DR. EVA UNTERSMAYR (Orcid ID : 0000-0002-1963-499X)
DR. ULRICH M ZISSLER (Orcid ID : 0000-0003-4826-3419)
PROF. KNUT BROCKOW (Orcid ID : 0000-0002-2775-3681)
PROF. OLIVER PFAAR (Orcid ID : 0000-0003-4374-9639)
PROF. HOLGER GARN (Orcid ID : 0000-0002-5178-4023)
PROF. CARSTEN SCHMIDT-WEBER (Orcid ID : 0000-0002-3203-8084)

Article type : EAACI Position Paper

Corresponding author mail id : csweber@tum.de

EAACI position paper

Non-invasive and minimally-invasive techniques for the diagnosis and management of allergic diseases

Baumann, R.(0000-0002-6523-1024)[1,2], Untersmayr, E. (0000-0002-1963-499X)[3], Zissler, UM. (0000-0003-4826-3419)[4], Eyerich, S.(0000-0002-1166-2355)[4], Adcock, IM.(0000-0003-2101-8843)[5], Brockow, K.(0000-0002-2775-3681) [6], Biedermann, T.(0000-0002-5352-5105) [6], Ollert, M.[7], Chaker, AM. (0000-0002-5117-4073)[4,8] ,Pfaar, O. (0000-0003-4374-9639)[9], Garn, H. (0000-0002-5178-4023) [10], Thwaites, RS.(0000-0003-3052-2793)[5], Togias, A.(0000-0001-9009-5717),[11] Kowalski, ML. (0000-0003-1852-047X)[12], Hansel, T. (0000-0003-3786-8105)[5], Jakwerth, C.(0000-0002-2902-0393)[4], Schmidt-Weber, CB.(0000-0002-3203-8084)[4, *]

1) Medical School Hamburg (MSH) – Medical University; Institute for Molecular Medicine; Medical Faculty; Hamburg, Germany;
2) RWTH Aachen University Hospital; Institute for Occupational, Social and Environmental Medicine; Aachen, Germany;

3) Institute of Pathophysiology and Allergy Research; Center of Pathophysiology, Infectiology and Immunology; Medical University of Vienna; Vienna, Austria;
4) Center of Allery and Environment (ZAUM); Technical University and Helmholtz Zentrum München; München, Germany; Member of the German Center of Lung Research (DZL) and the Helmholtz I&I Initiative
5) National Heart and Lung Institute, Imperial College London, and Royal Brompton and Harefield NHS Trust, London, United Kingdom
6) Department of Dermatology and Allergy Biederstein, School of Medicine, Technische Universität München, Germany
7) Department of Infection and Immunity, Luxembourg Institute of Health (LIH), Esch-sur-Alzette, Luxembourg and Department of Dermatology and Allergy Center, Odense Research Centre for Anaphylaxis (ORCA), University of Southern Denmark, Odense, Denmark
8) Department of Otolaryngology, Allergy Section, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany
9) Department of Otorhinolaryngology, Head and Neck Surgery,
University Hospital Marburg, Philipps-Universität Marburg, Germany
10) Institute of Laboratory Medicine and Pathobiochemistry - Molecular Diagnostics, Philipps University of Marburg - Medical Faculty, Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL), Marburg, Germany
11) Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
11) National Heart and Lung Institute, Imperial College London, United Kingdom
12) Department of Immunology and Allergy, Medical University of Lodz, Lodz, Poland
* Corresponding author

Key words: anaphylaxis, asthma, atopic dermatitis, food allergy, non-invasive diagnosis

Short title: Non-invasive diagnosis approaches in allergy management

Acknowledgements: We particularly thank Maryam Poorafshar, Magnus Molin and Jeronimo Carnes for their advice and experience from the diagnostic and pharmaceutical industry. The authors thank the European Academy of Allergy and Clinical Immunology (EAACI) for financial support of the task force. We also thank EIT Health for their visionary support of innovation projects in this area. EIT Health is a body of the EU and receives funding from the H2020 program. Dr. Pfaar reports grants and personal fees from ALK-Abelló, Allergopharma, Stallergenes Greer, HAL Allergy Holding B.V./HAL Allergie GmbH, Bencard Allergie GmbH/Allergy Therapeutics, Mobile Chamber Experts (a GA2LEN Partner), Anergis S.A., Laboratorios LETI/LETI Pharma, Biotech Tools S.A., Lofarma, grants from Biomay, Circassia, MEDA Pharma/MYLAN, Indoor Biotechnologies, grants from Glaxo Smith Kline, personal fees from Astellas Pharma Global, personal fees from EUFOREA, personal fees from ROXALL, personal fees from NOVARTIS, personal fees from SANOFI AVENTIS, personal fees from Med Update Europe GmbH, personal fees from streamedup! GmbH, outside the submitted work.

Disclaimer: Dr. Togias' authorship of this report does not constitute endorsement by the US National Institute of Allergy and Infectious Diseases or any other United States government agency.

Conflict of interest statement: Prof. Adcock, Prof. Brockow, Prof. Eyerich, Prof. Garn, Prof. Kowalski, Prof. Ollert, Prof. Untersmayr, Prof. Togias, Dr. Thwaites and Dr. Zissler have nothing to disclose. Prof. Schmidt-Weber and Dr. Jakwerth reports grants from Federal Ministery of Education and Research, grants from European Institute of Innovation & Technology (EIT), during the conduct of the study; Prof. Schmidt-Weber reports from personal fees from Allergopharma and Allergy Therapeutics. Dr. Pfaar reports the following conflicts outside the present publication: grants and personal fees from ALK-Abelló, Allergopharma, Stallergenes Greer, HAL Allergy Holding B.V./HAL Allergie GmbH, Bencard Allergie GmbH/Allergy Therapeutics, Lofarma, Biomay, Circassia, ASIT Biotech Tools S.A., Laboratorios LETI/LETI Pharma, Anergis S.A.; grants from Glaxo Smith Kline and personal fees from MEDA Pharma/MYLAN, Mobile Chamber Experts (a GA2LEN Partner), Indoor Biotechnologies, Astellas Pharma Global, EUFOREA, ROXALL, NOVARTIS, SANOFI AVENTIS, Med Update Europe GmbH, streamedup! GmbH. Prof. Baumann reports grants from Phadia, part of Thermofisher scientific, grants from European Institute of Innovation & Technology (EIT), during the conduct of the study; has a patent pending. Dr. Hansel holds shares of Mucosal Diagnostics. Prof. Biedermann reports grants and personal fees from Phadia-Thermo Fisher during the conduct of the study. Dr. Chaker reports grants for clinical studies and research and other from Allergopharma , ALK Abello, AstraZeneca, Bencard / Allergen Therapeutics, ASIT Biotech, Immunotek, Lofarma, GSK, Novartis, LETI, Roche, Sanofi Genzyme, Zeller and from the European Institute of Technology (EIT); has received travel support from the European Academy of Allergy and Clinical Immunology (EAACI), DGAKI, and SMI, all outside the submitted work.

Abbreviations:

Specific IgE (sIgE)

total IgE (tIgE)

dried blood spots (DBS)

bronchoalveolar lavage (BAL)

fraction of exhaled nitric oxide (FeNO)

serum periostin (POSTN)

eosinophil cationic protein (ECP)

exhaled breath condensate (EBC)

electronic nose (eNose)

skin prick test (SPT)

cysteinyl leukotrienes (Cys-LTs)

beta-platelet factor 4 (CXCL-4)

b-thromboglobulin, CCL-5 RANTES)

allergen immunotherapy (AIT)

C-reactive protein (CRP)

MicroRNA (miRNA)

transepidermal water loss (TEWL)

nerve growth factor (NGF)

artificial intelligence (AI)

8-hydroxydeoxyguanosine (8-OHdG)

inflammatory bowel disease (IBD)

irritable bowel syndrome (IBS)

eosinophil derived neurotoxin (EDN)

eosinophilic esophagitis (EoE)

matrix-assisted laser desorption/ionization (MALDI)

Leukotriene B4 receptor (LTB4R)

Peptidyl Arginine Deiminase 4 (PADI4)

Interleukin 1 Receptor Type 2 (IL1R2)

Protein Phosphatase 1 Regulatory Subunit 3D (PPP1R3D)

Kelch Like Family Member 2 (KLHL2)

Enoyl-CoA Hydratase Domain Containing 3 (ECHDC3)

C-C Motif Chemokine Ligand 2 also known as MCP1 (CCL-2)

Abstract:

Allergic diseases of the (upper and lower) airways, the skin as well as the gastrointestinal tract, are on the rise, resulting in impaired quality of life, decreased productivity and increased healthcare costs. As allergic diseases are mostly tissue specific, local sampling methods for respective biomarkers offer the potential for increased sensitivity and specificity. Additionally, local sampling using non-invasive or minimally-invasive methods can be cost-effective and well tolerated, which may even be suitable for primary or home care sampling.

Non- or minimally-invasive local sampling and diagnostics may enable a more thorough endotyping, may help to avoid under- or overdiagnosis, and may provide the possibility to approach precision prevention, due to early diagnosis of these local diseases even before they get systemically manifested and detectable. At the same time, dried blood samples may help to facilitate minimal-invasive primary or home care sampling for classical systemic diagnostic approaches.

This EAACI position paper contains a thorough review of the various technologies in allergy diagnosis available on the market, which analytes or biomarkers are employed, and which samples or matrices can be used. Based on this assessment, EAACIs position is to drive these developments to efficiently identify allergy and possibly later also viral epidemics and take advantage of comprehensive knowledge to initiate preventions and treatments.

