDM who suffer from concomitant rhinitis, NAC could precede BAC for the evaluation of the clinical relevance of HDM sensitisation. In the case of a negative NAC, a bronchial provocation with HDM can be considered. Figure 4 shows a proposal for a step-based approach for the diagnosis of HDM-driven AA.

The correlation between distinct organ-specific challenges might vary with different allergens. In this regard, a study performing nasal, bronchial and environmental challenges, found little correlation between upper and lower airway responses in cat-allergic individuals. Nevertheless, NAC in this study was not monitored by the objective measurement of nasal patency, and not all individuals included suffered from asthma (103). The relationship between CAC, NAC and BAC with different allergens should be investigated further to evaluate the capacity of each test to diagnose AA.

9 Unmet needs for the clinical implementation of BAC

Currently, BAC is only conducted in research settings for subjects with mild AA (14) where allergen doses usually different from natural exposure are administered. The allergen concentration eliciting EAR is patient-specific and sometimes achieved at supra-physiological doses (40, 104). Therefore, uniform dosing protocols and the use of ≥20% fall in FEV_1 as cut-off point for positivity might not be optimal to identify AA among the heterogeneous populations of asthmatics in the clinic. Thus, there is a need to validate BAC protocols administering allergen doses similar to natural exposure, and identify clinically relevant read-outs with corresponding cut-off points.

Allergen-induced responses can be life-threatening, and especially LAR might prove difficult to manage as it might occur after the patient is discharged. Because research BAC is more

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frequently performed in adults, safety information does not exist for children, although limited evidence is available (15). Moreover, the safety of BAC in moderate-to-severe asthmatics was not investigated. Withholding controller medication in moderate-to-severe asthmatics requires extreme caution, while not withholding medications might confound the interpretation of the test.

The number of centres regularly conducting research BAC is <20 world-wide. These centres have extensive experience and highly trained staff able to identify the early signs of EAR, LAR, and anaphylaxis, and to initiate immediate and adequate treatment. Given the risk of losing asthma control after BAC, these centres count also on high standards for patient follow-up and availability of medical assistance after the test. The translation of these requirements to a healthcare system and to a busy asthma clinic will pose many difficulties.

These limitations should be addressed carefully in order to support the implementation of clinical BAC.

Conclusion

The number of immunomodulatory therapies available for asthma is continuously increasing. However, these treatments are only cost-efficient in selected patients displaying specific features. Thus, there is a need to increase the number of clinical tools able to phenotype both mild and moderate-to-severe asthmatics in daily practice. An unmet need in the clinic is the correct diagnosis of AA, a step required to establish the indication of AIT. Moreover, the development of an objective tool to diagnose AA will support the reimbursement of AIT products by healthcare systems.

Due to both safety and cost-efficiency concerns the use of BAC has been restricted to research purposes to date. In this position paper, the EAACI Task Force reviewed existing literature to propose methodological modifications for BAC to be conducted with diagnostic purposes in the daily clinic. Given that the occurrence of EAR suffices to establish the positivity of BAC, the Task Force proposes to investigate the diagnostic performance for AA of clinical BAC protocols with a considerably shorter washout period for ICS. This approach represents a research priority to be

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evaluated in future studies. Moreover, the capacity of NAC and CAC to identify AA patients among atopic asthmatics is also worth of investigation.

In summary, the implementation of BAC protocols in the asthma clinic would support a more accurate characterisation of asthma patients with variable severity and control, potentially increasing the success rates of immunomodulatory therapies like AIT. However, before introduction, the safety profile evaluation should be prioritised and facilitated by a competent training curricula in allergen challenges for the clinician. Challenge protocols should be further aligned, standardised and validated, and tailored to the concept of precision medicine. Disease registries accumulating high-quality real world evidence both on the cost-effectiveness and the safety of the implementation of BAC protocols for phenotyping AA are of utmost importance to move the field forward.

References

1. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. *Nat Rev Dis Primers* 2015;**1**(1):15025.

This article is protected by copyright. All rights reserved

Accepted Article

2. Heffler E, Blasi F, Latorre M, Menzella F, Paggiaro P, Pelaia G, et al. The Severe Asthma Network in Italy: Findings and Perspectives. *J Allergy Clin Immunol Pract* 2019;**7**(5):1462-1468.

3. Del Giacco SR, Bakirtas A, Bel E, Custovic A, Diamant Z, Hamelmann E, et al. Allergy in severe asthma. *Allergy* 2017;**72**(2):207-220.

4. Sullivan PW, Lanz MJ, Ghushchyan VH, Kavati A, LeCocq J, Ortiz B, et al. Medication use and indicators of poor asthma control in patients with and without allergies. *Allergy Asthma Proc* 2019;**40**(4):221-229.

5. Sullivan PW, Lanz MJ, Ghushchyan VH, Kavati A, LeCocq J, Ortiz B, et al. Healthcare resource utilization, expenditures, and productivity in patients with asthma with and without allergies. *J Asthma* 2020;**57**(9):959-967.

6. Agache I, Bilò M, Braunstahl GJ, Delgado L, Demoly P, Eigenmann P, et al. In vivo diagnosis of allergic diseases--allergen provocation tests. *Allergy* 2015;**70**(4):355-365.

7. Dávila I, Domínguez-Ortega J, Navarro-Pulido A, Alonso A, Antolín-Amerigo D, González-Mancebo E, et al. Consensus document on dog and cat allergy. *Allergy* 2018;**73**(6):1206-1222.

8. Mosbech H, Deckelmann R, de Blay F, Pastorello EA, Trebas-Pietras E, Andres LP, et al. Standardized quality (SQ) house dust mite sublingual immunotherapy tablet (ALK) reduces inhaled corticosteroid use while maintaining asthma control: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2014;**134**(3):568-575.e567.

9. Virchow JC, Backer V, Kuna P, Prieto L, Nolte H, Villesen HH, et al. Efficacy of a House Dust Mite Sublingual Allergen Immunotherapy Tablet in Adults With Allergic Asthma: A Randomized Clinical Trial. *Jama* 2016;**315**(16):1715-1725.

10. Agache I, Lau S, Akdis CA, Smolinska S, Bonini M, Cavkaytar O, et al. EAACI Guidelines on Allergen Immunotherapy: House dust mite-driven allergic asthma. *Allergy* 2019;**74**(5):855-873.

11. Global initiative for asthma (GINA) 2021 update: https://ginasthma.org/gina-reports/ Last accessed 14.12.2021.

12. Agache I, Akdis CA, Akdis M, Canonica GW, Casale T, Chivato T, et al. EAACI Biologicals Guidelines-Recommendations for severe asthma. *Allergy* 2021;**76**(1):14-44.

13. Agache I, Rocha C, Beltran J, Song Y, Posso M, Solà I, et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab and omalizumab) for severe allergic asthma: A systematic review for the EAACI Guidelines - recommendations on the use of biologicals in severe asthma. *Allergy* 2020;**75**(5):1043-1057.

14. Diamant Z, Gauvreau GM, Cockcroft DW, Boulet LP, Sterk PJ, de Jongh FH, et al. Inhaled allergen bronchoprovocation tests. *J Allergy Clin Immunol* 2013;**132**(5):1045-1055.e1046.

