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Diagnostic and Therapeutic Procedures in Varying Allergy Types

Debates in allergy medicine: Molecular allergy diagnosis with ISAC will replace screenings by skin prick test in the future

Abstract

In today's clinical practice patients' skin is used as screening organ for diagnosing type 1 allergy. According to European guidelines skin prick testing with a panel of 18 allergen extracts is recommended, in the US between 10 to 50 allergens are used. The specificity and sensitivity of skin testing is individually highly variable depending on age, body mass, and skin barrier status. In atopic inflammation skin testing gives more false positive results. Smaller skin area and strain limits prick testing in small children. Although the risk for systemic reactions in skin prick testing is very small, emergency medications must be available. Considering the fact that IgE is the only reliable biomarker for type I allergy, upfront IgE screening with ISAC, followed by fewer skin tests to approve positive sensitizations, is proposed. It is time to arrive in the age of molecular allergy diagnosis in daily patient care.

Background

Since its detection, specific IgE represents the only diagnostic biomarker for exposure and sensitization in allergy [1] with predictive value in asthma [2], and of value for selecting patients for allergen immunotherapy [3]. It reliably correlates with clinical symptoms in respiratory allergies, less in food allergies, and is usually interpreted in the context of skin prick tests [4]. In most cases during daily clinical practice, IgE-determinations as well as skin prick tests are done with allergen extracts. In both cases, results must be interpreted considering the clinical symptoms and history of the patient, as even inhalant sensitization not necessarily correlates with symptoms [5]. Allergen extracts are produced under good laboratory practice (GLP) conditions by incubation of allergen sources (pollen from pollen farms, cultured house dust mites, food) in aqueous buffer solutions, followed by filtering and purification steps. As a result, the extracts contain a variety of allergens (e.g. Bet v 1 a), besides non-allergenic proteins or isoallergens non-relevant for IgE binding (e.g. Bet v 1d, e). Production severely depends on the allergen sources and associated environmental, culture and ripening conditions, which makes standardization of allergen extracts a difficult task. To improve the quality of extracts, it is accepted that they may sometimes be "spiked" with singe allergen molecules [6]. Variations in the biological activity of allergen extracts are decisive for in vitro IgE testing and skin prick tests, and even more when extracts are applied as therapeutics for allergen immunotherapy [7]. Comparisons to reference extracts have been requested for a long time [8]. In Europe, allergens for diagnostic application fall under the directives for therapeutics [9]. The cost-intensive procedures of approval and maintenance of approved products led to a dramatic reduction of diagnostic allergens available for intradermal use [10], but also for skin prick allergens a diagnostic bottle neck is to be expected. The improved standards of diagnostic and therapeutic allergens may critically affect allergy diagnosis in the near future and

prompts the critical evaluation of the fidelity and reliability of alternative methods.

Skin prick testing – allergy screening in the skin

Skin prick tests are regarded as means to determine sensitization and should be interpreted in the light of clinical history, clinical picture and results of testing for specific IgE. According to the American College of Allergy, Asthma and Immunology (ACAAI) [11], 10–50 allergen extracts are used for skin prick testing. The European guidelines propose a panel of 18 respiratory allergens of which, simultaneously, improved standardization is encouraged [12]. While skin prick testing in respiratory allergies is a reliable diagnostic tool, in food allergy more false positive results are seen on the one hand, while on the other hand over 95% of patients negative in skin prick tests with food do not present with immediate type symptoms [13]. The skin prick results should be compared to the positive control prick with histamine dihydrochloride 10 mg/ml [14]. The calculation of a histamine equivalent prick -index (HEP) area may be helpful, where the allergen prick size is correlated to the size of the histamine wheal to define a cut off value, but it is proposed that the true area of the wheal "is theoretically more accurate" than the diameter [15]. Like the Immuno solid-phase allergen chip (ISAC) -test, therefore, also the skin prick test (SPT) is a "semiquantitative" method. The wheal size of allergen skin prick tests has been associated with the extent of clinical reactivity especially in adults [16], and was suggested a predictive marker for clinical reactivity to specific food allergens, for instance for albumin at a diameter of 9 mm, for yolk 7, for cow's milk or fresh cow's milk 20 or 1 mm, respectively [17].

It should be noted that the histamine prick result itself is individually variable and depends on age and body mass index of the patient [18]. This finding was approved in a Korean study where obese children had significantly larger histamine wheals [19]. In contrast, the histamine tests in atopic children resulted in a significantly smaller flare, but longer itch reaction [20].

Importantly, the mean wheal diameter resulting from the prick has been shown to be affected by the personnel testing and by the lancet weight [21], and is differing between test centers, naturally depending on the concentration of the histamine solution used: a 1 mg/ml solution with wheals between 3 and 6.8 mm was found unacceptable, the form and size of lancets used resulted in comparable analytical sensitivities and specificities, and pain scores [22].

The collected data thus document continuous efforts to improve the fidelity of skin prick tests which individually vary depending not only on the patient, but even more on the assistants doing the test and on the exactness of the recording method.

There are disadvantages in skin prick testing

Anaphylactic side effects are a concern when testing with biologically active allergens in vivo and the possibility of emergency treatment must be provided [23]. In the largest cohort so far investigated with this specific question, 31,000 patients, in 0.077% systemic side reactions were recorded, with the highest risk with peanut and nuts when the wheal diameter was of over 8 mm [24]. The risk for systemic reactions due to skin tests treated by epinephrin i.m. evaluated in 1456 patients was totally 3.6% (intradermal testing: 3.1%; skin prick testing 0.41%), and highest in females [25]. A study in 20,530 patients reported that 80 patients tested experienced systemic reactions, 13 of them more severe, and calculated a risk of 0.009 and 0.003% for experiencing a major reaction during skin prick testing [26].

It is accepted that several conditions may elevate the risk for systemic reactions in skin prick testing, like previous anaphylactic events, testing in small children and in pregnancy with a risk for mother and child, and in uncontrolled asthma [27]. It is also known that the higher number of skin tests in polysensitized patients needed for diagnosis is associated with a higher risk for adverse reactions [28]. Larger skin prick test reactions and associated enhanced risk for adverse reactions have been explained by enhanced permeability of the skin [28].