Introduction

The prevalence of allergies is continuously increasing, along with associated difficulties in the appropriate treatment of patients [1]. Rapid, precise and pre-symptomatic diagnostic procedures are urgently needed to alleviate this burden. Many allergies are local diseases, and hence systemic diagnostic approaches often fail to detect disease before full manifestation. In contrast, local sampling methods and detection of characteristic biomarkers offer increased sensitivity and specificity, even before disease manifestation. Thus, non-invasive diagnostic approaches would enable earlier patient diagnosis, distinguish infections from allergies, and define endotype-specific prevention or treatment strategies. Additionally, local sampling using non-invasive or minimally-invasive methods can be cost-effective and well tolerated, which may even be suitable for primary or home care sampling. A rich portfolio of potential biomarkers that can be measured in non-invasive samples exists (Figure 1) but is currently not exploited by routine diagnostics. Here, we summarise the main systemic methods and biomarkers in allergic diseases of the airways (allergic rhinitis, asthma), circulation (anaphylaxis), skin (atopic dermatitis) and gastrointestinal tract (food allergy), before we explore possibilities for minimally- or non-invasive sampling methods for allergy management.

Due to the fact, that allergic diseases are mostly tissue specific, local sampling methods using non-invasive or minimally-invasive methods for respective biomarkers offer the potential for increased sensitivity and specificity. It is the goal of this article to illustrate the continually increasing number of biomarkers, and to facilitate the clinical management of allergic diseases in the near future.

This EAACI position paper aimed to anticipate the key challenges and identify opportunities in the future of allergy diagnosis. Ideal diagnostic biomarkers would preferably be based on non- or minimally-invasive sampling (Table 1 and Figure 1), as this enables home and repeated sampling with greater tolerability and reduced costs. Together with telemedicine, early biomarker assessment may allow personalized precision treatment and prevention, and may also enable doctors to avoid unnecessary treatments. To pave the way for this vision, substantial funding in the field is strongly recommended.

Method

A task force on non-invasive diagnosis of allergy was initiated and approved by the EAACI ExCom and a workshop was announced and held under the title "*Non-invasive diagnostic approaches in allergy*" on the 4th June 2019 at the annual EAACI congress in Lisbon. The meeting was chaired by the last author with the main objective to drive novel developments into clinical applications. The group was split into subgroups, who covered airways, skin, intestinal and systemic manifestation of the disease. The groups generated overviews, which were collated and discussed in detail on a separate manuscript drafting meeting. Following further online meetings for manuscript discussion and improvement of the manuscript, the Position Paper was then finalized by the first author together with last author and presented to the TF for final evaluation and approval.

Classical diagnosis: Minimally invasive options for systemic IgE in allergies

In allergy diagnostics, a main focus is the determination of specific IgE, although a positive IgE test does only imply previous contact and sensitization, but not a concise diagnosis of allergic disease. Multiple technologies are on the market to measure this parameter (Suppl. Tab. 1). *In vitro* detection of allergen-specific IgE (sIgE) against aeroallergens or food allergens and total IgE (tIgE) in human serum or plasma is a very useful tool for the diagnosis of allergic diseases at the clinics and in research. This is particularly helpful at the level of point of care, allowing physicians a quick insight into the sensitization condition of potential allergy patients. The early sensitization pattern to common allergens at the age of 4 years was shown to have a predictive value to the incidence and persistence of asthma and/or rhinitis at 16 years [2]. Although these studies were performed using serum samples, it was shown that in seasonal allergic rhinitis, specific nasal IgE reproduced in 99.7% the presence of the same specific IgE in the serum [3]. Currently, the clinical market for the measurement of sIgE can be divided into fully automated laboratory systems for whole allergens and components, multiplexed systems (microarrays) for component resolved diagnostics, screening tests (allergen mixtures and panels) and point of care systems (Suppl. table 1.). Current multiple simultaneous allergen tests offer simple screening methods, those platforms and tests also have limitations with respect to multiplex capacity, flexibility and analytical performance [4].

IgE in dried blood spot-based diagnosis

A recent development is the measurement of IgE levels in dried blood spots (DBS) and other capillary blood micro samples. In comparison to venous blood, DBS are considered to be less sensitive to storage and temperature conditions (ambient temperature), as well as transport processes (e.g. by mail), due to the inactivation of degrading enzymes during the drying process. Due to this reason, and as DBS are minimally invasive and require only small amounts of blood (±10 µL), DBS offer the possibility for decentralized sample collection and blood collection from small children and newborns [5] (Table 1). Recent reports showed that the determination of IgE and IgG reactivity to more than 170 allergen molecules has also been possible when using paper-DBS [6]. The paper-dried serum and blood spots could be stored at different temperatures (ie, +37°C, +22°C, +4°C, −20°C) delivering similar test results, indicating the possibility that shipment by mail without the need for cooling or express delivery in different seasons is possible and allows reliable and reproducible detection even of low levels of allergen-specific IgE [6].

Cytokines, Chemokines and RNAs in dried blood spots

In addition to IgE, cytokines and chemokines are detectable in DBS [7]. For example, chemokine levels in DBS were stable after different storage conditions such as room temperature for a week and multiple freeze/thaw cycles [7]. In addition, the DBS-technique allows the identification of viral RNA [8] or microRNA[5]. Whether cytokine, viral RNA and microRNA levels can indeed reliably be tested in DBS has to be further validated and would allow to transfer some of the blood-based biomarkers into a minimally-invasive DBS format.

Non-invasive and minimally-invasive diagnostics of allergic airway diseases

Allergies often start in upper airways and can progress to lower airways at all ages. Asthma can occur even in the first months of life. Therefore, allergic rhinitis and allergic asthma may considerably profit from an earlier accurate local diagnosis, before the disease gets systemically detectable. Current approaches and newer developments for both diseases are summarized in this section.

Classical and novel biomarkers in asthma

Asthma often starts in childhood and is the most common chronic disease among children worldwide, Biomarker development in asthma started with the observation that asthma comprises allergic eosinophilic, allergic non-eosinophilic, non-allergic (non-)eosinophilic and mixed granulocytic forms. Lung eosinophilia (>300 cells/μL) represents a major cellular biomarker for the diagnosis of asthma endotypes and is associated with increased corticosteroid responsiveness in asthma. However, quantification of lung eosinophils by bronchoalveolar lavage (BAL) and endobronchial biopsy is invasive. Alternatively, sputum induction and sputum eosinophil counting represents a useful biomarker (discussed below) but sputum induction and processing is laborious. Alternative (indirect) minimally-invasive methods and markers for lung eosinophilia include fraction of exhaled nitric oxide (FeNO), total IgE, and serum periostin (POSTN). [9]

As stated above, lung eosinophilia (>300 cells/μL) represents a major cellular biomarker for the diagnosis of asthma. To date, the blood eosinophil count as minimally-invasive biomarker for lung eosinophilia shows a modest diagnostic accuracy [9-12], and might therefore be best utilized for either excluding (high sensitivity) or including (high specificity) lung eosinophilia.[10] Moreover, the *blood* eosinophil count is a prerequisite for applying for treatment with anti-IL-5 or anti-IL-5R monoclonal antibodies for eosinophilic asthma. Blood eosinophil counts also characterize various atopic states and diseases, but are subject to significant daily fluctuations, are highly steroid sensitive, and increase after exercise [11]. As new biologics enter the market (e.g. anti-IgE, anti-IL-4Rα, anti-TSLP, IL-33 or anti-macrolides), the need for new and more accurate guiding of asthma therapy is substantial. FeNO represents an established non-invasive parameter that is easy-to-obtain and reflects T-helper cell type 2 (Th2) inflammation of the airways. FeNO may help to discriminate patients with controlled and uncontrolled asthma, predict asthma development and exacerbation, and monitor the efficacy of corticosteroid and biologic therapy. [13]

Eosinophil cationic protein (ECP) is an established clinical biomarker of eosinophil activity in asthma [14]. ECP is stored in specific granules in mature peripheral blood eosinophils, and released after stimuli such as IgG and IL-5, while ECP serum levels are increased in atopic asthma. ECP can also be measured in sputum, nasal secretions /nasal lavage fluid and urine.

An emerging biomarker in allergic diseases is serum Periostin, a "chronic" type 2 biomarker, in contrast to FeNO and possibly the eosinophil blood count as "acute" type 2 biomarkers (reviewed in [15]).

Recently, analysis of 73 studies identified 190 potential asthma biomarkers (proteomic, transcriptomic, genomic and/or epigenomic), revealing 10 potential biomarkers for asthma in at least 2 of 13 - omics levels[16], while IL-4, IL-13, STAT6 and VEGF-A were associated with asthma at 3 -omics levels. Ideally, these and other biomarkers should be detectable in non- or minimally-invasive samples. The rich list of potential non-cellular biomarkers has been previously summarized [17,18] (and Figure 1). Local sampling, using matrices such as urine, nasal secretions, tears, exhaled breath or exhaled breath condensate (EBC), may support the development of suitable biomarker tests.