This article is protected by copyright. All rights reserved

15. Rosewich M, Arendt S, El Moussaoui S, Schulze J, Schubert R, Zielen S. Bronchial allergen provocation: a useful method to assess the efficacy of specific immunotherapy in children. *Pediatr Allergy Immunol* 2013;**24**(5):434-440.

16. Eickmeier O, Zissler UM, Wittschorek J, Unger F, Schmitt-Grohé S, Schubert R, et al. Clinical relevance of Aspergillus fumigatus sensitization in cystic fibrosis. *Clin Exp Allergy* 2020;**50**(3):325-333.

17. Hallstrand TS, Leuppi JD, Joos G, Hall GL, Carlsen KH, Kaminsky DA, et al. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. *Eur Respir J* 2018;**52**(5).

18. Gauvreau GM, El-Gammal AI, O'Byrne PM. Allergen-induced airway responses. *Eur Respir J* 2015;**46**(3):819-831.

19. Boulet LP, Côté A, Abd-Elaziz K, Gauvreau G, Diamant Z. Allergen bronchoprovocation test: an important research tool supporting precision medicine. *Curr Opin Pulm Med* 2021;**27**(1):15-22.

20. Barnig C, Purohit A, Casset A, Sohy C, Lieutier-Colas F, Sauleau E, et al. Nonallergic airway hyperresponsiveness and allergen-specific IgE levels are the main determinants of the early and late asthmatic response to allergen. *J Investig Allergol Clin Immunol* 2013;**23**(4):267-274.

21. Schulze J, Reinmüller W, Herrmann E, Rosewich M, Rose MA, Zielen S. Bronchial allergen challenges in children - safety and predictors. *Pediatr Allergy Immunol* 2013;**24**(1):19-27.

22. Diamant Z, Timmers MC, van der Veen H, Friedman BS, De Smet M, Depré M, et al. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. *J Allergy Clin Immunol* 1995;**95**(1 Pt 1):42-51.

23. Dahlén B, Kumlin M, Ihre E, Zetterström O, Dahlén SE. Inhibition of allergen-induced airway obstruction and leukotriene generation in atopic asthmatic subjects by the leukotriene biosynthesis inhibitor BAYx 1005. *Thorax* 1997;**52**(4):342-347.

24. Kawayama T, O'Byrne PM, Watson RM, Killian KJ, Duong M, Yoshida M, et al. Effects of inhaled ciclesonide on circulating T-helper type 1/T-helper type 2 cells in atopic asthmatics after allergen challenge. *Clin Exp Allergy* 2006;**36**(11):1417-1424.

25. Gauvreau GM, Boulet LP, Cockcroft DW, Fitzgerald JM, Carlsten C, Davis BE, et al. Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 2011;**183**(8):1007-1014.

26. Gauvreau GM, Boulet LP, Cockcroft DW, Baatjes A, Cote J, Deschesnes F, et al. Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses. *Am J Respir Crit Care Med* 2008;**177**(9):952-958.

Accepted Article

This article is protected by copyright. All rights reserved

27. Davis BE, Illamperuma C, Gauvreau GM, Watson RM, O'Byrne PM, Deschesnes F, et al. Single-dose desloratadine and montelukast and allergen-induced late airway responses. *Eur Respir J* 2009;**33**(6):1302-1308.

28. Kelly MM, O'Connor TM, Leigh R, Otis J, Gwozd C, Gauvreau GM, et al. Effects of budesonide and formoterol on allergen-induced airway responses, inflammation, and airway remodeling in asthma. *J Allergy Clin Immunol* 2010;**125**(2):349-356.e313.

29. Singh D, Petavy F, Macdonald AJ, Lazaar AL, O'Connor BJ. The inhaled phosphodiesterase 4 inhibitor GSK256066 reduces allergen challenge responses in asthma. *Respir Res* 2010;**11**(1):26.

30. Gauvreau GM, Pageau R, Séguin R, Carballo D, Gauthier J, D'Anjou H, et al. Dose-response effects of TPI ASM8 in asthmatics after allergen. *Allergy* 2011;**66**(9):1242-1248.

31. Gauvreau GM, Boulet LP, Schmid-Wirlitsch C, Côté J, Duong M, Killian KJ, et al. Roflumilast attenuates allergen-induced inflammation in mild asthmatic subjects. *Respir Res* 2011;**12**(1):140.

32. Kent SE, Boyce M, Diamant Z, Singh D, O'Connor BJ, Saggu PS, et al. The 5-lipoxygenase-activating protein inhibitor, GSK2190915, attenuates the early and late responses to inhaled allergen in mild asthma. *Clin Exp Allergy* 2013;**43**(2):177-186.

33. Singh D, Boyce M, Norris V, Kent SE, Bentley JH. Inhibition of the early asthmatic response to inhaled allergen by the 5-lipoxygenase activating protein inhibitor GSK2190915: a dose-response study. *Int J Gen Med* 2013;**6**:897-903.

34. Singh D, Cadden P, Hunter M, Pearce Collins L, Perkins M, Pettipher R, et al. Inhibition of the asthmatic allergen challenge response by the CRTH2 antagonist OC000459. *Eur Respir J* 2013;**41**(1):46-52.

35. Diamant Z, Sidharta PN, Singh D, O'Connor BJ, Zuiker R, Leaker BR, et al. Setipiprant, a selective CRTH2 antagonist, reduces allergen-induced airway responses in allergic asthmatics. *Clin Exp Allergy* 2014;**44**(8):1044-1052.

36. Gauvreau GM, O'Byrne PM, Boulet LP, Wang Y, Cockcroft D, Bigler J, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014;**370**(22):2102-2110.

37. Leaker BR, Singh D, Ali FY, Barnes PJ, O'Connor B. The effect of the novel phosphodiesterase-4 inhibitor MEM 1414 on the allergen induced responses in mild asthma. *BMC Pulm Med* 2014;**14**:166.

38. Scheerens H, Arron JR, Zheng Y, Putnam WS, Erickson RW, Choy DF, et al. The effects of lebrikizumab in patients with mild asthma following whole lung allergen challenge. *Clin Exp Allergy* 2014;**44**(1):38-46.

39. Gauvreau GM, Boulet LP, Leigh R, Cockcroft DW, Killian KJ, Davis BE, et al. A nonsteroidal glucocorticoid receptor agonist inhibits allergen-induced late asthmatic responses. *Am J Respir Crit Care Med* 2015;**191**(2):161-167.

This article is protected by copyright. All rights reserved

40. Gauvreau GM, Arm JP, Boulet LP, Leigh R, Cockcroft DW, Davis BE, et al. Efficacy and safety of multiple doses of QGE031 (ligelizumab) versus omalizumab and placebo in inhibiting allergen-induced early asthmatic responses. *J Allergy Clin Immunol* 2016;**138**(4):1051-1059.

41. Gauvreau GM, Boulet LP, FitzGerald JM, Cockcroft DW, Davis BE, Leigh R, et al. A dual CysLT(1/2) antagonist attenuates allergen-induced airway responses in subjects with mild allergic asthma. *Allergy* 2016;**71**(12):1721-1727.

42. Singh D, Leaker B, Boyce M, Nandeuil MA, Collarini S, Mariotti F, et al. A novel inhaled phosphodiesterase 4 inhibitor (CHF6001) reduces the allergen challenge response in asthmatic patients. *Pulm Pharmacol Ther* 2016;**40**:1-6.