Conditions reducing skin prick test reliability

It has been reported that stress in the patient may sporadically lead to false positive skin reactivity [29], but more studies are needed to support these observations. However, what is much more important in clinics is that the intake of numerous medications may interfere with skin prick reactivity. This was recently analysed in detail in a large retrospective study [30]. Tricyclic antidepressants, benzodiazepines, quetiapine, and mirtazapine should be discontinued 1 week before testing, H(1)-blockers 3 days [30]. The risk for a negative histamine test was not elevated for selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors and proton pump inhibitors [30]. Therefore, the abstinence from drugs needs to be planned in advance of skin prick testing, but it is often a difficult task to take patients off their medications even over a few days. IgE testing is not dependent on any interferences with medications.

Why doctors and patients like skin prick testing

Albeit the many practical limitations as reviewed above, it seems impossible to abstain from skin prick tests in daily management of allergic patients. In most incidences skin prick tests are done upfront further allergy diagnosis as they allow a readout within 15–20 min. Physicians of any specialty apply skin prick tests,

especially as costs are refunded by health insurances and they do not require expensive devices but only well-trained personnel. They know well that skin prick tests visually and dramatically document to the patients the existing hypersensitivity. This is very useful for patient compliance concerning further allergy tests and therapy. A patient who has undergone repeated skin tests is no longer fond of this method and tends to reject repeated testing.

Skin prick testing in an epidemiological study achieved a 90% compliance in school children when re-testing after 10 years [31] and allowed an estimation of prevalence of sensitization rising from 30 to 41%. Positive skin prick tests in newborns predicted an allergic career to early adult age [32]. However, when skin test with house dust mite allergens was evaluated in 692 patients, it was found most reliable only in patients below 50 years of age [33]. This is problematic as allergies occur in elderly to a similar rate as in younger adults, and must be diagnosed, as reviewed previously [34].

The interpretation of skin prick test results on atopic skin, which may be false positive, actually requires an expert in order to prevent unnecessary avoidance diets [35]. When Foong et al**.** compared head-to-head skin prick testing and IgE testing in atopic children, there was no difference in food-specific results, but in respiratory allergies specific IgE testing resulted in more (false) positive results than prick testing or ISAC IgE testing [36]. Also before, ISAC testing in atopic children has been found to be a promising alternative overcoming problems of testing in the hypersensitive atopic skin, but still correlating well with the skin tests [37].

Molecular allergy diagnosis goes global

In contrast to natural allergen extracts and purified allergens, recombinant allergens can be expressed under standardized conditions without undesired contamination, with an exactness matching the today's requirements of diagnostic allergens.

Overall, the accumulating knowledge on molecular allergens has changed our understanding of allergic mechanisms and helped to design sensitization maps all over the world [38], and even establish correlations with climate change [39]. Equally important, molecular allergy, particularly multiplex allergen microarray diagnosis proved successful globally, such as in Spain [40], Italy [41], in the overall Mediterranean area [42], Iran [43], South Africa [44, 45], Brazil [46], and in China [47].

Molecular allergy diagnosis using singleplex allergens or multiplex allergen microarrays are typical methods of precision medicine [48] and they enhance the specificity of IgE-diagnosis in polysensitized respiratory allergies [49], can be applied in food allergies [36, 50] and atopic eczema [36, 37], and may even reveal unexplained anaphylaxis [3]. A strong correlation was found between results with the ISAC112 microarray test, and SPT and other specific IgE tests [51, 52], with a particularly good correlation in allergies to pollen [53] and to house dust mites [54]. It is accepted that molecular allergy diagnosis improves the risk evaluation, sorts out genuine from cross-reactive sensitizations, improves the overall predictive value of the diagnostic results, as well as the accuracy of the resulting allergen immunotherapy. In daily routine maximally 112 allergens can be tested at a time, but in experimental approaches more than 170 molecules have proven possible [55]. Technically much more will be possible in the future, considering that impressively half of the published 3000 allergens in the Allergome data base (www.allergome.org) are available in natural or recombinant form.

Considering the rapid development of molecular allergy during the past 3 decades, and relating it to the complexity of nature, we may only asymptotically approach harboring "all" allergens for diagnosis. This is even more true for therapeutic allergens. In terms of clinical diagnosis, this limitation may for the time being be circumvented by prick-to-prick testing with suspected (and suspicious) substances brought by the patient.

Recommendations and praxis: molecular allergy entered clinics

As a shift in paradigm, the WAO-ARIA-GA2LEN consensus document [56], which is presently updated, states that molecular-based allergy diagnostics, may be used by the expert in the second-line diagnostic workup, thus equivalent with extract-based skin prick- and IgE-testing. It has to be emphasized that any allergy diagnostic method, including IgE and SPT screening, may render unexpected results, which have to be handled in the light of the patient's history and clinical picture. For the less experienced allergists, automated tools were developed to support the complex interpretations of over 100 results [57], whereas the classical method due to the subjective bias in the doctor's investigation renders a simplified, but possibly incomplete view. Hence, the diagnostic allergy field is in transition at the moment, and a first "Molecular Allergology User's Guide" was urgently needed as recently published by the European Academy of Allergy and Clinical Immunology (EAACI) [58]. In this handbook, besides the classical diagnostic work-up "from symptoms to molecules" (Top-down) starting off with extract-based skin prick screening and IgE-testing, the procedure "from molecules to clinic" (bottom-up) is discussed, which starts with allergen molecule-related information followed by the other tests. Considering that most doctors in allergy diagnosis will not leave the skin prick method as a primary screening approach, the authors proposed the "U-shaped" approach as a compromise, integrating both methods [58].

One major argument against the bottom-up approach is usually the economic constraint.

Are the economic concerns against ISAC relevant?

At present few clinics routinely apply component-resolved diagnosis using allergen microarrays. In most cases this method is offered to the patient as a private service when all other diagnostic workup has been completed. This is on the one hand due to economic restrictions as most health insurances do not cover the costs of the ISAC allergen microarray testing. Therefore, the ISAC test is offered to more affluent patients. This economic perspective is the likely reason for the gender bias towards more male patients visiting the a private allergy center offering ISAC as first line diagnostics. It is well known that the socioeconomic and health insurance status affects the access to medical care also in totally other fields of medicine [59]. A recent meta-analysis predicted that microarray testing could be cost-saving only if a substantial reduction of single IgE testing and oral food challenge tests could be achieved. Simultaneously, the authors could not identify microarray studies resulting in changes in patient management significant enough to render cost-reductions [60].

Cost disadvantages of ISAC may have to do with i) multiplex IgE testing taking more time to interpret and communicate the results to the patient, but also by ii) the general habit of using the microarray as the final allergy diagnosis method, instead of using it for screening. This results in an enhancement of the cumulative costs.