Asthma: Exhaled breath

The assessment of exhaled gases *in vivo* or from biofluids using electronic noses (eNose) has developed as a possible non-invasive point-of-care tool for the diagnosis and phenotyping of patients with different respiratory diseases including allergic asthma (Table 1). Some eNOSEs detect specific volatile organic compounds (VOCs) without identifying their molecular entities by utilising multiple cross-reactive sensors (reviewed in [19]). Other eNoses detect the specific molecular entities using LC-MS/MS but this currently requires sending samples to central laboratories. Both types of eNoses enable pattern recognition and the detection of unique breath profiles for individual patients. Clustering on these eNose breath profiles in children and adults and asthmatics has identified differences in atopy [20], inflammatory biomarkers [21] and symptom control asthmatic subjects [22]. There are limited studies investigating the relationship between exhaled VOCs and atopic asthma. VOCs profiles captured by the Cyranose C320 only reported a trend for classification between atopic and non-atopic asthmatics in children [23]. The study was limited by the small sample size (n=31) and potentially better eNOSEs or combinations of eNOSEs would provide better discrimination. The direct detection of viruses in exhaled breath has not been realized and only after sampling of about 300l of air, viral DNA can be captured using various capturing systems [24].

Brinkman et al recently suggested breath analysis can indeed contribute to the management of severe asthma. The same study that identified and followed up severe asthma phenotypes using metabolomic phenotypes obtained from a composite of eNoses, which were associated with changing inflammatory profiles and oral steroid use of severe asthma [21]. Future studies should further address the stability of eNOSE signatures and their mechanistic link with Gold standard skin prick tests (SPT) or allergen-specific IgE levels.

Induced sputum cell counts and mediators

Hypertonic saline-induced sputum can be used as a source of secreted biomarkers from the lower airways. Sputum cell counts generate reproducible results and can be used as a validated marker of lower airway inflammation, when standardized protocols are applied and thoroughly followed [17,25]. Besides sputum neutrophils, sputum eosinophil numbers are related to disease severity in adults or children [26-29] and distinguish between eosinophilic and neutrophilic asthma [30]. Higher numbers of over 300 eosinophils/µL occur during exacerbations, allergen-induced late-phase responses, or tapering of corticosteroids [31-34]. Sputum eosinophils are a useful tool for disease management, and can be used to improve the prevention of asthma exacerbations [35]. Furthermore, secreted mediators like cysteinyl leukotrienes (Cys-LTs), beta-platelet factor 4 (CXCL-4), b-thromboglobulin, CCL-5 (RANTES), thromboxane or serotonin are directly correlated with severe asthma, and therefore may serve as biomarkers for this condition [36-40]. Sputum Cys-LTs are recognized as central mediators in neonatal and childhood asthma phenotypes and may be particularly promising to monitor the development of asthmatic inflammation in early life [41,42]. The association between sputum markers and asthma control needs further attention and validation.

Allergic rhinitis, chronic rhinosinusitis and biomarkers of the upper airways as proxy for the lung

Allergic rhinitis often starts during childhood. Its early diagnosis is an unmet need, as viral rhinitis or other chronic, often referred to as “idiopathic” rhinitis, can cause similar and equally bothersome symptoms. Non-invasive sampling for distinctive and prognostic biomarkers would

help to increase acceptance for early testing within high-risk groups (e.g. children with atopic dermatitis or uncharacterized wheeze). A proportion of patients with chronic rhinitis and allergic rhinitis progress to allergic asthma; earlier detection may therefore prove beneficial in allergy *and* asthma management and prevention. Monitoring of allergen immunotherapy (AIT), and preferably prediction of response to treatment, represent clinically meaningful outcomes but are yet not supported by validated biomarkers. Furthermore, especially for the application of biologics in chronic rhinosinusitis with nasal polyps non-invasive sampling can reveal meaningful information about type and severity of inflammation and thus may help to tailor an appropriate treatment strategy.

A comprehensive overview of upper airway biomarkers that are non-invasively accessible in nasal secretions were previously summarized and are shown in the figure (Figure 1)[17,18].

According to the united airway concept, upper and lower airways are in connection to each other and share many similar inflammatory responses. As a practical consequence of this, upper airway biomarkers may be supportive to diagnose processes of the lower airways. Indeed, studies using optimized protocols and selected biomarkers such as IL-24 show a moderate to strong correlation between upper and lower airways using nasal secretions and induced sputa [17]. Moreover, nasal CCL-26 is significantly correlated with sputum CCL-26 (and IL-13 and IL-5) levels, which in turn is linked tightly with airway eosinophilia [17]. Minimally invasive sampling using nasosorption® and sputosorption® have offered some promise in providing surrogate markers of eosinophilic asthma, though these techniques require further study [43]. They are based on adsorptive materials, which allow efficient recovery of analytes (Table 1). Whether nasal secretions may therefore have the potential to replace invasive eosinophil counts, requires verification.

In the context of non-invasive diagnosis, local nasal inflammatory responses to airborne stimuli are particularly important and may be even more sensitive than systemic responses. In support of this assumption, human nasal mucosal C-reactive protein (CRP) responses after the inflammatory stimulus of a 6h-inhalation of ultrafine welding fume particles were found to be not only highly correlated to systemic CRP responses, but also showed increased and earlier reactivity [44]. Moreover, nasal levels of human ST2 and IL-5 were more strongly upregulated than systemic levels after a single nasal allergen challenge [45]. Furthermore, nasal periostin was clearly

more discriminatory for asthma than systemic periostin, and the same was true for IgE [46]. In addition, potentially pathogenic bacteria were identified by DNA-PCR test in nasal samples of children with chronic cough, enabling specific antibiotic treatment [47]. To enable reliable and reproducible detection of nasal inflammatory responses, standardization of collection materials, elution and laboratory protocols as well as storage and temperature conditions[512] is required [48].

Saliva and tear fluid

Saliva DNA methylation may represent a helpful tool to mirror associations between wheezing and asthma development, as PM20D1 hypermethylation was reported to be associated with early childhood wheezing.[49] Tear sampling is quite difficult, especially if not preceded by a positive conjunctival allergen challenge. Therefore, tear sampling is not within the scope of this position paper

Urine

Urine would represent an ideal matrix for the non-invasive sampling and monitoring of allergic diseases and asthma. Promising preliminary findings from research studies suggest that there may be a place for routine urine sampling in asthma in the future, particularly for the measurement of eicosanoids and their metabolites.

Urinary LTE4 is the stable metabolite of LTC4 and LTD4 and thus represents a biomarker of increased Cys-LT activity [50,51]. Sources of LTE4 include many inflammatory cells types of the upper and lower airways such as eosinophils, mast cells, basophils, macrophages, platelets, and neutrophils [52,53]. Increased urinary LTE4 excretion has been demonstrated in allergen-induced asthma, asthma exacerbations, aspirin challenge, and increased basal excretion in AERD [54-57]. Furthermore, it may represent a biomarker of the inflammatory state in chronic rhinosinusitis, but comorbid asthma and atopy can also contribute to elevated urinary LTE4 levels [58]. Several drugs are able to block Cys-LT synthesis significantly decreasing urinary LTE4 levels [59], whereas corticosteroids do not affect urinary LTE4 [60].

A recent study showed that concentrations of urinary adenosine are complementary to peripheral airway NO in diagnosing pediatric exercise-induced bronchoconstriction reflecting poor asthma control [61]. Another class of mediators that may be measured in the urine is

eosinophil-derived proteins such as EDN. A recent meta-analysis taking into account 27 pediatric studies conducted over a period of 20 years concluded that over 70% of these studies revealed an association between eosinophil-derived proteins and childhood asthma, thus suggesting a diagnostic role for eosinophil-derived protein measurements [62].

miRNA

MicroRNAs (miRNAs) are small non-coding RNA molecules that are 18–22 nucleotides long and play an important regulatory role in numerous immunological and inflammatory disorders, including allergic diseases (reviewed in [63]). Due to their surprisingly high stability, miRNAs may represent potential biomarkers for allergic diseases. In patients with allergic rhinitis, serum miR-181a levels were reduced, which correlated with disease severity and Th2 cytokines [64]. In addition, local miRNAs in extracellular vesicles isolated from the supernatant of nasal mucus from allergic rhinitis patients showed significant differential expression in comparison to healthy controls [65]. In allergic asthma, a combination of five serum miRNA ratios (miR-21-5p/miR-15a-5p; miR-27a-3p/miR-15a-5p; miR-29c-3p/miR-15a-5p; miR-223-3p/miR-425-5p; miR-15a/miR-342-3p) has been described as a reliable biomarker signature, which correlated well with clinical characteristics such as lung function and therapy regimen [66]. Of note, the use of ratios compensates substantial variation in miRNA levels and contributes to normalization of the result. In addition, single nucleotide polymorphisms in serum pre-miRNAs has been described in connection with the development of allergic asthma [67].

Microbiome

While gut and skin microbiome are obvious biomarker sources for allergy diagnosis (please see below), also the airways are colonized at least in the upper airways (reviewed in [68]), however the diagnostic value is still under investigation.

Non-invasive and minimally-invasive diagnostics of skin diseases

Atopic dermatitis usually begins during the first 6 months of life. It often precedes allergic rhinits and asthma (atopic march) [69], but can also spontaneously resolve. Non-allergic skin diseases other than atopic dermatitis such as systemic sclerosis, SLE, lupus erythematosus and psoriasis

are beyond the scope of this manuscript. Due to its accessibility, non-invasive diagnosis in skin has a longstanding tradition and even in times of big data and molecular pathology, the eye of a dermatologist is one of the most important diagnostic tools. The visual diagnosis is supported today by several invasive techniques (punch biopsies, histology, lesional gene expression) and more and more non-invasive or minimally invasive methods that are summarized in this section.