43. Leaker BR, Singh D, Lindgren S, Almqvist G, Eriksson L, Young B, et al. Effects of the Toll-like receptor 7 (TLR7) agonist, AZD8848, on allergen-induced responses in patients with mild asthma: a double-blind, randomised, parallel-group study. *Respir Res* 2019;**20**(1):288.

44. Schulze J, Rosewich M, Dressler M, Riemer C, Rose MA, Zielen S. Bronchial allergen challenge using the Medicaid dosimeter. *Int Arch Allergy Immunol* 2012;**157**(1):89-97.

45. Eguiluz-Gracia I, Pérez-Sánchez N, Bogas G, Campo P, Rondón C. How to Diagnose and Treat Local Allergic Rhinitis: A Challenge for Clinicians. *J Clin Med* 2019;**8**(7).

46. Campo P, Eguiluz-Gracia I, Plaza-Serón MC, Salas M, José Rodríguez M, Pérez-Sánchez N, et al. Bronchial asthma triggered by house dust mites in patients with local allergic rhinitis. *Allergy* 2019;**74**(8):1502-1510.

47. Bozek A, Winterstein J, Galuszka B, Jarzab J. Different Development Forms of Local Allergic Rhinitis towards Birch. *Biomed Res Int* 2020;**2020**:3408561.

48. Melillo G, Bonini S, Cocco G, Davies RJ, de Monchy JG, Frolund L, et al. EAACI provocation tests with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. *Allergy* 1997;**52**(35 Suppl):1-35.

49. Kowalski ML, Ansotegui I, Aberer W, Al-Ahmad M, Akdis M, Ballmer-Weber BK, et al. Risk and safety requirements for diagnostic and therapeutic procedures in allergology: World Allergy Organization Statement. *World Allergy Organ J* 2016;**9**(1):33.

50. Vandenplas O, Suojalehto H, Aasen TB, Baur X, Burge PS, de Blay F, et al. Specific inhalation challenge in the diagnosis of occupational asthma: consensus statement. *Eur Respir J* 2014;**43**(6):1573-1587.

51. Pizzichini MM, Kidney JC, Wong BJ, Morris MM, Efthimiadis A, Dolovich J, et al. Effect of salmeterol compared with beclomethasone on allergen-induced asthmatic and inflammatory responses. *Eur Respir J* 1996;**9**(3):449-455.

This article is protected by copyright. All rights reserved

52. Gauvreau GM, Doctor J, Watson RM, Jordana M, O'Byrne PM. Effects of inhaled budesonide on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1996;**154**(5):1267-1271.

53. Kelly EA, Busse WW, Jarjour NN. Inhaled budesonide decreases airway inflammatory response to allergen. *Am J Respir Crit Care Med* 2000;**162**(3 Pt 1):883-890.

54. Inman MD, Watson RM, Rerecich T, Gauvreau GM, Lutsky BN, Stryszak P, et al. Dose-dependent effects of inhaled mometasone furoate on airway function and inflammation after allergen inhalation challenge. *Am J Respir Crit Care Med* 2001;**164**(4):569-574.

55. Subbarao P, Dorman SC, Rerecich T, Watson RM, Gauvreau GM, O'Byrne PM. Protection by budesonide and fluticasone on allergen-induced airway responses after discontinuation of therapy. *J Allergy Clin Immunol* 2005;**115**(4):745-750.

56. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;**342**(8875):833-837.

57. Klimek L, Hoffmann HJ, Kalpaklioglu AF, Demoly P, Agache I, Popov TA, et al. In-vivo diagnostic test allergens in Europe: A call to action and proposal for recovery plan-An EAACI position paper. *Allergy* 2020;**75**(9):2161-2169.

58. Augé J, Vent J, Agache I, Airaksinen L, Campo Mozo P, Chaker A, et al. EAACI Position paper on the standardization of nasal allergen challenges. *Allergy* 2018;**73**(8):1597-1608.

59. Eifan AO, Fedina A, Durham SR, Scadding GW. Comparison of nasal allergen challenges with dissolved Timothy Grass pollen tablets and aqueous extract. *Allergy* 2020.

60. Frølund L, Svendsen UG, Nielsen NH, Weeke B, Madsen F. Bronchial allergen challenge: comparison between two different methods of provocation. *Clin Allergy* 1987;**17**(5):439-448.

61. Taylor DA, Harris JG, O'Connor BJ. Comparison of incremental and bolus dose inhaled allergen challenge in asthmatic patients. *Clin Exp Allergy* 2000;**30**(1):56-63.

62. Diamant Z, Timmers MC, van der Veen H, Page CP, van der Meer FJ, Sterk PJ. Effect of inhaled heparin on allergen-induced early and late asthmatic responses in patients with atopic asthma. *Am J Respir Crit Care Med* 1996;**153**(6 Pt 1):1790-1795.

63. Ihre E, Gyllfors P, Gustafsson LE, Kumlin M, Dahlén B. Early rise in exhaled nitric oxide and mast cell activation in repeated low-dose allergen challenge. *Eur Respir J* 2006;**27**(6):1152-1159.

64. Gauvreau GM, Boulet LP, Postma DS, Kawayama T, Watson RM, Duong M, et al. Effect of low-dose ciclesonide on allergen-induced responses in subjects with mild allergic asthma. *J Allergy Clin Immunol* 2005;**116**(2):285-291.

Accepted Article

This article is protected by copyright. All rights reserved

65. Gauvreau G, Watson R, Rerecich T, Baswick E, Inman M, O'Byrne P. Repeatability of allergen-induced airway inflammation. *J Allergy Clin Immunol* 1999;**104**:66-71.

66. Ali FR, Oldfield WL, Higashi N, Larché M, Kay AB. Late asthmatic reactions induced by inhalation of allergen-derived T cell peptides. *Am J Respir Crit Care Med* 2004;**169**(1):20-26.

67. Bérubé D, Cartier A, L'Archevêque J, Ghezzo H, Malo JL. Comparison of peak expiratory flow rate and FEV1 in assessing bronchomotor tone after challenges with occupational sensitizers. *Chest* 1991;**99**(4):831-836.

68. Giannini D, Paggiaro PL, Moscato G, Gherson G, Bacci E, Bancalari L, et al. Comparison between peak expiratory flow and forced expiratory volume in one second (FEV1) during bronchoconstriction induced by different stimuli. *J Asthma* 1997;**34**(2):105-111.

69. Moscato G, Dellabianca A, Paggiaro P, Bertoletti R, Corsico A, Perfetti L. Peak expiratory flow monitoring and airway response to specific bronchial provocation tests in asthmatics. *Monaldi Arch Chest Dis* 1993;**48**(1):23-28.

70. Weytjens K, Malo JL, Cartier A, Ghezzo H, Delwiche JP, Vandenplas O. Comparison of peak expiratory flows and FEV1 in assessing immediate asthmatic reactions due to occupational agents. *Allergy* 1999;**54**(6):621-625.

71. Stenberg H, Diamant Z, Ankerst J, Bjermer L, Tufvesson E. Small airway involvement in the late allergic response in asthma. *Clin Exp Allergy* 2017;**47**(12):1555-1565.

72. Lieutier-Colas F, Purohit A, Meyer P, Fabries JF, Kopferschmitt MC, Dessanges JF, et al. Bronchial challenge tests in patients with asthma sensitized to cats: the importance of large particles in the immediate response. *Am J Respir Crit Care Med* 2003;**167**(8):1077-1082.