Especially in polysensitized patients the ISAC allergen microarray could lead to a cost reduction [58]. In contrast to the procedure "from symptoms to molecules", starting at the skin as primary screening organ followed by 2-step IgE screening, the "from molecules to clinic" approach is more timely and therefore economically interesting for patients, doctors and health insurances.

Conclusion

From the above research it becomes apparent that skin prick testing is a historic compromise and has many disadvantages, such as impreciseness, operator- and patient- dependency, and the risk for systemic reactions, albeit in the % to ‰ range. Nobody has so far dared to address any potential de novo sensitization through skin prick testing. This is remarkable since it has been known for a long time [61], and new evidence is accumulating that the skin is a highly effective route for sensitization, even more so in settings of barrier disruption, sometimes even rendering anaphylaxis [62].

Furthermore, we conclude that allergy screening with the ISAC multiplex allergen array not only with a similar fidelity leads to allergy diagnosis, but is favorable in

- polysensitized patients
- in small children with limited skin area, but higher strain
- in elderly when skin tests get less reliable [34].
- In all settings of inflamed or atopic skin
- when medications interfering with skin prick testing cannot be discontinued

ISAC testing has a high sensitivity and specificity [38], and showed a strong correlation with singleplex tests including IgE and skin prick testing with extracts [51, 52], specifically for respiratory allergens [53, 54], with slight alterations from allergen to allergen.

We strongly believe that in the future, skin prick screening will no longer be acceptable for allergy diagnosis, considering the more stringent recent regulations. Allergy diagnosis should finally arrive in the twenty-first century and start with ISAC as one of the most comprehensive methods and using IgE as the unique biomarker for allergies. It is clear that – in analogy to the classical procedure starting with skin prick test screening, results must under any circumstances be aligned with the clinical picture. However, upfront IgE screening followed by fewer selected SPTs in relation to the clinical phenotype, will reduce the strain in the tested patient, whilst still following the international standards.

Debates in Allergy Medicine: Allergy skin testing cannot be replaced by molecular diagnosis in the near future

Abstract

Percutaneous skin prick tests (SPT) have been considered the preferred method for confirming IgE-mediated sensitization. This reliable and minimally invasive technique correlates with in vivo challenges, has good reproducibility, is easily quantified, and allows analyzing multiple allergens simultaneously. Potent extracts and a proficient tester improve its accuracy. Molecular-based allergy diagnostics (MA-Dx) quantifies allergenic components obtained either from purification of natural sources or recombinant technology to identify the patient's reactivity to those specific allergenic protein components. For a correct allergy diagnosis, the patient selection is crucial. MA-Dx has been shown to have a high specificity, however, as MA-Dx testing can be ordered by any physician, the pre-selection of patients might not always be optimal, reducing test specificity. Also, MA-Dx is less sensitive than in vitro testing with the whole allergen or SPT. Secondly, no allergen-specific immunotherapy (AIT) trial has yet shown efficacy with patients selected on the basis of their MA-Dx results. Thirdly, why would we need molecular diagnosis, as no molecular treatment can yet be offered? Then there are the practical arguments of costs (SPT highly cost-efficient), test availability for MA-Dx still lacking in wide areas of the world and scarce in others. As such, it is hard physicians can build confidence in the test and their interpretation of the MA-Dx results. In conclusion: as of now these techniques should be reserved for situations of complex allergies and polysensitization; in the future MA-Dx might help to reduce the number of allergens for AIT, but trials are needed to prove this concept.

Keywords: Allergens, Skin testing, Prick testing, Specific IgE, Molecular allergy diagnostics, Peanut, Oral allergy syndrome

Background

The increased prevalence of allergic diseases makes it mandatory to use quick, precise, and reliable diagnostic tools. To make the diagnosis of a specific allergy, several components are needed: a subject with symptoms corresponding to an allergic disease, a physician knowledgeable of allergic disorders and specific allergy tests, the availability of quality allergy testing instruments—in vitro and/or in vivo—and finally, and perhaps most importantly, a physician capable of interpreting the test results in light of the patient's symptoms. Only if all of the above components are "checked off the list" is it very likely for a correct allergy diagnosis to be made. In this article, our objective is to discuss the part of "allergy testing," but right from the start it can already be assumed that a discussion of allergy testing is more useful, proactive, and better assessed when the complete context of allergy diagnosis is considered. It all starts with a patient presenting with symptoms and signs suggestive of allergic diseases, particularly allergic rhinitis (with or without allergic conjunctivitis), allergic asthma, food allergy, or even anaphylaxis. A positive personal and family history of allergic diseases, together with a clinical history of fluctuating symptoms over time, sometimes within the course of a day, or even within the course of a year, makes the diagnosis of allergy more plausible. An exacerbation of the

symptoms following exposure to triggers could add further clinical support to the suspicion that we are dealing with an allergy, mainly if symptoms exacerbate on exposure to a certain potential allergen (e.g., cat, dog, horse, house dust mite) or year after year during the same months (pollen season). However, determining which of the patient's allergen(s) might be based on the clinical history only is not considered adequate, as clinical observations are subject to a high degree of error; [1] hence the relevance of having accurate and confident specific allergy testing available [2] (Tables 1 and 2).

Table 1 summarizes in a non-exhaustive way some characteristics of specific allergen tests to help the reader differentiate between the methods used and some practical details of each one of the tests. Molecular-based allergy diagnostics (MA-Dx) is a variant for determining specific IgE (sIgE) in serum (or any other body fluid tested) that quantifies allergenic components obtained either from the purification of natural sources or recombinant technology in order to identify the patient's reactivity to specific allergenic proteins (rather than the whole allergen). As such, MA-Dx is able to discriminate between allergy to the major allergen from house dust mite Der p 1, or Der p 2 or Der p 21, for example, as opposed to the traditional IgE testing (in vivo or in vitro) that typically reports positivity to *Dermatophagoides pteronyssinus* in general.

Two modalities of the microarray technique are commonly recognized: ImmunoCAP, which uses panels of single allergens together with the corresponding allergen extract, and Immuno-Solid phase Allergen Chip (ISAC), which enables testing for specific IgE against multiple allergen components in a multiplex assay [3, 4]. Although MA-Dx undoubtedly constitutes a promising tool in allergy diagnosis, its current use in clinical practice is still highly selective and only considered as a complementary diagnostic test, when a detailed clinical history and traditional extract-based IgE tests (such as SPT or in vitro sIgE tests) are inconclusive or contraindicated.