Non-invasive methods to assess skin functionality

Type-2 inflammation is associated with barrier dysfunction, but also with alterations in the skin's innate immune function; therefore, the assessment of skin function can play an important role in the diagnosis and clinical management of atopic dermatitis. One hallmark of a disturbed epidermal barrier is *dry skin*, that occurs due to an increased loss of water. This transepidermal water loss (TEWL) can be measured with a Tewameter that detects the rate of evaporation (Table 1). TEWL measurements are often used to determine the grade of barrier disruption in eczema and most importantly to monitor barrier restoration under therapy [70]. *Hydration* of the skin can be also measured using corneometry. Here, the skin serves as dielectric medium and the capacitance is measured as indicator of stratum corneum thickness and water content. Skin hydration represents not an indicator for barrier function, but is an indicator for injury, metabolic alterations and effective topical treatment. Skin vascularization can be measured by laser Doppler perfusion imaging [71]. Currently it is explored whether histamine pharmacodynamic response phenotypes can be distinguished by this technique [72].

Another skin parameter, *skin elasticity*, can be measured with a Cutameter that uses negative pressure to aspirate a skin area. This technique is used to evaluate the skin stiffness in scleroderma and systemic sclerosis [70,73]. In addition, *sebum lipids* can be quantified by sebumetry where a semi-transparent tape is applied to the skin that turns transparent after contact with sebum. The level of transparency can then be quantified and indicates the overall lipid content of skin. The exact lipid composition can then be identified by down-stream analyses such as mass spectrometry to provide information on disease-specific lipid alterations [74]. The pH level is another parameter of the skin barrier and is increased in situations of infection [75].

Minimally-invasive techniques to obtain skin samples

Tape-stripping is a well-established technique to obtain information on the outermost layer of the skin, the stratum corneum. Here, adhesive tapes are applied with a bit of pressure on the skin and removed again to generate an outside-inside view on skin layers (Table 1). The tapes can then be used to analyze mRNA, DNA or proteins and lipids. Whereas the biggest advantage of tape-stripping is its low invasiveness, the disadvantage is the standardization of sampling and the leakage of serum proteins on the tapes in diseases with disrupted epidermal barrier. In addition, human skin is rich in RNAses and molecular diagnostics on expression of disease-specific genes has to be thoroughly validated to avoid negative results due to RNA digestion [76]. In contrast to RNA analysis, protein sampling by tape-stripping is robust. Here, mechanistic insights have been obtained for psoriasis and eczema-associated proteins, anti-microbial peptides and also itch-associated proteins such as neurotrophic nerve growth factor (NGF) and IL-31 [77,78].

Scraping the skin surface with a surgical blade is another method to obtain minimally-invasive skin samples without inducing a scar, but this method has also difficulties in being standardized [79]. Proteins and mRNA can also be obtained furthermore from the interstitial fluid of blisters that have been induced by applying a vacuum (suction blister). Compared to tape-stripping, the amount of proteins and mRNA is much higher in blister fluids [80], however, this method is very time consuming and therefore not suited for clinical routine diagnostics.

Punch biopsies deliver, without any doubt, the maximum of information concerning gene expression, protein, content, tissue architecture and composition of inflammatory infiltrates. However, with diameters of 2-4 mm, local anesthesia and the requirement of sutures, patient's acceptability is low and samples cannot be obtained from children. The development of microbiopsies with a diameter less than 0.5 mm can overcome these disadvantages of common punch biopsies and allows profound RNA and DNA analysis from lesional tissue, even in babies [81]. On the basis of mRNA extracted from the lesional skin biopsies it is possible to reliably distinguish atopic eczema from psoriasis in case where visual anamnesis fails (e.g. on the scalp or hands). Specifically, abundant expression of iNos in the biopsies identifies psoriasis and is low in atopic eczema, whereas CCL27 is low in psoriasis and high in atopic eczema [82,83]. Furthermore, symptoms that occur in multiple skin diseases, such as acanthosis, can be defined on inflammatory mediators such as IL-17 that could be specifically treated with biologics [84].

New developments in non-invasive skin diagnostics

Probably the most fascinating development in non-invasive skin diagnostics are small microfluidic chips or patches that are applied onto the skin and measure a panel of parameters, such as pH, volume of sweat, lactate, glucose and chloride. Such chips/patches can thereby rapidly deliver information on the level of skin, electrolyte levels, hydration and even markers for cystic fibrosis [85,86]. These chips/patches do not need batteries as they gain their energy from radio waves that are emitted from electronic devices and directly transmit the information to analysis apps. Moreover, electrical impedance spectroscopy has been identified as a rapid and reliable diagnostic tool to detect skin barrier defects, which have been associated with many skin inflammatory disorders such as atopic dermatitis [87]. Together with the aforementioned artificial intelligence (AI) algorithms used for picture diagnosis, these non-invasive techniques will revolutionize diagnostics, not only in skin.

Urine

In atopic dermatitis, aquaporin-2, EDN and LTE4 are elevated in the urine [88,89] [90]. Also indicators of oxidation such as nitrate and 8-hydroxydeoxyguanosine (8-OHdG) are elevated in urine of AD patients [91].

miRNA

The serum levels of four miRNAs, including miR-21-5p, were reported to be elevated in experimental allergy models [66]. miRNAs were also found in human breast milk and were associated with early development of AD in the offspring [92]. In addition, miR-203a-3p, measured in the urine of children with AD, can be considered as a biomarker for the degree of inflammation and additional atopic diseases [93]. Moreover, miR-146a-5p [94] and miR-720 [95] were found to be increased in keratinocytes from AD patients and ıncreased serum levels of miR-483-5p [93] and miR-155-5p were described in AD [96]. The latter suppresses CTLA-4 that otherwise has a suppressive effect on T cells. Whether promising systemic miRNAs could also be used in a non- or minimal-invasive manner using DBS, has yet to be explored. The location of some of described miRNAs in breast milk, skin and urine basically paves the way for non-invasive sampling and diagnosis of specific allergic diseases.

Microbiome

The composition of the microbiota of the skin can also be analyzed by (bacterial) 16S ribosomal DNA [97] or by metagenomics [98]. Today we understand that compositions of the cutaneous microbiota are also determined by the local nature of the skin, and as such cebaceous, oily, moist and dry habitats have been defined [99]. Microbiota of the skin shape the barrier and immunity of the skin; inversely, and barrier and cutaneous immunity determine the composition of the cutaneous microbiota [100]. Early colonization with commensal *Staphylococci* at two months of age lowers the risk of atopic dermatitis at one year, indicating that microbiota analysis has the potential to be used as a biomarkers of disease development [101]. Together with imaging data, functional analysis of the skin, and minimally invasive sample analysis, these analyses offer a holistic view of barrier dysfunction.

Non-invasive and minimally-invasive diagnostics of food allergy, food intolerance and other gastrointestinal diseases

Due to the inaccessibility of the organ, diseases associated with gastrointestinal inflammation are a diagnostic challenge, with the need for invasive diagnostic procedures. In food allergy, which commonly presents in childhood, accurate non-invasive diagnostics and its discrimination from inflammatory or functional bowel diseases would represent a major advantage. Moreover, the physiological conditions of the gastrointestinal tract associated with changing intraluminal pH levels and the presence of a large variety of enzymes and microorganisms impacts on the stability of available potential biomarkers. Liquid biopsies represent a promising approach, thus far especially in advanced cancer diagnosis [102]. Diagnostic and prognostic biomarker characterization is still limited in food allergy [103] and blinded placebo-controlled oral food challenges remain the gold standard for diagnosis of food allergy.

Intestinal biomarkers

For non-invasive gastrointestinal diagnostics, stool samples containing inflammatory mediators, specific antibodies or markers associated with disease mechanisms may be used. Even though IgE is known to be present in stool samples of food sensitized patients [104], its value in food allergy diagnosis remains to be determined. In gastroenterological routine evaluation of patients with

inflammatory bowel disease (IBD), fecal calprotectin (S100A8/S100A9 dimer), which is indicative for neutrophils, plays an essential role for monitoring treatment efficacy and remission of inflammation. Of interest, fecal calprotectin levels were repeatedly reported to be elevated in food allergic children [105,106], but their diagnostic value as a biomarker in IgE-mediated food allergy still needs to be confirmed. Calprotectin is an antimicrobial peptide of the S100 family and is expressed by a large number of cells including monocytes and granulocytes and therefore represents a general inflammation marker (reviewed in [107]). Calprotectin measurements were suggested to be utilized for discrimination between allergic and non-allergic intestinal inflammation. For example, in non-IgE mediated cow's milk allergy, fecal calprotectin levels were significantly elevated in children with positive oral food challenge results even under elimination diet suggesting an ongoing intestinal low-grade inflammation [108]. In irritable bowel syndrome (IBS) patients with food hypersensitivities showing a positive oral food challenge after elimination diet, ECP and tryptase levels in fecal samples were found to be significantly higher compared to non-responders in challenge tests [109]. As serial serum ECP measurement has proven diagnostic value in eosinophilic esophagitis (EoE) [110], it is tempting to speculate that serial fecal testing could be an non-invasive alternative since ECP was described to be relative stable even under conditions of gastric acids [111]. In infant non-IgE mediated cow's milk allergy, fecal levels of another eosinophilic granule component, the eosinophil derived neurotoxin (EDN), showed the highest disease specificity (besides intestinal permeability tests) compared to all other determined parameters [112]. This knowledge might be useful for future definition of a minimally-invasive diagnostic panel for eosinophilic gastrointestinal diseases. For EoE sponge or string sample collection, as well as breath condensates are new methods under investigation that could replace endoscopic evaluations in the future [113]. The fraction of FeNO was proposed to prospectively correlate with peanut oral food challenge outcome if assessed together with Ara h2-specific IgE antibodies [114]. This correlation shows a high reproducibility in repeated measurements even after 1 year intervals [115]. However, another study performed in cow's milk allergic children did not observe a difference regarding FeNO levels in children with positive oral food challenge tests and in children without clinical reactions [116].