73. Naji N, Keung E, Kane J, Watson RM, Killian KJ, Gauvreau GM. Comparison of changes in lung function measured by plethymography and IOS after bronchoprovocation. *Respir Med* 2013;**107**(4):503-510.

74. Amakye DO, Davis BE, Martin AL, Peters GE, Cockcroft DW. Refractoriness to inhaled mannitol 3 hours after allergen challenge. *Ann Allergy Asthma Immunol* 2013;**111**(3):182-184.

75. Davis BE, Amakye DO, Cockcroft DW. Airway responsiveness to mannitol 24 h after allergen challenge in atopic asthmatics. *Allergy* 2015;**70**(6):682-688.

76. Lemiere C, S NG, Sava F, D'Alpaos V, Huaux F, Vandenplas O. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. *J Allergy Clin Immunol* 2014;**134**(5):1063-1067.

77. Betancor D, Villalobos V, Valverde-Monge M, Barroso B, Sastre J. Exhaled nitric oxide is of limited value in the diagnosis of occupational asthma.J Allergy Clin Immunol Pract. 2021;9(4):1726-1727.e2.

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78. Pin I, Freitag A, O'Byrne P, Girgis-Gavardo A, Watson R, Dolovich J, et al. Changes in the cellular profile of induced plutum after allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992;**145**:1265-1269.

79. Lemière C, D'Alpaos V, Chaboillez S, César M, Wattiez M, Chiry S, et al. Investigation of occupational asthma: sputum cell counts or exhaled nitric oxide? *Chest* 2010;**137**(3):617-622.

80. Racine G, Castano R, Cartier A, Lemiere C. Diagnostic Accuracy of Inflammatory Markers for Diagnosing Occupational Asthma. *J Allergy Clin Immunol Pract* 2017;**5**(5):1371-1377.e1371.

81. Vandenplas O, D'Alpaos V, Evrard G, Jamart J. Incidence of severe asthmatic reactions after challenge exposure to occupational agents. *Chest* 2013;**143**(5):1261-1268.

82. Sompornrattanaphan M, Jitvanitchakul Y, Malainual N, Wongsa C, Jameekornrak A, Theankeaw O, et al. Dust mite ingestion-associated, exercise-induced anaphylaxis: a case report and literature review. *Allergy Asthma Clin Immunol* 2020;**16**:2.

83. Coates AL, Wanger J, Cockcroft DW, Culver BH, Diamant Z, Gauvreau G, et al. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J* 2017;**49**(5).

84. Olaguibel JM, Alobid I, Alvarez Puebla M, Crespo-Lessmann A, Domínguez Ortega J, García-Rio F, et al. Functional Examination of the Upper and Lower Airways in Asthma and Respiratory Allergic Diseases: Considerations in the Post-SARS-CoV-2 Era. *J Investig Allergol Clin Immunol* 2021;**31**(1):17-35.

85. Eguiluz-Gracia I, Mathioudakis AG, Bartel S, Vijverberg SJH, Fuertes E, Comberiati P, et al. The need for clean air: The way air pollution and climate change affect allergic rhinitis and asthma. *Allergy* 2020;**75**(9):2170-2184.

86. Dente FL, Bacci E, di Franco A, Giannini D, Conti I, Macchioni P, et al. Reproducibility of early and late asthmatic responses to allergen challenge in a large group of asthmatics. *Respir Med* 2000;**94**(5):441-447.

87. Kopferschmitt-Kubler MC, Bigot H, Pauli G. Allergen bronchial challenge tests: variability and reproducibility of the early response. *J Allergy Clin Immunol* 1987;**80**(5):730-740.

88. Fauquert JL, Jedrzejczak-Czechowicz M, Rondon C, Calder V, Silva D, Kvenshagen BK, et al. Conjunctival allergen provocation test : guidelines for daily practice. *Allergy* 2017;**72**(1):43-54.

89. Lee WY, Southworth T, Singh D. Different inhaled allergen challenge models give reproducible results. *Pulm Pharmacol Ther* 2015;**33**:57-58.

90. Gauvreau GM, Watson RM, Rerecich TJ, Baswick E, Inman MD, O'Byrne PM. Repeatability of allergen-induced airway inflammation. *J Allergy Clin Immunol* 1999;**104**(1):66-71.

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91. Minami T, Fukutomi Y, Lidholm J, Yasueda H, Saito A, Sekiya K, et al. IgE Abs to Der p 1 and Der p 2 as diagnostic markers of house dust mite allergy as defined by a bronchoprovocation test. *Allergol Int* 2015;**64**(1):90-95.

92. Schulze J, Leberkuehne L, Salzmann-Manrique E, Schubert R, Zielen S, Rosewich M. Comparison of two different assays and the predictive value of allergen components in house dust mite allergy. *Immunotherapy* 2017;**9**(15):1253-1262.

93. Shaaban R, Zureik M, Soussan D, Neukirch C, Heinrich J, Sunyer J, et al. Rhinitis and onset of asthma: a longitudinal population-based study. *Lancet* 2008;**372**(9643):1049-1057.

94. Linneberg A, Henrik Nielsen N, Frølund L, Madsen F, Dirksen A, Jørgensen T. The link between allergic rhinitis and allergic asthma: a prospective population-based study. The Copenhagen Allergy Study. *Allergy* 2002;**57**(11):1048-1052.

95. Braunstahl GJ, Overbeek SE, Kleinjan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol* 2001;**107**(3):469-476.

96. Fischl A, Eckrich J, Passlack V, Klenke SK, Hartmann D, Herrmann E, et al. Comparison of bronchial and nasal allergen provocation in children and adolescents with bronchial asthma and house dust mite sensitization. *Pediatr Allergy Immunol* 2020;**31**(2):143-149.

97. Eguiluz-Gracia I, Testera-Montes A, González M, Pérez-Sánchez N, Ariza A, Salas M, et al. Safety and reproducibility of nasal allergen challenge. *Allergy* 2019;**74**(6):1125-1134.

98. Pantin CT, Southworth T, Wetzel K, Singh D. Reproducibility of nasal allergen challenge responses in adults with allergic rhinitis. *Clin Pharmacol* 2019;**11**:67-76.

99. Eguiluz-Gracia I, Testera-Montes A, Salas M, Perez-Sanchez N, Ariza A, Bogas G, et al. Comparison of diagnostic accuracy of acoustic rhinometry and symptoms score for nasal allergen challenge monitoring. *Allergy* 2021;**76**(1):371-375.

100. Pepper AN, Ledford DK. Nasal and ocular challenges. *J Allergy Clin Immunol* 2018;**141**(5):1570-1577.

101. Schröder J, Mösges R. Conjunctival provocation tests: prediction of seasonal allergy. *Curr Opin Allergy Clin Immunol* 2018;**18**(5):393-397.

102. Pfaar O, Claßen DP, Astvatsatourov A, Klimek L, Mösges R.Reliability of a New Symptom Score in a Titrated Quantitative Conjunctival Provocation Test Supported by an Objective Photodocumentation.Int Arch Allergy Immunol. 2018;176(3-4):215-224.

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103. Sicherer SH, Wood RA, Eggleston PA. Determinants of airway responses to cat allergen: comparison of environmental challenge to quantitative nasal and bronchial allergen challenge.J Allergy Clin Immunol. 1997;99(6 Pt 1):798-805.