In this review, we shall discuss several evidence-based and practical arguments to establish that, in most cases, conventional in vivo methods to confirm allergy sensitivity (such as SPT) should not currently, nor in the near future, be replaced by MA-Dx. However, they could be very useful as a complementary diagnostic modality in selected cases. For practical reasons, other skin testing modalities (i.e., intracutaneaous tests) or older in vitro sIgE techniques (i.e., RAST) are not included in this debate.

Arguments

As diagnostic reliability to confirm allergic sensitization is mandatory, it is very important to emphasize that these tests should always be considered as complements to the prime diagnostic tool: a careful medical history and physical examination. Moreover, both, SPT and MA-Dx require skill and knowledge for a correct interpretation of results, [5] and accurate application to the clinical entity of the patient. Both exhibit diagnostic advantages and limitations (Table 2). Although promising, MA-Dx is not currently substituting traditional SPT, and in most cases is considered as a third-line approach, after the clinical history and SPT or sIgE

Table 1 Characteristics of various specific allergen tests

Test	Substance tested	Number of allergens tested per test	Readout
Allergy tests in vivo			
• Skin prick test	sIgE to whole natural allergen	On average 40 allergens	Semi-objective (physician measures wheal/flare)
• Nasal provocation test	sIgE to whole natural allergen	1 allergen at a time (maximum 3–4 per session)	Subjective/Objective
• Conjunctival provocation test	sIgE to whole natural allergen	1 allergen at a time (maximum 3–4 per session)	Subjective/Objective
Allergy tests in vitro			
• sIgE to a batch of allergens (Immulite, Microtest, RAST)	sIgE to whole natural allergen (s)	On average 20–60 allergens	Objective
• Molecular-based allergy diagnostics: sIgE to microarray-based allergen protein components (ImmunoCAP [single allergen assay], ISAC [multiplex assay])	sIgE to allergen protein components	1 to >100 allergen protein components	Objective
• Basophil/histamine release, BAT	Effect of allergen on patient's basophils	1 allergen at a time (maximum several allergens/session)	Objective

sIgE specific IgE, *ISAC* immuno solid-phase allergen chip, *RAST* radioallergosorbent test, *BAT* Basophil activation test

Table 2 Comparison of some advantages and limitations of skin prick test and molecular-based allergy diagnostics for allergy confirmation (adapted from 3,5)

Skin tests (extract based)	Molecular-based sIgE tests (component based)
Available only where equipment, reagents and trained staff are on hand.	Available only in laboratories with high-end machinery where specific reactives and trained staff are on hand.
Moderately costly.	High cost.
Minor discomfort for scratching, itch if positive.	Minor pain. Venesection may be painful or anxiety-provoking (particularly in children),
Requires patient cooperation. Performance in small children may be limited.	Little patient effort or cooperation required.
Slight risk of systemic allergic reaction (more so in some special situations). A convincing recent history of anaphylaxis represents a contra-indication.	No risk to patient; may be first line with certain high-risk allergens.
Require areas of normal skin for testing.	Can be done regardless of extensive skin disease.
Must stop antihistamines and some other drugs several days before test.	Can be done regardless of taken medications.
Methodology and result quality variable, standardization not always possible. No formal quality control at the current time.	Laboratory test subject to strict quality control, reagent availability and technique standardization.
Results in 30 min	Results may take days/weeks.
Results are visible and compelling to patients; may have value in ensuring compliance with allergen avoidance measures.	Results are not directly meaningful to patients.
Can extemporaneously prepare allergens (with appropriate considerations; specialist practice).	Some food allergens, drugs and pollens not available for testing.
In most cases have better sensitivity for clinically relevant allergies.	Reasonably good sensitivity.
Fresh food allergens (prick-to-prick) available with good sensitivity.	Fresh allergens not available.
No interference from high total IgE.	False positives possible with high total IgE levels.
Numerical measurements may vary by different operators.	Numerical results obtained on different types of equipment are not directly comparable.

testings, as has been clearly stated on evidence-based consensus such as the WAO-ARIA-GA2LEN consensus on MA-Dx [3] This can be sustained considering several scientific and practical arguments.

Scientific arguments

Outside the context of clinical trials and medical research, tests should only be run in day-to-day medical practice if their results lead to a certain action. In allergy in particular, testing is carried out with a triple objective: to confirm the diagnosis of allergy (A); to suggest specific avoidance measures to the patient (B); and to guide the preparation of specific-allergen immunotherapy (AIT) (C).

We shall argue below how these three objectives are better met by classic allergy testing, as compared with MA-Dx.

Determination of sIgE in vivo *to the whole allergen is more sensitive than MA-Dx*

To date, very few studies have compared the accuracy of MA-Dx to traditional in vivo tests in allergic patients, mainly in the context of food allergy and with an oral food challenge as the reference standard. In general, MA-Dx tended to have higher specificity, but lower sensitivity relative to the extract-based whole allergen SPT for the prediction of allergic response, but the diagnostic performance of the in vitro tests varied largely between studies, depending on the allergens investigated and the way in which MA-Dx testing was used.

Ott, et al. [6] compared the accuracy of ISAC containing eight individual components (α, β and κ casein, Bos d4, Bos d5, Gal d1, Gal d2, Gal d4) with the accuracy of SPT (native hen's egg or native cow's milk). SPT had the highest sensitivity for cow's milk allergy, 93.6% (95% CI: 78.5–99%), whilst all five ISAC components assessed had low sensitivity for cow's milk allergy (range: 23.9–50%). On the contrary, all five ISAC 51 components had high specificity for cow's milk allergy (range 88.4–97.7%), whereas SPT had low specificity, 48.2% (95% CI: 28.7–68%). Similarly, Alessandri et al. [7] assessed allergy to raw and boiled egg, concluding that SPT had the highest sensitivity for predicting allergic response to raw egg white, 88% (95% CI: 71.8–96.6%), while Gal d3 measured using ISAC had the highest specificity, 100% (95% CI: 90–100%). Results using boiled egg were very similar to raw egg for both testing modalities. Perhaps the more promising results of MA-Dx in the field of food allergy come from peanut allergy, by recognizing sIgE antibodies to Ara h2 as the most common peanut allergen associated with clinical reactivity, and that sensitization to Ara h1, 2, or 3 has been related with more severe clinical reactions in some subjects [8]. However, studies in this matter have shown several

limitations and inconsistencies, as has been pointed out in the most recent AAAAI/ACAAI/JCAAI food allergy position paper [9]. For hazelnut allergy, Albarini et al. [10] compared four components measured by ISAC (Cor a1 1010, Cor a1 0401, Cor a8 and Cor a9) to SPT, which had 100% sensitivity, while the ISAC components had low sensitivity (range: 6.3–56.3%). In this study, the ISAC components had higher specificity (range: 73.7–100%) than SPT (52.6%).