Although non-invasive biomarkers for food allergy are still in the early phase, the need to replace the troublesome oral food challenge is very high.

Urine

Food allergy has been associated with an apparent defect of the intestinal barrier function, which may in turn be associated with the flux of immunologically intact allergens across the intestinal epithelium. The carbohydrate uptake quantified in urine for measurement of intestinal permeability has high relevance in food allergy (and in IBD, IBS and colon cancer) [117]. Taken together, promising urinary biomarker candidates are already known and require further validation for allergic diseases.

miRNA

While various miRNAs play a role in diabetes mellitus, there are hardly any miRNA studies on food allergy or food intolerance. No differences in the miRNA expression profile of mast cells were found in a single publication on peanut allergy [118]. However, (extracellular) blood levels of miR-150 and miR-342-3p [119], but also of miR-106b-5p, miR-26a-5p and miR-29b-3p [120] were increased in IBS patients. miR-150-3p and -5p are said to be involved in inflammatory processes, whereas miR-342-3p plays a role in intestinal motility and the control of smooth muscle cells [119]. In addition, miR-490-5p plays a role in the mast cells in patients with the same clinical picture [121].

Microbiome

Specific fecal microbiota and metabolite signatures were suggested as early diagnostic markers [122] thus far in colon cancer. Preliminary specific microbial signatures have also been suggested for the diagnosis of food allergy [123] potentially enabling non-invasive diagnosis. For further investigation of microbial pattern, large-scale studies considering standardized pre-analytic sample preparation are required.

Non-invasive and minimally-invasive diagnostics of systemic allergies and anaphylaxis

Anaphylaxis, the most severe manifestation of an IgE-mediated allergic reaction, is an acute emergency [124]. The diagnosis is often straightforward based on clinical manifestations and exposure to a potential triggering agent. However, the diagnosis may be difficult when presentation is atypical (e.g. skin symptoms are absent in about 20% of patients), clinical

development is more gradual, and/or for health care workers who are not familiar with the presentation [125]. Thus, there is a high need for validated biomarkers that can be used to identify individuals at high risk for anaphylaxis, as well as for rapid confirmation of the diagnosis upon presentation to an urgent care facility.

Current biomarkers of anaphylaxis

Biomarkers in the blood, urine or in other tissue fluids are already available for anaphylaxis such as plasma histamine (or its metabolite, methylhistamine in urine) and total serum mast cell tryptase [126]. The rationale to use these mediators for diagnosis is based on the fact that tryptase and histamine are released from cellular granules upon activation [127]. The available diagnostic tryptase assay measures all forms of circulating tryptase. After anaphylaxis, levels are increased from 15-30 min to several hours after onset. As mature tryptase has to diffuse from the tissue to the blood, elevation of serum tryptase may not be detectable during the first 15 to 30 minutes [128].

Need for novel biomarkers and experimental biomarkers in anaphylaxis

The tryptase determination is the current gold standard for anaphylaxis diagnosis however, with limited sensitivity. Therefore, sequential analysis is necessary in mild forms of food anaphylaxis, where levels are low with the caveat that baseline samples are not available[129]. The assay is laborious, has no immediate result and difficult to realize in 'point of care' settings.

In the field of metabolomics and systems biology, novel early biomarkers of anaphylaxis may be identifiable using highly sensitive techniques such as matrix-assisted laser desorption/ionization (MALDI) in studies with well-phenotyped patients. Also, peripheral blood cell transcriptomics have been analyzed in patients with positive oral food challenges and potential signatures of six genes (LTB4R, PADI4, IL1R2, PPP1R3D, KLHL2, and ECHDC3) have been identified [130].

Urine

After anaphylaxis increased urinary concentrations of mast cell carboxypeptidase A3, chymase, cytokines, platelet-activating factor, CCL-2, and prostaglandins or sulfidoleukotrienes were found [131,132]. A multiplex approach in patients with anaphylaxis may identify further biomarker candidates using a small amount of serum and urine.

miRNA or microbiome

Currently both miRNA or microbiome were not explored as biomarker for anaphylaxis risks. However, miRNA-155 was shown to control mast cell activation and anaphylaxis in a mouse model [133] and may therefore offer future options of diagnostic characterization of anaphylaxis.

Outlook and EAACI position

The article highlights multiple opportunities that allow non- or minimally-invasive diagnosis of allergic diseases. The Covid-19 pandemic illustrates that the distinction of allergic diseases from other diseases such as viral infections is a key demand in medicine. As viral airway diseases such as Covid-19 affects the airway epithelium and thus the same organ and the same cells as it is the case in allergic airway diseases, these could potentially also be diagnosed with the same technology, which deserves further research. Most of the recently developed techniques along with the steadily increasing number of cytokine, chemokine, lipid mediator and miRNA biomarkers shows that the diagnosis and management of allergic diseases could be revolutionized in the coming years. In addition, the systematic use of multiple omic technologies combined with machine learning or artificial intelligence (reviewed in [134] [135] [136]) paves the way for further opportunities of non-invasive diagnosis in allergy. Examples for progress in this area are the discovery of DNA-methylation pattern in food allergy [137], or IgE pattern in large cohorts [138]. Moreover the assessment of multi-omics analysis in the U-BIOPRED cohort [139] and even in multi-cohort analysis of [140] have demonstrated the potential of computer-aided omic analysis. These future biomarkers are important drivers for precision medicine in the field of allergic diseases. To demonstrate the robustness of each biomarker and their predictive power, the task force strongly recommends support of research and innovation investments for sampling of well-defined patient cohorts to enable head-to-head testing, in order to make biomarker tests available for precision prevention and treatment of allergic diseases.

Figure Legend

Figure 1 summarizes the five main matrices of non-invasive diagnosis. Excretion such as urine and faeces are promising sources for food allergy diagnosis, while breath exhalates and nasal secretions (mucosal lining fluids) are key for airway diseases. Serum and microbiopsies are more universal matrices, however usually more invasive to obtain than the other matrices. In the inner circle, the cellular sources of biomarker are depicted, while the outer circle shows typical analytes implicated for the respective matrices. An excerpt of biomarkers discussed in the article is shown.

Table 1 Legend

The table 1 is summarizing the key methodologies used for the none- and minimally-invasive diagnosis of allergic diseases.

Suppl. table 1 Legend

The table displays current technology platforms available for diagnosis of allergy.

References

1. Pawankar R, Canonica, GR., Holgate ST, Lockey, RF. White Book on Allergy 2011-2012 Excecutive summary. *World Health Organization.* 2012.

2. Wickman M, Lupinek C, Andersson N, et al. Detection of IgE Reactivity to a Handful of Allergen Molecules in Early Childhood Predicts Respiratory Allergy in Adolescence. *EBioMedicine.* 2017;26:91-99.

3. Castelli S, Arasi S, Tripodi S, et al. IgE antibody repertoire in nasal secretions of children and adults with seasonal allergic rhinitis: a molecular analysis. *Pediatr Allergy Immunol.* 2019.

4. Jeon H, Jung JH, Kim Y, Kwon Y, Kim ST. Allergen Microarrays for In Vitro Diagnostics of Allergies: Comparison with ImmunoCAP and AdvanSure. *Ann Lab Med.* 2018;38(4):338-347.

5. Diener C, Galata V, Keller A, Meese E. MicroRNA profiling from dried blood samples. *Critical reviews in clinical laboratory sciences.* 2019;56(2):111-117.

6. Garib V, Rigler E, Gastager F, et al. Determination of IgE and IgG reactivity to more than 170 allergen molecules in paper-dried blood spots. *J Allergy Clin Immunol.* 2019;143(1):437-440.

7. Thijs JL, Fiechter R, Giovannone B, de Bruin-Weller MS. Biomarkers detected in dried blood spots from atopic dermatitis patients strongly correlate with disease severity. 2019;74(11):2240-2243.

8. Vazquez-Moron S, Ardizone Jimenez B, Jimenez-Sousa MA, Bellon JM, Ryan P, Resino S. Evaluation of the diagnostic accuracy of laboratory-based screening for hepatitis C in dried blood spot samples: A systematic review and meta-analysis. *Sci Rep.* 2019;9(1):7316.

9. Diamant Z, Vijverberg S, Alving K, et al. Toward clinically applicable biomarkers for asthma: An EAACI position paper. *Allergy.* 2019;74(10):1835-1851.

10. Korevaar DA, Westerhof GA, Wang J, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *Lancet Respir Med.* 2015;3(4):290-300.

11. Spector SL, Tan RA. Is a single blood eosinophil count a reliable marker for "eosinophilic asthma?". *J Asthma.* 2012;49(8):807-810.

12. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. *Allergy.* 2013;68(3):402-406.

13. Ciprandi G, Gallo F, Ricciardolo FL, Cirillo I. Fractional Exhaled Nitric Oxide: A Potential Biomarker in Allergic Rhinitis? *Int Arch Allergy Immunol.* 2017;172(2):99-105.