104. Cockcroft DW, Davis BE, Boulet LP, Deschesnes F, Gauvreau GM, O'Byrne PM, et al. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. *Allergy* 2005;**60**(1):56-59.

Figure legends

Figure 1: Early and late asthmatic responses during a positive bronchial allergen challenge and the different drugs with a known effect (or lack of effect) on both responses, airway hyperresponivenes and sputum eosinophilia. Allergic subjects experience a drop in FEV1 10-15 minutes after allergen inhalation. This early response is driven by the activation of mast cells and basophils (through IgE-medicated mechanisms) and neutrophils, which leads to the constriction of smooth muscle in the large airways. Early asthmatic response usually resolves within 3 hours. Some allergic patients also develop a later drop in FEV1 which is driven by inflammatory phenomena (recruitment and activation of group 2 innate lymphoid cells (ILC2), Th2 cells, IgE-producing B cells and eosinophils) together by the activation of the bronchial epithelium. The frequency of the late asthmatic response is allergen-specific and ranges from 15% for seasonal pollens to 85-90% for house dust mites. The late asthmatic response usually peaks 7 hours after allergen inhalation and the fall in FEV1 can be more profound than during the early response. LTC4: leukotriene C4; PGD2: prostaglandin D2; TSLP; thymic stromal lymphopoietin.

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Figure 2: Proposed management of early asthmatic responses after bronchial allergen challenge. This algorithm is also amenable for patients experiencing positive diluent challenge or symptoms after a negative bronchial allergen challenge. Bpm: beats per minute; ERT: emergency response team; MDI: metered-dose inhaler; PEF: peak expiratory flow; FEV1: forced expiratory volume in the 1st second

Figure 3: Proposed protocol for bronchial allergen challenge and subsequent observation periods. FeNO: fractional exhaled nitric oxide; ICS: inhaled corticosteroids; ERT: emergency response team; FEV1: forced expiratory volume in the 1st second; MDI: metered-dose inhaler; PEF: peak expiratory flow; SABA: short-acting β2 agonist.

Figure 4: Proposed algorithm for the diagnosis of house dust mite-driven allergic asthma. AIT: allergen immunotherapy; FeNO: fractional exhaled nitric oxide; HDM: house dust mites. Modified from reference number 10 with permission from the author.

Table 1: Comparison of the different bronchial challenges

Table 2: Absolute and relative contraindications for clinical bronchial allergen challenge. AIT: allergen immunotherapy.

Table 3: Optimal and minimal washout periods for all relevant medications before bronchial allergen challenge. IL-5R: receptor for IL-5; IL-4/13R: receptor for IL-4 and IL-13. *The periods indicated are mainly based on expert consensus.

Table 4: Comparison of the different allergen challenges in the clinic. FeNO: fractional exhaled nitric oxide; PNIF: peak nasal inspiratory flow.

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Table 1

	Procedure	Method	Indication	Strength	Weakness
Methacoline or histamine challenge	Inhalation of incremental doses of methacholine or histamine until the concentration (PC20) or the effective delivered dose (PD20) inducing a ≥20% fall in FEV1 is reached, or the maximal dose has been inhaled.	Direct	Diagnosis of airway hyperresponsiveness, defined as an increased sensitivity and exaggerated response to non-allergenic stimuli that cause airway narrowing	High sensitivity and negative predictive value for asthma diagnosis: very useful to rule out asthma	Sensitivity decreases with inhaled corticosteroids. Lower specifity than mannitol for asthma diagnosis: not as good to confirm asthma Lower sensitivity for exercise-induced bronchoconstriction. Cannot differentiate between inflammation-induced and intrinsic bronchoconstriction.
Exercise challenge	Exercise through an ergometer or treadmill while breathing dry cold air (eg performing the test in an air-conditioned room at 22°C and a relative humidity of <50%), until the patient reaches the target heart rate/minute ventilation in order to check whether there is a decline in FEV1 during the 30 minutes following the test	Indirect	Diagnosis of exercise-induced bronchoconstriction Differential diagnosis of exercise-related symptoms	High specificity for exercise-induced bronchoconstriction	Lower sensitivity than direct challenges for asthma diagnosis
Eucapnic voluntary hyperpnoea	The patient performs voluntary hyperpnoea for 6 minutes while inhaling dry air supplemented with 4.9% carbon dioxide to prevent hypocapnia	Indirect	Diagnosis of exercise-induced bronchoconstriction Differential diagnosis of exercise-related symptoms	High specificity for exercise-induced bronchoconstriction	Lower sensitivity than direct challenges for asthma diagnosis.
Hypertonic saline challenge	Inhalation of 4.5% sterile saline which is given for increasing durations of time until the longest duration is administered, or there is a ≥15% fall in FEV1 (PD15)	Indirect	Diagnosis of airway hyperresponsiveness	High specificity	Lower sensitivity than direct challenges
Adenosine monophospate (AMP) challenge	Inhalation of incremental AMP solutions during 2 minutes at tidal breathing, or through a 5-breath dosimeter until a ≥20% fall in FEV1 occurs (PC20), or the solution with the maximal concentration has been inhaled	Indirect	Diagnosis of airway hyperresponsiveness	High specificity Stronger relatinship with bronchodilator response and inflammation-induced bronchoconstriction	Lower sensitivity than direct challenges
Mannitol challenge	Capsule-based dry powered inhaler device that is used to deliver increasing doses of mannitol until the effective delivered dose of mannitol causing a ≥15% fall in FEV1 (PD15) is reached, or the maximal dose has been inhaled.	Indirect	Assessment of the severity of airway hyperresponsiveness	High specificity Stronger relationship with inflammation-induced bronchoconstriction Can be used to monitor treatment effect	Lower sensitivity than direct challenges
Allergen challenge	Inhalation of incremental doses of an allergen until the dose inducing an early ≥20% fall in FEV1 is reached or the maximal allergen dose has been inhaled	Indirect	Diagnosis of allergic asthma	Gold standard for the identification of the allergic triggers of asthma symptoms	Insufficient standardisation High frequency of late asthmatic responses Not evaluated in moderate and severe asthma

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Table 2

Absolute contraindications	Relative contraindications
Patients with uncontrolled or partially controlled asthma (diurnal symptoms ≥2 days/week, nocturnal symptoms/awakenings ≥1 day/week, use of rescue medication ≥2 days/week, any limitation to regular activities, exacerbation requiring oral corticosteroids for ≥5 days in the previous three months) (48, 49).	Patients who cannot discontinue temporarily the intake of non-selective β blockers. Patients who cannot perform reproducible spirometry manoeuvres (alternatively, impulse oscillometry can be considered).
Patients with FEV_1<70% (50) despite optimized anti-asthma therapy.	Patients with uncontrolled and/or unstable cardiac, respiratory (other than asthma), metabolic, immunologic, oncologic or systemic disease.
Patients who lose control or cannot keep a FEV_1 ≥70% during the minimal washout period for antiasthma drugs.	History of anaphylactic reaction to subcutaneous AIT or following the ingestion of aeroallergen (allergen-specific contraindication).
Pregnant women	
Patients who have an absolute contraindication for the administration of inhaled β2 agonists, corticosteroids or epinephrine.	
Inability to cooperate (21) (only age-related contraindication).	
Confirmed or suspected COVID-19 or other respiratory infections	