As far as we can ascertain, only two comparative studies investigating the accuracy of MA-Dx for aeroallergen mediated allergy have been published [11, 12], and both used SPT as the reference standard. Conversely, De Swert et al. [13] investigated soy flour allergy, comparing the measurement of the soy flour component rGly m4 by using ISAC to serum IgE to the same component and to SPT for soy flour. ISAC reported the highest sensitivity, 86% (95% CI: 42–100%), but also the lowest specificity, 80% (95% CI: 28–100%). Single sIgE ImmunoCAP testing and SPT had similar sensitivity (75%) and specificity (100%).

All aforementioned studies investigated the diagnostic performance of a relatively limited range of MA-Dx components of a specific allergen. Thus, these studies are somehow unable to provide any information on the sensitivity/specificity of the whole allergen panel. We consider this shortcoming to be a serious limitation, because, for example, it remains unclear to what degree MA-Dx testing may produce false-positive results by detecting sensitizations, which are not always clinically relevant.

Some evidence suggests that MA-Dx can be useful for distinguishing between structurally similar allergens that cross-react with the same IgE antibody [3]. This knowledge can be used to specifically avoid contact with the causative allergen in food allergy and idiopathic anaphylaxis, but its use also has been associated with large numbers of clinically false–positive test results. For example, a study from the UK [14] showed that the addition of ImmunoCAP and ISAC to standard diagnostic work-up could identify a potentially causative allergen in previously undiagnosed patients. At the same time, however, using MA-Dx also resulted in the identification of a large amount of sensitizations that were not considered to be clinically associated with the anaphylaxis. Thus, MA-Dx results still need to be taken with caution in order to limit potentially unnecessary allergen avoidance strategies.

Preparation of AIT based on skin test vs. MA-Dx results

The selection of the allergen(s) for use in AIT has historically been based on the results of skin testing. Until today, no clinical trial has shown the efficacy of AIT by selecting the patients and allergens solely on the basis of MA-Dx test results. Much less has it been shown that selecting allergen(s) for AIT based on MA-Dx could lead to a more efficient or safer AIT, as opposed to AIT with allergen(s) selected according to SPT results.

In vitro diagnosis, when combined with a positive SPT in selecting patients for grass pollen sublingual AIT with tablets resulted in enhanced clinical efficacy in one study [15]. Again, the primary patient selection criterion for inclusion in this trial was the SPT. The determination of the exact allergen(s) for AIT can be facilitated using a secondary test, but the preferable option should be an end-organ challenge test: nasal or conjunctival challenge testing is used by many allergists in Europe to reduce the number of allergens for AIT to one or very few [16].

Some published evidence tends to favor MA-Dx as a more adequate tool than traditional skin testing to decide which allergens to use in AIT [17], but in general their results could not be considered fully definitive. In a trial involving 141 patients with respiratory allergy in Spain, Sastre et al. [18] showed that the number of allergens to be applied in AIT could be reduced considerably or modified when using MA-Dx (with disagreements on AIT prescription when ImmunoCAP results were assessed vs. SPT up to 79 [54%] of cases), implying that this molecular approach can be considered more accurate than the in vivo test. However, in this study's result, no details were given regarding which specific AIT prescriptions were actually used. Moreover, they based their results by measuring interobserver agreement, which is an approach we consider highly prone to suffering subjective biases. More importantly, the authors did not go on to show a hypothetical higher efficacy in the sense of symptoms or medication reduction of such an MA-Dx-based AIT.

Published evidence favoring MA-Dx has been found to have more value regarding *Hymenoptera* venom allergens, where the selection of the correct allergens for venom immunotherapy (VIT) has proven to be truly enhanced by molecular diagnosis. Furthermore, in vivo sting testing can potentially induce systemic reactions, but even in this subgroup of allergic patients, the benefit of MA-Dx applies exclusively to some very selected cases of multiple venom allergen positivity, or to those with a history of an adverse reaction to a *Hymenoptera* sting with negative SPT results [19]. Regardless of all these considerations, the only currently recommended diagnostic strategy for predicting the success of VIT is the sting challenge with a living insect [20]. Since these sting challenge tests can induce severe systemic reactions, in vitro methods for predicting the success of VIT would be preferable, but the evidence supporting this strategy is still limited.

Molecular diagnosis without molecular treatment

AIT is done with extracts of whole allergens. Some groups have investigated AIT with (modified) peptides

for cat [21], birch [22], or a mix of several house dust mite molecular allergens in one report to date [23], but these treatments are still considered experimental. Moreover, molecular treatment has still not been developed for most allergens. MA-Dx is said to be more accurate, and thus could represent a better guide for determining which specific allergens should be selected for AIT administration [3, 17], but some very recent evidence strongly recommends single allergen AIT in polyallergic patients in whom one of the relevant allergens is clearly responsible for the symptoms [24]. It seems perfectly plausible to achieve this based on SPT results alone. If this is the preferred practice, the reality of patient tailored AIT exclusively based on MA-Dx indeed seems still a distant prospect.

Absence of natural adjuvants in molecular AIT

There might be another (albeit hypothetical) argument against purified molecular AIT. The efficacy of AIT can be enhanced by some adjuvants, for example, some toll-like receptor (TLR) ligands, such as lipopolysaccharides [25]. Some natural allergens have been shown to contain TLR stimulating capacity [26], and this important potential effect would be lost if only a certain protein or protein component were used for molecular AIT.

Practical arguments

The cost of MA-Dx is too high

When considering test costs, service and maintenance costs, and personnel costs for performing and interpreting the results, it is easy to recognize that MA-Dx tests are onerous, and can carry a substantial financial burden for laboratories, patients and/or insurance companies. As a clear example, a recent UK-based comparative cost analysis [27] reports a per person cost of £219.51 for an ISAC microarray panel (using a LuxScan 10 k reader, allowing 4 allergens per kit), £136.37 for sIgE testing (on average 8 allergens measured per patient), and £62.28 for SPT, respectively. In the US, the cost of a complete 112 microarray-based allergen molecule ISAC panel is about $300 [28]. In Latin-American countries this is about 600 USD, 8.25 times the Mexican minimum monthly salary, and more than ten times the cost of a 30 allergen SPT.