14. Nair P, Ochkur SI, Protheroe C, et al. Eosinophil peroxidase in sputum represents a unique biomarker of airway eosinophilia. *Allergy.* 2013;68(9):1177-1184.

15. Izuhara K, Nunomura S, Nanri Y, Ono J, Takai M, Kawaguchi A. Periostin: An emerging biomarker for allergic diseases. *Allergy.* 2019;74(11):2116-2128.

16. Pecak M, Korosec P, Kunej T. Multiomics Data Triangulation for Asthma Candidate Biomarkers and Precision Medicine. *OMICS.* 2018;22(6):392-409.

17. Zissler UM, Ulrich M, Jakwerth CA, et al. Biomatrix for upper and lower airway biomarkers in patients with allergic asthma. *J Allergy Clin Immunol.* 2018;142(6):1980-1983.

18. Zissler UM, Esser-von Bieren J, Jakwerth CA, Chaker AM, Schmidt-Weber CB. Current and future biomarkers in allergic asthma. *Allergy.* 2016;71(4):475-494.

19. Catino A, de Gennaro G, Di Gilio A, et al. Breath Analysis: A Systematic Review of Volatile Organic Compounds (VOCs) in Diagnostic and Therapeutic Management of Pleural Mesothelioma. *Cancers (Basel).* 2019;11(6).

20. de Vries R, Dagelet YWF, Spoor P, et al. Clinical and inflammatory phenotyping by breathomics in chronic airway diseases irrespective of the diagnostic label. *Eur Respir J.* 2018;51(1).

21. Brinkman P, Wagener AH, Hekking PP, et al. Identification and prospective stability of electronic nose (eNose)-derived inflammatory phenotypes in patients with severe asthma. *J Allergy Clin Immunol.* 2019;143(5):1811-1820 e1817.

22. Farraia M, Cavaleiro Rufo J, Paciencia I, et al. Human volatilome analysis using eNose to assess uncontrolled asthma in a clinical setting. *Allergy.* 2020.

23. Fuglsbjerg MG, Rasmussen MA, Hansen KS, et al. Limited clinical value of exhaled volatile organic compound measurements in childhood asthma. *ERJ Open Res.* 2018;4(4).

24. Alsved M, Fraenkel CJ, Bohgard M, et al. Sources of Airborne Norovirus in Hospital Outbreaks. *Clin Infect Dis.* 2020;70(10):2023-2028.

25. Efthimiadis A, Spanevello A, Hamid Q, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur Respir J Suppl.* 2002;37:19s-23s.

26. Pedersen F, Zissler UM, Watz H, Rabe KF, Hohlfeld JM, Holz O. Rating sputum cell quality in clinical trials for asthma and COPD treatment. *Int J Chron Obstruct Pulmon Dis.* 2019;14:195-198.

27. Spanevello A, Migliori GB, Sharara A, et al. Induced sputum to assess airway inflammation: a study of reproducibility. *Clin Exp Allergy.* 1997;27(10):1138-1144.

28. Gibson PG, Grootendor DC, Henry RL, et al. Sputum induction in children. *Eur Respir J Suppl.* 2002;37:44s-46s.

29. in 't Veen JC, de Gouw HW, Smits HH, et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. *Eur Respir J.* 1996;9(12):2441-2447.

30. Baigelman W, Chodosh S, Pizzuto D, Cupples LA. Sputum and blood eosinophils during corticosteroid treatment of acute exacerbations of asthma. *Am J Med.* 1983;75(6):929-936.

31. Louis R, Sele J, Henket M, et al. Sputum eosinophil count in a large population of patients with mild to moderate steroid-naive asthma: distribution and relationship with methacholine bronchial hyperresponsiveness. *Allergy.* 2002;57(10):907-912.

32. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med.* 2000;161(1):9-16.

33. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med.* 2000;161(1):64-72.

34. in't Veen JC, Smits HH, Hiemstra PS, Zwinderman AE, Sterk PJ, Bel EH. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. *Am J Respir Crit Care Med.* 1999;160(1):93-99.

35. Demarche SF, Schleich FN, Henket MA, Paulus VA, Van Hees TJ, Louis RE. Effectiveness of inhaled corticosteroids in real life on clinical outcomes, sputum cells and systemic inflammation in asthmatics: a retrospective cohort study in a secondary care centre. *BMJ Open.* 2017;7(11):e018186.

36. Idzko M, Pitchford S, Page C. Role of platelets in allergic airway inflammation. *J Allergy Clin Immunol.* 2015;135(6):1416-1423.

37. Durk T, Duerschmied D, Muller T, et al. Production of serotonin by tryptophan hydroxylase 1 and release via platelets contribute to allergic airway inflammation. *Am J Respir Crit Care Med.* 2013;187(5):476-485.

38. Johansson MW, Han ST, Gunderson KA, Busse WW, Jarjour NN, Mosher DF. Platelet activation, P-selectin, and eosinophil beta1-integrin activation in asthma. *Am J Respir Crit Care Med.* 2012;185(5):498-507.

39. Tornhamre S, Ehnhage A, Kolbeck KG, Edenius C, Lindgren JA. Uncoupled regulation of leukotriene C4 synthase in platelets from aspirin-intolerant asthmatics and healthy volunteers after aspirin treatment. *Clin Exp Allergy.* 2002;32(11):1566-1573.

40. Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepko R, Mroczko B, Szmitkowski M. Changes in RANTES and beta-thromboglobulin after intensive exercise in patients with allergic asthma. *Int Arch Allergy Immunol.* 2009;148(1):31-40.

41. Macfarlane AJ, Dworski R, Sheller JR, Pavord ID, Kay AB, Barnes NC. Sputum cysteinyl leukotrienes increase 24 hours after allergen inhalation in atopic asthmatics. *Am J Respir Crit Care Med.* 2000;161(5):1553-1558.

42. Schauer U, Alefsen S, Jager R, Riedel F, Rieger CH. Blood eosinophils, leukotriene C4 generation, and bronchial hyperreactivity in formerly preterm infants. *Arch Dis Child.* 1994;71(6):506-510.

43. Melo JT, Jr., Tunstall T, Pizzichini MMM, et al. IL-5 Levels in Nasosorption and Sputosorption Correlate with Sputum Eosinophilia in Allergic Asthma. *Am J Respir Crit Care Med.* 2019;199(2):240-243.

44. Baumann R, Brand P, Chaker A, et al. Human nasal mucosal C-reactive protein responses after inhalation of ultrafine welding fume particles: positive correlation to systemic C-reactive protein responses. *Nanotoxicology.* 2018;12(10):1130-1147.

45. Baumann R, Rabaszowski M, Stenin I, et al. Nasal levels of soluble IL-33R ST2 and IL-16 in allergic rhinitis: inverse correlation trends with disease severity. *Clin Exp Allergy.* 2013;43(10):1134-1143.

46. Cai F, Abreu F, Ding H, Yen K, Matthews J, Scheerens H. Nasal Biomarkers Characterization In Lebrikizumab Bronchoscopy Study (CLAVIER). *J Allergy Clin Immunol.* 2018;141(5):AB118.

47. Song C, Chorath J, Pak Y, Redjal N. Use of Dipstick Assay and Rapid PCR-DNA Analysis of Nasal Secretions for Diagnosis of Bacterial Sinusitis in Children With Chronic Cough. *Allergy Rhinol (Providence).* 2019;10:2152656718821281.

48. Thwaites RS, Jarvis HC, Singh N, et al. Absorption of Nasal and Bronchial Fluids: Precision Sampling of the Human Respiratory Mucosa and Laboratory Processing of Samples. *Journal of visualized experiments : JoVE.* 2018(131).

49. Popovic M, Fiano V, Fasanelli F, et al. Differentially methylated DNA regions in early childhood wheezing: An epigenome-wide study using saliva. *Pediatr Allergy Immunol.* 2019;30(3):305-314.

50. Divekar R, Hagan J, Rank M, et al. Diagnostic Utility of Urinary LTE4 in Asthma, Allergic Rhinitis, Chronic Rhinosinusitis, Nasal Polyps, and Aspirin Sensitivity. *J Allergy Clin Immunol Pract.* 2016;4(4):665-670.

51. Szefler SJ, Wenzel S, Brown R, et al. Asthma outcomes: biomarkers. *J Allergy Clin Immunol.* 2012;129(3 Suppl):S9-23.

52. Lee CW, Lewis RA, Corey EJ, Austen KF. Conversion of leukotriene D4 to leukotriene E4 by a dipeptidase released from the specific granule of human polymorphonuclear leucocytes. *Immunology.* 1983;48(1):27-35.

53. Laidlaw TM, Kidder MS, Bhattacharyya N, et al. Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. *Blood.* 2012;119(16):3790-3798.

54. Celejewska-Wojcik N, Mastalerz L, Wojcik K, et al. Incidence of aspirin hypersensitivity in patients with chronic rhinosinusitis and diagnostic value of urinary leukotriene E4. *Pol Arch Med Wewn.* 2012;122(9):422-427.

55. Yamaguchi H, Higashi N, Mita H, et al. Urinary concentrations of 15-epimer of lipoxin A(4) are lower in patients with aspirin-intolerant compared with aspirin-tolerant asthma. *Clin Exp Allergy.* 2011;41(12):1711-1718.

56. Sanak M, Bochenek G, Faber J, Plutecka H, Szczeklik A. Elevated urinary leukotriene E excretion in asthma: a comparison of HPLC-mass spectrometry and ELISA. *Allergy.* 2010;65(5):663-664.