Table 3

Inhaled medications	Minimal withdrawal time*	Optimal withdrawal time*
Short-acting β2-agonists	6 hours	8 hours
Long-acting β2-agonists	48 hours	72 hours
Ultra-long-acting β2-agonists	72 hours	72 hours
Short-acting anticholinergics	6 hours	8 hours
Long-acting anticholinergics	72 hours	72 hours
Corticosteroids	24 hours	4 weeks
Cromones	4 hours	24 hours
Systemic medications	**Minimal withdrawal time**	**Optimal withdrawal time**
Corticosteroids	8 weeks	8 weeks
Antihistamines	7 days	10 days
Leukotriene receptor antagonists or leukotriene synthesis inhibitors	72 hours	8 weeks
Metylxanthines	24 hours	24 hours
Anti-IgE monoclonal antibodies	6 months	6 months
Anti-IL-5, anti-IL-5R and anti-IL-4/13R monoclonal antibodies	8 weeks	6 months

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Table 4

	Conjunctival allergen challenge	Nasal allergen challenge	Bronchial allergen challenge
Indications	Diagnosis of allergic conjunctivitis in patients with a mismatch between symptoms and IgE-sensitisation tests. Choice of allergen immunotherapy (AIT) extract in polysensitized allergic patients Monitoring the efficacy of AIT Identification of allergic triggers in patients with atopic or vernal keratoconjunctivitis	Diagnosis of allergic rhinitis in patients with a mismatch between symptoms and IgE-sensitisation tests. Choice of AIT extract in polysensitized allergic patients Monitoring the efficacy of AIT Diagnosis of local allergic rhinitis and occupational allergic rhinitis	Diagnosis of allergic asthma in patients with a mismatch between symptoms and IgE-sensitisation tests. Diagnosis of occupational allergic asthma
Washout period for relevant drugs	Topical medications: 48 hours-4 weeks Systemic medications: 5 hours-4 weeks	Nasal medications: 48 hours-5 days Systemic medications: 48 hours-3 weeks	Inhaled medications Minimal: 6-24 hours Optimal: 8 hours-4 weeks Systemic medications Minimal: 4 hours-8 weeks Optimal: 24 hours-6 months
Room conditions	Controlled environment (20±1.5°C and 40-60% humidity) not contaminated by other substances (e.g. methacholine, other allergens, etc.) and precautions for infectious aerosols		
Control test	Unilateral instillation of saline solution (NaCl 0.9%) while allergen solution is instilled in the contralateral eye	Bilateral application of isotonic pH neutral non-irritant solution prior to allergen administration Usually, the same diluent used to prepare the allergen solutions is administered.	Inhalation of isotonic pH neutral non-irritant solution prior to allergen inhalation Usually, the same diluent used to prepare the allergen solutions is administered
Delivery of the allergen	Instillation of eyedrops (alternatively a micropipette can be used) in the inferior external quadrant of bulbar conjunctiva of 1 eye while occluding the nasolacrimal duct The instillation of several increasing allergen concentrations is recommended. The instillation of 1 allergen/session is recommended.	Bilateral application through pump-metered aerosol spray, micropipette or impregnated disk The administration of a single dose of undiluted allergen is recommended for the daily practice, except for the monitoring of AIT efficacy The administration of one allergen/session is usually recommended, yet there is a validated protocol for multiple allergen provocation.	Inhalation using a dosimeter at tidal breathing or through counted deep breaths. The inhalation of 5 increasing allergen concentrations is recommended. Only 1 allergen/session can be administered
Monitoring	Evaluation of conjunctival redness, itching, tearing and edema by Abelson score or total ocular symptom score (TOSS)	Combination: Symptom score: visual analogue scale, total nasal symptom score, Linder score, Lebel score Objective evaluation of nasal patency: PNIF, acoustic rhinometry, active anterior rhinomanometry, 4-phase rhinomanometry	FEV1 as measured by forced spirometry (alternatively impulse oscillometry indexes) Inflammatory biomarkers (FeNO) can assist in some cases
Safety parameters	Extremely safe technique with adverse reactions restricted to nasal symptoms Late reactions are very rare A 2-hour observation period at the hospital is recommended after a positive test	When the allergen is administered by aerosol spray or micropipette, the test is an extremely safe technique, even in asthmatic patients Late reactions are very rare 1-hour observation period at the hospital is recommended after a positive test	Profound and treatment-resistant bronchoconstriction can occur, even though the procedure is generally well-tolerated. 15-90% of patients develop late reactions (the frequency is allergen-specific) 7-hour observation period at the hospital is recommended after a positive test
Reproducibility	92% for house dust mites and grass pollen[1] Not measured for other allergens	Interclass correlation coefficient (ICC) for TNSS: 0.78. ICC for acoustic rhinometry: 0.66-0.89[2]	ICC for FEV1: 0.80[3]

References:

1. Lougnon Z P-TC, Peireira B, Michaud E, Gourdon Dubois N, Montaudié I, Labbé G, Merlin E, Chiambaretta F, Fauquert JL. Scoring Conjunctival Provocation Test.. EAACI Congress 2016 Helsinki Allergy 2016;71(S102):421-22.
2. Pantin CT, Southworth T, Wetzel K, Singh D. Reproducibility of nasal allergen challenge responses in adults with allergic rhinitis. Clin Pharmacol. 2019;11:67-76.
3. Dente FL, Bacci E, di Franco A, Giannini D, Conti I, Macchioni P, Scuotri L, Taccola M, Vagaggini B, Paggiaro PL. Reproducibility of early and late asthmatic responses to allergen challenge in a large group of asthmatics. Respir Med. 2000;94(5):441-7.

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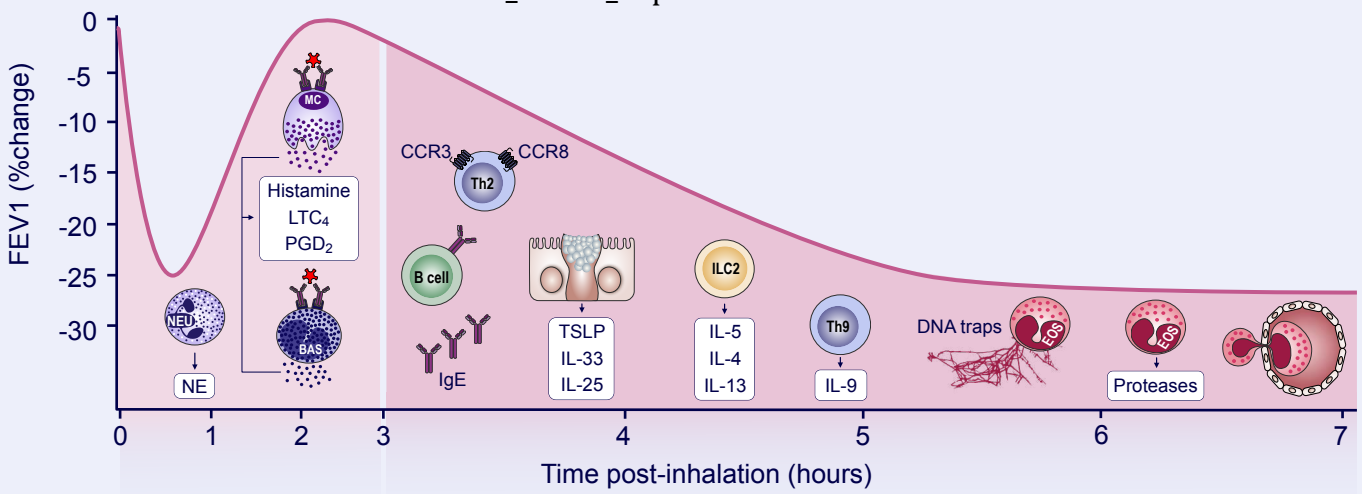