Limited availability of MA-Dx tests

A very practical argument is that, in many parts of the world, MA-Dx tests for allergy are not yet available, neither the necessary laboratory equipment nor the trained personnel to adequately run the kits, which greatly limits the possibility for allergy care physicians to gain experience with such diagnostic techniques. To run microarray plates for MA-Dx, a special microchip reader machine is needed, and trained personnel capable of managing and maintaining it is mandatory. The reagents and consumables usually have to be imported, raising the maintenance costs. Consequently, many laboratories are reluctant to venture into the MA-Dx territory, as the cost-benefit ratio can only be balanced to the benefit side when enough tests are run.

Physicians confidence in the test: correct interpretation

As we set out to emphasize from the start: the final step in allergy diagnosis is the correct interpretation of the test results. Thus, to get MA-Dx well established as a routine diagnostic tool, physicians need to become acquainted with it and be able to gain confidence in the correct interpretation of the results. Microarray analyses are also prone to limitations and errors due to imprecise technical material and imperfections in the technique for hybridization and scanning (i.e., deviations in the amount of biologic material printed in each microarray spot, variations in the amount of the fluorescent reactive used to mark samples, errors inherent to the light measurement by the scanner, among others), in addition to the inherent difficulties, related to gene material stability and its processing per se. In many parts of the world, allergists do not feel comfortable (yet) interpreting MA-Dx results and are even less acquainted with how to put these results into practice. As long as only a few allergists use these tests for a very limited number of their patients, it does not seem that this apparent lack of confidence is going to change in the near future.

Conclusion

Even though MA-Dx technology constitutes an innovative and promising area, such techniques should be considered a complementary, more selective, third-line diagnostic modality reserved for very specific cases, such as complex allergies and polysensitization. Furthermore, it should be regarded as an add-on diagnostic approach that might help to identify homologous allergens that by their cross-reactivity might explain the clinical symptoms of oral allergy syndrome linked to respiratory allergy to pollen, and as a tool to predict the risk for more severe adverse food allergy reactions (i.e., Ara h 2 versus Ara h 8 positivity).

In the future, it is probable that MA-Dx will help to reduce the number of allergens to be administered in AIT, but efficacy data in this regard are still absent. Moreover, no molecular tools are available to date, which allow the prediction of AIT outcomes. The cost-benefit is another very important problem regarding MA-Dx. In countries with a low gross domestic product, the decision to recommend an expensive test, such as MA-Dx, should be made carefully and, again, limited to very specific cases. In more wealthy countries or communities, issues such as access and insurance coverage would be important to consider.

We can conclude that, until new and better-designed investigations provide more solid evidence in this regard, MA-Dx shall not completely replace traditional SPT or challenge tests as the first-line approach to confirm specific allergy at present nor in the near future. Nevertheless, performing both in vitro and in vivo tests may undoubtedly contribute to improve sensitivity/specificity and the overall allergic diagnostic accuracy under specific circumstances.

Risk and safety requirements for diagnostic and therapeutic procedures in allergology: World Allergy Organization Statement

Abstract

One of the major concerns in the practice of allergy is related to the safety of procedures for the diagnosis and treatment of allergic disease. Management (diagnosis and treatment) of hypersensitivity disorders involves often intentional exposure to potentially allergenic substances (during skin testing), deliberate induction in the office of allergic symptoms to offending compounds (provocation tests) or intentional application of potentially dangerous substances (allergy vaccine) to sensitized patients. These situations may be associated with a significant risk of unwanted, excessive or even dangerous reactions, which in many instances cannot be completely avoided. However, adverse reactions can be minimized or even avoided if a physician is fully aware of potential risk and is prepared to appropriately handle the situation.

Information on the risk of diagnostic and therapeutic procedures in allergic diseases has been accumulated in the medical literature for decades; however, except for allergen specific immunotherapy, it has never been presented in a systematic fashion. Up to now no single document addressed the risk of the most commonly used medical procedures in the allergy office nor attempted to present general requirements necessary to assure the safety of these procedures.

Following review of available literature a group of allergy experts within the World Allergy Organization (WAO), representing various continents and areas of allergy expertise, presents this report on risk associated with diagnostic and therapeutic procedures in allergology and proposes a consensus on safety requirements for performing procedures in allergy offices. Optimal safety measures including appropriate location, type and required time of supervision, availability of safety equipment, access to specialized emergency services, etc. for various procedures have been recommended.

This document should be useful for allergists with already established practices and experience as well as to other specialists taking care of patients with allergies.

Introduction

Over the last several decades allergy practice has been expanding worldwide in parallel with the increasing number of patients suffering from allergic diseases. It has been widely accepted that appropriate training and certification are necessary for the physician to correctly diagnose and manage allergic diseases. However, in some countries the allergy specialty is still poorly developed or does not exist. Allergy practice, therefore, varies from country to country and according to local regulations or traditions both trained allergists or physicians with other specialties are performing allergy procedures such as skin testing or immunotherapy. Furthermore, in several regions of the world the increasing number of allergy sufferers has not been matched by an appropriate supply of trained specialists; as a result, physicians without training in allergy including general practitioners and pediatricians will be assisting allergic patients more and more.

Doctors dealing with allergic diseases (certified allergists or other specialists trained in allergy) are employing, in the office, various diagnostic and therapeutic procedures which are associated with a significant risk of unwanted reactions for a patient. The reactions, depending on the procedure, may vary from local discomfort to severe anaphylaxis and death. Most unwanted reactions can be either avoided or the risk/symptom intensity may be minimized if the procedures are performed in an appropriate manner. However, certain risk of unwanted and or excessive reaction remains even if all precautions are respected. During some diagnostic procedures, called provocations (e.g. oral drug or food challenges), the allergist deliberately aims at inducing adverse symptoms which are mimicking those occurring at natural exposure and sometimes may be associated with a significant discomfort and even with some risk to the patient. In such situations unpleasant or even potentially dangerous symptoms are inherent to the procedure and cannot be completely avoided. Thus, it is critical that well established inclusion/exclusion criteria for the challenge are considered and the protocols of provocations are strictly followed. Furthermore the patient should be appropriately monitored by trained and experienced medical staff not only during the whole procedure but also for an appropriate time after its completion. Such precautions usually allow for a significant reduction of risk of unwanted or excessive symptoms.