57. Bancalari L, Conti I, Giannessi D, et al. Early increase in urinary leukotriene E4 (LTE4) is dependent on allergen dose inhaled during bronchial challenge in asthmatic subjects. *Allergy.* 1999;54(12):1278-1285.

58. Santarelli GD, Lam KK, Han JK. Establishing Urinary Leukotriene E4 as a Diagnostic Biomarker for Chronic Rhinosinusitis with Comorbid Asthma and Atopy. *Otolaryngol Head Neck Surg.* 2019;161(5):764-769.

59. Liu MC, Dube LM, Lancaster J. Acute and chronic effects of a 5-lipoxygenase inhibitor in asthma: a 6-month randomized multicenter trial. Zileuton Study Group. *J Allergy Clin Immunol.* 1996;98(5 Pt 1):859-871.

60. O'Shaughnessy KM, Wellings R, Gillies B, Fuller RW. Differential effects of fluticasone propionate on allergen-evoked bronchoconstriction and increased urinary leukotriene E4 excretion. *Am Rev Respir Dis.* 1993;147(6 Pt 1):1472-1476.

61. Barreto M, Capi M, Lionetto L, et al. Urinary and exhaled biomarkers of exercise-induced bronchoconstriction in atopic asthmatic children. *Pediatr Pulmonol.* 2019;54(9):1447-1456.

62. Klonoff-Cohen H, Polavarapu M. Eosinophil protein X and childhood asthma: A systematic review and meta-analysis. *Immun Inflamm Dis.* 2016;4(2):114-134.

63. Weidener J, Bartel S, Zissler UM, et al. Spotlight on microRNAs in allergy and asthma *Allergy.* in press.

64. Liu W, Zeng Q, Luo R. Correlation between Serum Osteopontin and miR-181a Levels in Allergic Rhinitis Children. *Mediators Inflamm.* 2016;2016:9471215.

65. Wu G, Yang G, Zhang R, et al. Altered microRNA Expression Profiles of Extracellular Vesicles in Nasal Mucus From Patients With Allergic Rhinitis. *Allergy Asthma Immunol Res.* 2015;7(5):449-457.

66. Milger K, Gotschke J, Krause L, et al. Identification of a plasma miRNA biomarker signature for allergic asthma: A translational approach. *Allergy.* 2017;72(12):1962-1971.

67. Su XW, Yang Y, Lv ML, et al. Association between single-nucleotide polymorphisms in pre-miRNAs and the risk of asthma in a Chinese population. *DNA Cell Biol.* 2011;30(11):919-923.

68. Dimitri-Pinheiro S, Soares R, Barata P. The Microbiome of the Nose-Friend or Foe? *Allergy Rhinol (Providence).* 2020;11:2152656720911605.

69. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance. *Ann Allergy Asthma Immunol.* 2018;120(2):131-137.

70. Polanska A, Danczak-Pazdrowska A, Silny W, Jenerowicz D, Olek-Hrab K, Osmola-Mankowska A. Nonlesional skin in atopic dermatitis is seemingly healthy skin - observations using noninvasive methods. *Wideochir Inne Tech Maloinwazyjne.* 2013;8(3):192-199.

71. Fullerton A, Stucker M, Wilhelm KP, et al. Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging. A report from the Standardization Group of the European Society of Contact Dermatitis based upon the HIRELADO European community project. *Contact Dermatitis.* 2002;46(3):129-140.

72. Kumar SS, Liu X, Sherwin CM, Jones BL. The Reliability of Histamine Pharmacodynamic Response Phenotype Classification in Children With Allergic Disease. *Front Pharmacol.* 2020;11:227.

73. Dobrev HP. In vivo study of skin mechanical properties in patients with systemic sclerosis. *J Am Acad Dermatol.* 1999;40(3):436-442.

74. Berdyshev E, Goleva E, Bronova I, et al. Lipid abnormalities in atopic skin are driven by type 2 cytokines. *JCI Insight.* 2018;3(4).

75. Hulpusch C, Tremmel K, Hammel G, et al. Skin pH-dependent Staphylococcus aureus abundance as predictor for increasing atopic dermatitis severity. *Allergy.* 2020.

76. Dyjack N, Goleva E, Rios C, et al. Minimally invasive skin tape strip RNA sequencing identifies novel characteristics of the type 2-high atopic dermatitis disease endotype. *J Allergy Clin Immunol.* 2018;141(4):1298-1309.

77. Yamaguchi J, Aihara M, Kobayashi Y, Kambara T, Ikezawa Z. Quantitative analysis of nerve growth factor (NGF) in the atopic dermatitis and psoriasis horny layer and effect of treatment on NGF in atopic dermatitis. *J Dermatol Sci.* 2009;53(1):48-54.

78. Mehul B, Laffet G, Seraidaris A, et al. Noninvasive proteome analysis of psoriatic stratum corneum reflects pathophysiological pathways and is useful for drug profiling. *Br J Dermatol.* 2017;177(2):470-488.

79. Kang H, Li X, Zhou Q, et al. Exploration of candidate biomarkers for human psoriasis based on gas chromatography-mass spectrometry serum metabolomics. *Br J Dermatol.* 2017;176(3):713-722.

80. Svoboda M, Hlobilova M, Maresova M, et al. Comparison of suction blistering and tape stripping for analysis of epidermal genes, proteins and lipids. *Arch Dermatol Res.* 2017;309(9):757-765.

81. Lin LL, Prow TW, Raphael AP, et al. Microbiopsy engineered for minimally invasive and suture-free sub-millimetre skin sampling. *F1000Res.* 2013;2:120.

82. Garzorz-Stark N, Krause L, Lauffer F, et al. A novel molecular disease classifier for psoriasis and eczema. *Exp Dermatol.* 2016;25(10):767-774.

83. Quaranta M, Knapp B, Garzorz N, et al. Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. *Sci Transl Med.* 2014;6(244):244ra290.

84. Eyerich K, Eyerich S. Immune response patterns in non-communicable inflammatory skin diseases. *J Eur Acad Dermatol Venereol.* 2018;32(5):692-703.

85. Reeder JT, Xue Y, Franklin D, et al. Resettable skin interfaced microfluidic sweat collection devices with chemesthetic hydration feedback. *Nat Commun.* 2019;10(1):5513.

86. Reeder JT, Choi J, Xue Y, et al. Waterproof, electronics-enabled, epidermal microfluidic devices for sweat collection, biomarker analysis, and thermography in aquatic settings. *Sci Adv.* 2019;5(1):eaau6356.

87. Rinaldi AO, Morita H, Wawrzyniak P, et al. Direct assessment of skin epithelial barrier by electrical impedance spectroscopy. *Allergy.* 2019;74(10):1934-1944.

88. Di ZH, Lv YN, Zhang L, et al. Urinary aquaporin-2 is elevated in infant atopic dermatitis. *Br J Dermatol.* 2010;163(5):1132-1134.

89. Goto T, Morioka J, Inamura H, et al. Urinary eosinophil-derived neurotoxin concentrations in patients with atopic dermatitis: a useful clinical marker for disease activity. *Allergol Int.* 2007;56(4):433-438.

90. Hon KL, Leung TF, Ma KC, et al. Urinary leukotriene E4 correlates with severity of atopic dermatitis in children. *Clin Exp Dermatol.* 2004;29(3):277-281.

91. Nakai K, Yoneda K, Maeda R, et al. Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis. *J Eur Acad Dermatol Venereol.* 2009;23(12):1405-1408.

92. Simpson MR, Brede G, Johansen J, et al. Human Breast Milk miRNA, Maternal Probiotic Supplementation and Atopic Dermatitis in Offspring. *PLoS One.* 2015;10(12):e0143496.

93. Lv Y, Qi R, Xu J, et al. Profiling of serum and urinary microRNAs in children with atopic dermatitis. *PLoS One.* 2014;9(12):e115448.

94. Rebane A, Runnel T, Aab A, et al. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. *J Allergy Clin Immunol.* 2014;134(4):836-847 e811.

95. Chikh A, Matin RN, Senatore V, et al. iASPP/p63 autoregulatory feedback loop is required for the homeostasis of stratified epithelia. *EMBO J.* 2011;30(20):4261-4273.

96. Sonkoly E, Janson P, Majuri ML, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. *J Allergy Clin Immunol.* 2010;126(3):581-589 e581-520.

97. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science.* 2009;326(5960):1694-1697.

98. Emiola A, Oh J. High throughput in situ metagenomic measurement of bacterial replication at ultra-low sequencing coverage. *Nat Commun.* 2018;9(1):4956.

99. Oh J, Byrd AL, Park M, Program NCS, Kong HH, Segre JA. Temporal Stability of the Human Skin Microbiome. *Cell.* 2016;165(4):854-866.

100. Eyerich S, Eyerich K, Traidl-Hoffmann C, Biedermann T. Cutaneous Barriers and Skin Immunity: Differentiating A Connected Network. *Trends Immunol.* 2018;39(4):315-327.

101. Kennedy EA, Connolly J, Hourihane JO, et al. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. *J Allergy Clin Immunol.* 2017;139(1):166-172.

102. Bellosillo B, Montagut C. High-accuracy liquid biopsies. *Nat Med.* 2019;25(12):1820-1821.

103. Muraro A, Arasi S. Biomarkers in Food Allergy. *Curr Allergy Asthma Rep.* 2018;18(11):64.

104. Andre F, Andre C, Colin L, Cavagna S. IgE in stools as indicator of food sensitization. *Allergy.* 1995;50(4):328-333.