		Early response	Late response	Airway hyperresponsiveness	Sputum eosinophila
ATTENUATION — Indicated for asthma	Inhaled corticosteroids	▪ Budesonide ▪ Fluticasone furoate ▪ Mometasone furoate ▪ Ciclesonide ▪ Beclomethasone	▪ Budesonide ▪ Fluticasone furoate ▪ Mometasone furoate ▪ Ciclesonide ▪ Beclomethasone	▪ Budesonide ▪ Fluticasone furoate ▪ Mometasone furoate ▪ Ciclesonide ▪ Beclomethasone	▪ Budesonide ▪ Fluticasone furoate ▪ Mometasone furoate ▪ Ciclesonide ▪ Beclomethasone
	Short-acting β_2 agonists	▪ Salbutamol ▪ Albuterol			
	Long-acting and ultra long-acting β_2 agonists	▪ Salmeterol ▪ Formoterol ▪ Vilanterol ▪ Indacaterol	▪ Salmeterol ▪ Formoterol ▪ Vilanterol ▪ Indacaterol	▪ Salmeterol ▪ Formoterol ▪ Vilanterol ▪ Indacaterol	
	Antileukotrienes	▪ Montelukast	▪ Montelukast	▪ Montelukast	▪ Montelukast
	Methylxanthines	▪ Theophylline	▪ Theophylline		
	Cromones	▪ Cromoglycante	▪ Cromoglycante		
	mABs	▪ Omalizumab	▪ Omalizumab		▪ Mepolizumab ▪ Benralizumab ▪ Reslizumab
Indicated for other diseases	Antihistamines	▪ Desloratadine ▪ Cetirizine ▪ Terfenadine			
	Loop diuretic	▪ Furosemide	▪ Furosemide		
	NSAIDs	▪ Lysine-ASA	▪ Lysine-ASA		
LACK OF EFFECT — Indicated for asthma	Short-acting β_2 agonists			▪ Salbutamol ▪ Albuterol	
	Long-acting and ultra long-acting β_2 agonists				▪ Salmeterol ▪ Formoterol ▪ Vilanterol
	Methylxanthines			▪ Theophylline	
	mABs	▪ Mepolizumab	▪ Mepolizumab	▪ Mepolizumab	
Indicated for other diseases	Loop diuretic		▪ Furosemide	▪ Furosemide	
	NSAIDs			▪ Lysine-ASA	
	mAbs	▪ Efalizumab	▪ Efalizumab	▪ Efalizumab	▪ Efalizumab
ENHANCED — Indicated for asthma	Short-acting β_2 agonists		▪ Salbutamol ▪ Albuterol		▪ Salbutamol ▪ Albuterol