The literature on the risk of allergic procedures exists, but it has not been recently reviewed in a systematic way. Furthermore, there is no available consensus on safety requirements for performing specific diagnostic procedures. Thus it is important to reach the consensus on optimal safety measures (e.g. appropriate location, type and required time of supervision, availability of safety equipment, access to specialized emergency services, etc.) for various procedures.

An international group of experts collaborating within the World Allergy Organization (WAO) presents this consensus report assessing risk and proposing safety requirements for performing procedures in allergy offices. This document refers to available literature and also to other documents and resources (e.g. local regulations) available to experts. Since for the majority of reviewed procedures no formal recommendations were available, the experts had to reach the consensus with regard to proposed recommendations. As a result, optimal safety measures for various procedures have been proposed.

This consensus, which is based on the recommendations of international experts, provides useful information for allergy specialists and all doctors who diagnoses and treat allergy patients. Moreover, the consensus has value for general allergy practice worldwide; thus WAO is an appropriate organization to provide it.

The following grading of recommendations on the safety measures has been presented based on the consensus reached by the expert panel:

A. Mandatory
B. Recommended
C. Suggested
D. non-required

Recommendations reported in the sections below have been summarized in Table 1 and 2.

Diagnostic procedures

Skin testing for inhalant and food allergens – Skin Prick Test (SPT) and Intradermal Skin Test (IDST)

Skin testing with inhalant and food allergens

Definition and short technical description These in vivo tests are used for the detection of allergen specific IgE on the skin mast cells and confirmation of sensitization to a specific allergen [1].

Skin Prick Test (SPT)

Skin prick testing relies on the introduction of a very small amount of allergen extract into the epidermis using a disposable fine needle or lancet device, which is changed with each test allergen [2]. Besides metal devices, there are other varieties of commercially available skin prick devices. The incorporation of these devices into the protocol may require prior evaluation [3]. Skin prick should be applied carefully, as insufficient prick may produce false negative results, and induction of bleeding (too deep) may produce false positive results and bear the risk of systemic reactions. Allergens should

Table 1 Summary of Safety Recommendations for Diagnostic Allergy Procedures *(for details, see the text)*

Section subtitle	Recommended site	Emergency equipment availability	Emergency staff (ICU) availability	Duration of supervised follow-up in the office after procedure	Comments
Skin testing (SPT and IDST)					
With Inhalant and food allergens	Both O and H	(a) mandatory	(c) not required	20 min	Field skin testing (prick test only), for epidemiology studies, may also be carried out by trained medical personnel.
Skin testing with hymenoptera venoms	Both O and H	(a) mandatory	(c) not required	20 min	
Skin testing with drugs	O or H depending on the risk assessment	(a) mandatory Comment: not applicable for patch testing	available on site (a) or available within 30 min (b) depending on the risk assessment	20 min	Patients at risk: Patients who are tested for anaphylactic reactions, or with a history of complicating conditions such as asthma, mastocytosis and severe cardiac disease
Skin testing with occupational allergens	Both O and H	(a) mandatory	(c) not required	20 min	
Skin testing with latex	Both O and H	(a) mandatory	(b) available within 30 min	20 min	For some patients waiting time should be extended to 40 min
Bronchial challenge with allergen	Both O and H[a]	(a) mandatory	(b) available within 30 min	7 h	[a]although outpatient clinic is acceptable the hospital setting is recommended
Bronchial challenge with lysine aspirin (Lys ASA BPT)	Both O and H	(a) mandatory	(b) available within 30 min	1 h	
Nonspecific bronchial provocation tests (NS-BPT)	Both O and H	(a) mandatory	(c) not required	not required	
Nasal allergen provocation tests	Both O and H	(a) mandatory	(b) available within 30 min	30 min	
Nasal aspirin provocation tests	Both O and H	(a) mandatory	(b) available within 30 min	30 min	
Nasal endoscopy	Both O and H	(d) not required	(c) not required	not required unless complications occur	
Food challenges	Both O and H	(a) mandatory	(a) available on site or (b) available within 30 min, depending on risk assessment	1-2 h after negative and 4 h after positive food challenge	
Oral drug provocation test	O or H depending on risk assessment	(a) mandatory	(a) available within 5 min, or (b) available within 30 min, depending on risk assessment	at least 2 h; hospitalization is recommended after severe reaction	
Insect sting challenge	Both O and H	(a) mandatory	(b) available within 30 min	at least 2 h until symptoms have disappeared	

O: Outpatient clinic
(a) mandatory
(b) recommended
(c) suggested
(d) not required
H: Hospital setting
(a) available on site (in less than 5 min)
(b) available within 30 min
(c) not required

be placed at least 2 cm apart to avoid overlapping responses between allergens tested. The results of skin prick testing are read at 15 min and the diameter of the resulting weal is recorded in two dimensions (longest and its orthogonal diameter). By convention, a positive test is one in which the mean of the two weal diameters

Table 2 Summary of Safety Recommendations for Therapeutic Allergy Procedures *(for details, see the text)*

Section subtitle	Recommended site	Emergency equipment availability	Emergency Staff (ICU) Availability	Duration of supervised follow-up in the office after procedure	Comments
Subcutaneous immunotherapy with inhalant allergens	Both O and H	(a) mandatory	(a) available on site (in less than 5 min), or (b) available within 30 min, depending on risk assessment and the immunotherapy protocol used	30 min	
Venom immunotherapy	Both O and H	(a) mandatory	(b) should be available on site, (in less than 5 min) or within 30 min, depending on the risk assessment and the immunotherapy protocol used	30 min	
Drug desensitization procedures	O or H depending on risk assessment	(a) mandatory	(a) available on site (in less than 5 min)	30 min after acute reactions	Waiting time can be extended to 24-98 h for delayed reactions
Oral immunotherapy for food allergy	Both O and H	(a) mandatory	available within 5 minutess (a) or available within 30 min (b) depending on risk assessment	2 h	Currently not recommended for routine clinical use
Treatment with Anti-IgE and other biologicals		(a) mandatory	(b) available within 30 min	2 h for the first administration;30 min for succesive administration	
Treatment with products from human plasma		(a) mandatory	(b) available within 30 min	30 min	

O: Outpatient clinic
(a) mandatory
(b) recommended
(c) suggested
(d) not required
H: Hospital setting
(a) available on site (in less than 5 min)
(b) available within 30 min
(c) not required

is at least 3 mm greater than the negative control (saline) [4]. Positive and negative controls are critical to enable interpretation of test results. Ideally, the histamine control is read at 10 min. The test is usually performed on the volar aspect of the forearm but it is also performed on the back, especially in young children. There is a gradient of response when using the back – with larger responses in the lower third compared to the upper third [5]. Interpretation of results should consider the following factors: the allergen extracts used (standardized when available), the type of lancet device, the skin site chosen for testing, the clinical state of the patient and the medications used by the patient.