105. Beser OF, Sancak S, Erkan T, Kutlu T, Cokugras H, Cokugras FC. Can Fecal Calprotectin Level Be Used as a Markers of Inflammation in the Diagnosis and Follow-Up of Cow's Milk Protein Allergy? *Allergy Asthma Immunol Res.* 2014;6(1):33-38.

106. Kukkonen K, Kuitunen M, Haahtela T, Korpela R, Poussa T, Savilahti E. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. *Pediatr Allergy Immunol.* 2010;21(1 Pt 1):67-73.

107. Ayling RM, Kok K. Fecal Calprotectin. *Adv Clin Chem.* 2018;87:161-190.

108. Merras-Salmio L, Kolho KL, Pelkonen AS, Kuitunen M, Makela MJ, Savilahti E. Markers of gut mucosal inflammation and cow's milk specific immunoglobulins in non-IgE cow's milk allergy. *Clin Transl Allergy.* 2014;4(1):8.

109. Carroccio A, Brusca I, Mansueto P, et al. Fecal assays detect hypersensitivity to cow's milk protein and gluten in adults with irritable bowel syndrome. *Clin Gastroenterol Hepatol.* 2011;9(11):965-971 e963.

110. Domenech Witek J, Jover Cerda V, Gil Guillen V, Domenech Clar JB, Rodriguez Pacheco R. Assessing eosinophilic cationic protein as a biomarker for monitoring patients with eosinophilic esophagitis treated with specific exclusion diets. *World Allergy Organ J.* 2017;10(1):12.

111. Berstad K, Berstad A, Jr., Sjodahl R, Weberg R, Berstad A. Eosinophil cationic protein and phospholipase A2 activity in human gastric juice. With emphasis on Helicobacter pylori status and effects of antacids. *Scand J Gastroenterol.* 1992;27(12):1011-1017.

112. Kalach N, Kapel N, Waligora-Dupriet AJ, et al. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin Chem Lab Med.* 2013;51(2):351-361.

113. Hines BT, Rank MA, Wright BL, et al. Minimally invasive biomarker studies in eosinophilic esophagitis: A systematic review. *Ann Allergy Asthma Immunol.* 2018;121(2):218-228.

114. Preece K, Bhatia R, Belcher J, et al. The fraction of exhaled nitric oxide improves prediction of clinical allergic reaction to peanut challenge in children. *Clin Exp Allergy.* 2014;44(3):371-380.

115. Percival E, Bhatia R, Preece K, et al. Reproducibility of serum IgE, Ara h2 skin prick testing and fraction of exhaled nitric oxide for predicting clinical peanut allergy in children. *Allergy Asthma Clin Immunol.* 2016;12:35.

116. Gabriele C, Hol J, Kerkhof E, et al. Fractional exhaled nitric oxide in infants during cow's milk food challenge. *Pediatr Allergy Immunol.* 2008;19(5):420-425.

117. Samadi N, Klems M, Untersmayr E. The role of gastrointestinal permeability in food allergy. *Ann Allergy Asthma Immunol.* 2018;121(2):168-173.

118. Larsen LF, Juel-Berg N, Hansen A, et al. No difference in human mast cells derived from peanut allergic versus non-allergic subjects. *Immun Inflamm Dis.* 2018;6(4):416-427.

119. Fourie NH, Peace RM, Abey SK, et al. Elevated circulating miR-150 and miR-342-3p in patients with irritable bowel syndrome. *Exp Mol Pathol.* 2014;96(3):422-425.

120. Tao W, Dong X, Kong G, Fang P, Huang X, Bo P. Elevated Circulating hsa-miR-106b, hsa-miR-26a, and hsa-miR-29b in Type 2 Diabetes Mellitus with Diarrhea-Predominant Irritable Bowel Syndrome. *Gastroenterol Res Pract.* 2016;2016:9256209.

121. Ren HX, Zhang FC, Luo HS, Zhang G, Liang LX. Role of mast cell-miR-490-5p in irritable bowel syndrome. *World J Gastroenterol.* 2017;23(1):93-102.

122. Yachida S, Mizutani S, Shiroma H, et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med.* 2019;25(6):968-976.

123. Feehley T, Plunkett CH, Bao R, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med.* 2019;25(3):448-453.

124. Muraro A, Roberts G, Worm M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy.* 2014;69(8):1026-1045.

125. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol.* 2004;114(2):371-376.

126. Simons FE. Anaphylaxis: Recent advances in assessment and treatment. *J Allergy Clin Immunol.* 2009;124(4):625-636; quiz 637-628.

127. Metcalfe DD, Pawankar R, Ackerman SJ, et al. Biomarkers of the involvement of mast cells, basophils and eosinophils in asthma and allergic diseases. *World Allergy Organ J.* 2016;9:7.

128. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest.* 1989;83(5):1551-1555.

129. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci.* 1991;36(3):857-865.

130. Watson CT, Cohain AT, Griffin RS, et al. Integrative transcriptomic analysis reveals key drivers of acute peanut allergic reactions. *Nat Commun.* 2017;8(1):1943.

131. Vadas P, Gold M, Perelman B, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med.* 2008;358(1):28-35.

132. Beck SC, Wilding T, Buka RJ, Baretto RL, Huissoon AP, Krishna MT. Biomarkers in Human Anaphylaxis: A Critical Appraisal of Current Evidence and Perspectives. *Front Immunol.* 2019;10:494.

133. Biethahn K, Orinska Z, Vigorito E, et al. miRNA-155 controls mast cell activation by regulating the PI3Kgamma pathway and anaphylaxis in a mouse model. *Allergy.* 2014;69(6):752-762.

134. Baurley JW, McMahan CS, Ervin CM, Pardamean B, Bergen AW. Biosignature Discovery for Substance Use Disorders Using Statistical Learning. *Trends Mol Med.* 2018;24(2):221-235.

135. Tartarisco G, Tonacci A, Minciullo PL, et al. The soft computing-based approach to investigate allergic diseases: a systematic review. *Clin Mol Allergy.* 2017;15:10.

136. Eguiluz-Gracia I, Tay TR, Hew M, et al. Recent developments and highlights in biomarkers in allergic diseases and asthma. *Allergy.* 2018;73(12):2290-2305.

137. Alag A. Machine learning approach yields epigenetic biomarkers of food allergy: A novel 13-gene signature to diagnose clinical reactivity. *PLoS One.* 2019;14(6):e0218253.

138. Fontanella S, Frainay C, Murray CS, Simpson A, Custovic A. Machine learning to identify pairwise interactions between specific IgE antibodies and their association with asthma: A cross-sectional analysis within a population-based birth cohort. *PLoS Med.* 2018;15(11):e1002691.

139. De Meulder B, Lefaudeux D, Bansal AT, et al. A computational framework for complex disease stratification from multiple large-scale datasets. *BMC Syst Biol.* 2018;12(1):60.

140. Loza MJ, Djukanovic R, Chung KF, et al. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respir Res.* 2016;17(1):165.

Indication	Device / Tool or methodology	Description
Allergic airway diseases: Allergic rhinitis, Asthma	**Dried blood spots**	Dried blood spots (DBS) allow sampling of capillary blood micro samples in very small volumes (±10 µL).
	Nasal absorption sampling	Nasal absorption sampling allows the collection of nasal lining fluids (±40 µL) and determination of epithelial cytokines in a standardized manner.
	Nitric oxide analyzers	Nitric oxide analyzers for the determination of exhaled fraction nitric oxide (FeNO) level.
	CT-imaging	CT-imaging technology allows to identify intra- or extra-thoracic airway thickening.
	eNoses	eNOSEs detect specific volatile organic compounds (VOCs) without identifying their molecular entities. Other eNoses detect the specific molecular entities using LC-MS/MS but this currently requires sending samples to central laboratories.
Skin (Atopic dermatitis)	**Tewameter**	TEWL measurements are often used to determine the grade of barrier disruption in eczema by determination of the rate of evaporation.
	Cutameter	Cutameter measure *skin elasticity* to evaluate the skin stiffness in scleroderma and systemic sclerosis.
	Sebumetry	Sebumetry is using a semi-transparent tape which is applied to the skin that turns transparent after contact with sebum for the quantification of the overall lipid content of skin. Potential down-stream analyses such as MS to determine disease-specific lipid alterations.
	Corneometry	Corneometry allows the measurement of the hydration of the skin as indicator of stratum corneum thickness and water content as an indicator for injury, metabolic alterations and effective topical treatment.
	Microfluidic chips or patches	Microfluidic chips or patches on the skin allow the measurement of pH, volume of sweat, lactate, glucose and electrolyte levels.
	Tape-stripping	Tape-stripping is a well-established technique to obtain information on the outermost layer of the skin, the stratum

		corneum. The tapes can then be used to analyze mRNA, DNA or proteins and lipids.
	Scraping the skin surface	minimally-invasive skin samples without inducing a scar
	Electrical impedance spectroscopy	Electrical impedance spectroscopy allows the detection of skin barrier defects, which have been associated with many skin inflammatory disorders such as atopic dermatitis.
	Suction blister	Proteins and mRNA can also be obtained furthermore from the interstitial fluid of blisters that have been induced by applying a vacuum (suction blister).
Food allergy	**Urine**	Excretion for monitoring of allergic diseases and asthma by measurement of e.g. eicosanoids and their metabolites.