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Figure 1_Agache

Figure 2

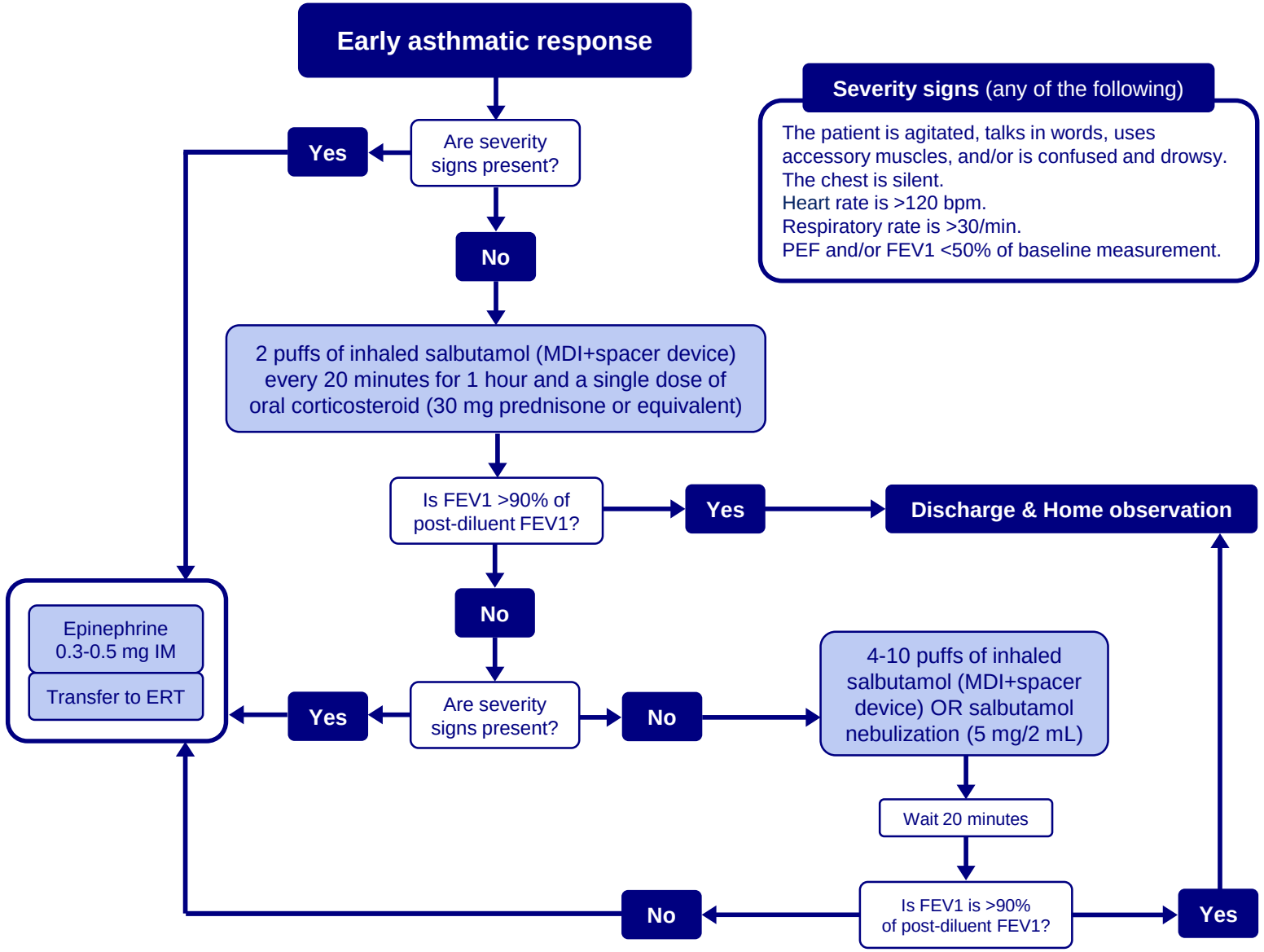

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Figure 3

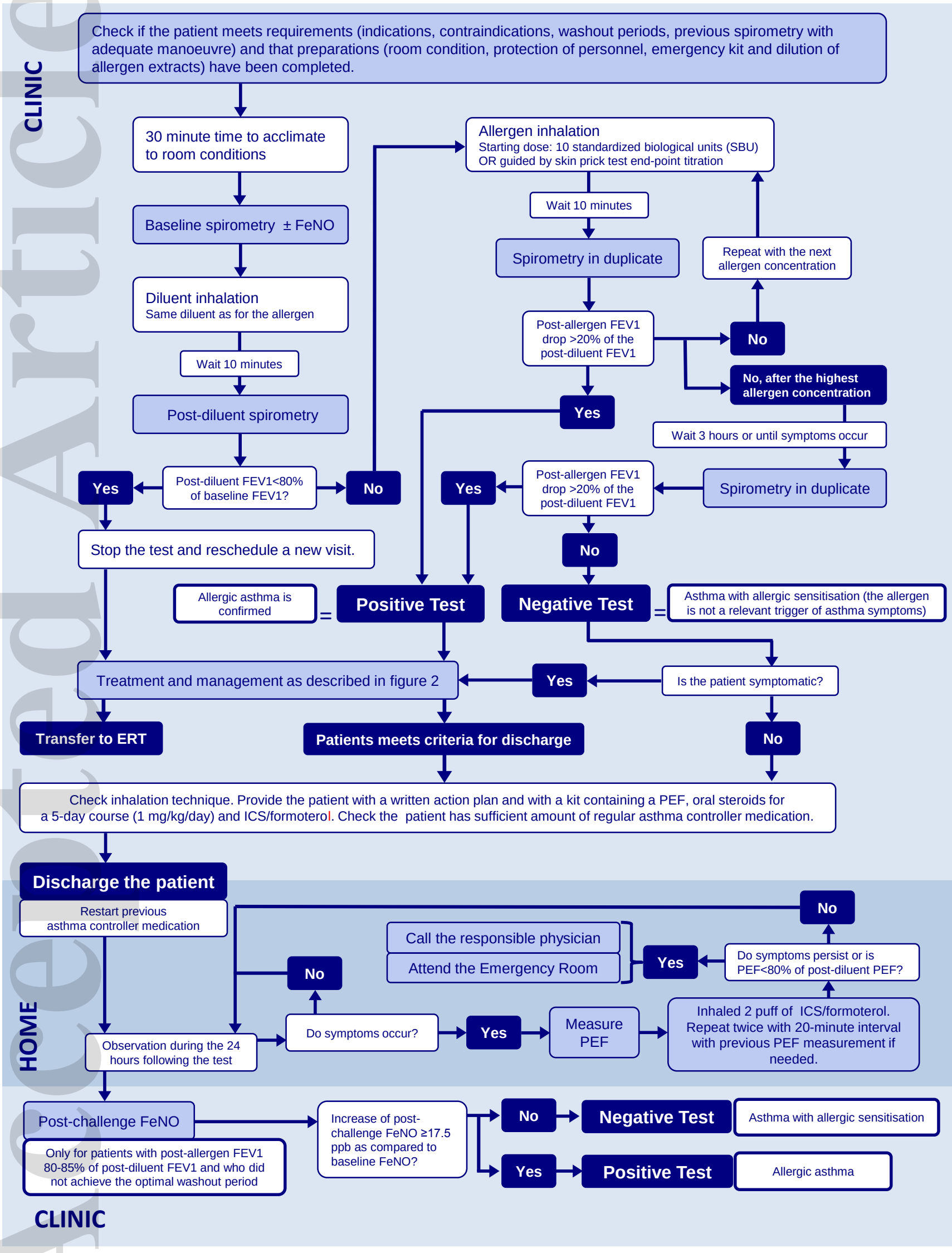

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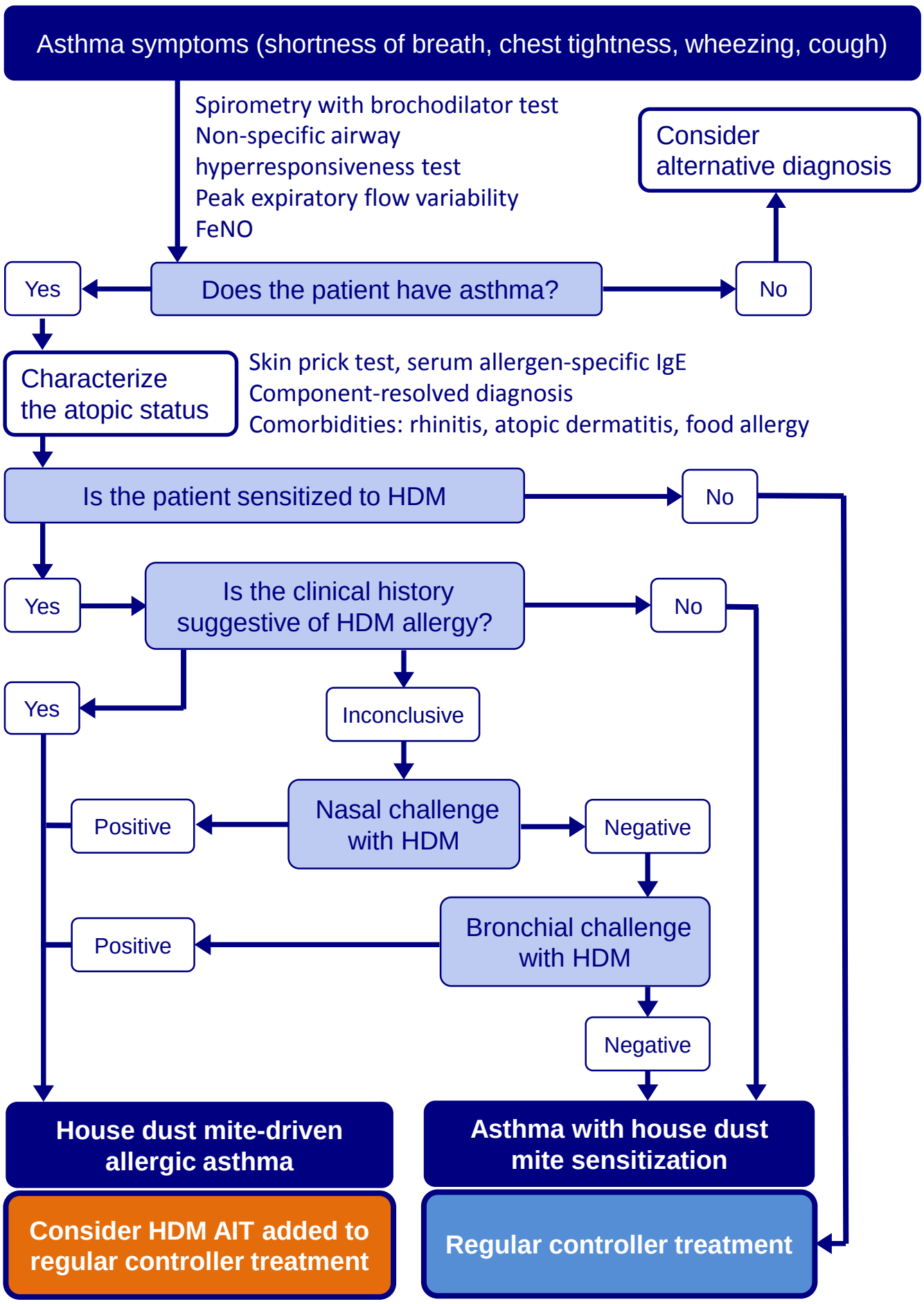
Figure 4



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