Intradermal allergy testing (IDST)

Intradermal allergy testing is a procedure where a small amount of diluted allergen is injected into the dermis. It increases the sensitivity but decreases the specificity of the test and is carried out with allergen concentrations 100 to 1000 times less than that used for skin prick tests. It has no place in aeroallergen (other than for research) and food allergen testing. It is most commonly used in testing for drug and venom allergy.

Clinical indications SPTs may be used for the evaluation of allergen-specific IgE to inhalants, foods, drugs and venom in the following conditions: respiratory/inhalant allergy, food allergy, venom allergy, drug allergy.

IDSTs have a very high non-specific reaction rate and are not recommended for testing with inhalants or foods [6] and food allergens [7]. Moreover, intradermal tests carry a higher risk of adverse reactions than SPTs.

Age limitation Testing can be performed from infants to the elderly. Infants and the elderly have smaller SPT weal responses, and prominent flare responses [8].

Description of adverse/unintended reactions associated with the procedure SPT is considered a safe procedure, with minimal discomfort. Adverse events can occur but rarely. These are classified as allergic or non-allergic.

1. Type and spectrum of unintended allergic reactions
   - Local:
     In some patients with marked sensitivity late phase local skin swelling (the IgE late phase

response) consisting of tender and painful swelling may occur (seen more commonly with intradermal testing). Rarely, it could cause quite marked swelling and discomfort, but does not usually last more than 36 h [9].

- Systemic:
  Systemic reactions associated with SPTs, usually starting within 15 to 30 min, have been reported as case reports [10], in surveys and in prospective studies. Although systemic reactions may occur in any individual undergoing skin testing (both adults and children), specific risk factors should be taken into consideration when performing these tests (see section III).
- Fatal Reactions:
  Few fatal reactions as a result of skin testing have been described in the literature [11, 12]. Based on two large retrospective surveys by the American Academy of Allergy and Immunology in the US, seven fatalities have been described involving older children and adults. Six of these deaths involved intradermal testing to inhalants and food and one death involving skin prick testing performed with 90 allergens.

2. Type and spectrum of non-allergic reactions.
   These may include syncope (vasovagal syncope) and headache, Based on a prospective study in children [13] and a retrospective survey [11], all reported systemic and vasovagal syncope reactions related to skin testing occurred within 15 to 30 min of the test.
3. Prevalence and risk associated with the procedure.
   The prevalence of systemic reactions related to skin prick testing with inhalant and food allergens is low but not absent. It was estimated to be less than 0,055 % [14, 15]. The rate of systemic reactions requiring epinephrine was reported as 20 per 100,000 SPT visits [16]. The prevalence in young children appears to be higher with a reported rate of systemic reactions of 0.12 % [13] and 6.5 % in infants less than 6 months of age [17].
4. Risk factors for adverse/unintended reactions
   - Systemic Reactions: [7, 9, 11, 12, 17]
     - Infants especially <6 months
     - Multiple allergens
     - Previous history of anaphylaxis to food when testing for incriminating food
     - Testing with fresh food (non-commercial extracts)
     - Testing with non-standardized latex extracts
     - Extensive eczema
     - Uncontrolled asthma
     - Intradermal Testing
     - Vasovagal syncope [13]:
     - Female sex
     - Testing with multiple allergens

Institutional/organizational safety recommendations Several guidelines for performing skin tests have been published:

- Allergy diagnostic testing: an updated practice parameter (Bernstein et al, 2008) [18]
  - Skin Prick Testing for the diagnosis of allergic diseases – A manual for practitioners (Australasian Society of Clinical Immunology and Allergy, 2013) [9]
  - Allergic Rhinitis and its Impact on Asthma; Practical guide to skin prick tests in allergy to aeroallergens. Allergy 2012;67:18-24 [19].

WAO safety recommendations These recommendations are based on the rare occurrence of severe systemic reactions reported in retrospective surveys, one prospective study, and several case reports. Quality of evidence is high regarding the rare occurrence of systemic life threatening and fatal (1 case in the literature with skin prick test without intradermal test) reactions justify the need for facilities offering skin prick testing to have the following prerequisites for safety. There are no recommendations for intradermal testing as they are not indicated for inhalant and food testing [20].

1. Site:
   - Both a hospital and outpatient clinic setting
   - Field (skin prick test only), e.g. epidemiology studies, may also be carried out by trained medical personnel.
2. Personnel:
   Can be performed by trained nurse/technician under supervision of experienced physician
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Not required
5. Pretreatment:
   Not applicable
6. Duration of supervised follow up after the procedure:
   It is recommended that patients who have undergone skin prick testing and have positive results, who have asthma or a history of anaphylaxis, should remain in the centre for at least 20 min following completion of the skin prick test [9].
7. Contra-indications:
   Contraindications to skin prick testing may be categorized into:

- Clinical situations which interfere with the procedure or its interpretation. These include absence of normal skin, including dermatographism, use of medication that might inhibit skin prick responses.
- Relative contraindications related to safety/high risk situations. These include severe or unstable asthma and patients on beta-blockers. If SPT is considered to carry a significant risk e.g. in a highly sensitized patient or in a woman with unstable asthma in pregnancy then avoid performing the test.

8. Other considerations:
   In patients with a history of anaphylaxis, skin prick tests should be initiated with several serial 10-fold dilutions of the usual test concentration.

Skin testing with hymenoptera venoms

Definition and short technical description Immediate hypersensitivity skin testing is performed with standard techniques and standard reporting of results. Venom skin testing may begin with prick/puncture tests using a venom concentration of 1,0- 100 mcg/ml, or with intradermal tests using venom concentration of 0.001 -1 mcg/ml. If the puncture test is negative, it is followed by an intradermal test using venom concentration of 0.01 mcg/ml. If the intradermal test using venom concentration of 0.01 mcg/ml is negative, it is repeated using concentrations of 0.1 mcg/ml, and then if necessary 1.0 mcg/ml. A positive puncture test has a wheal diameter at least 3 mm larger than the diluent (negative) control. Intradermal tests should introduce sufficient volume to give a 3–4 mm bleb (usually 0.02–0.03 ml). A positive intradermal test has a wheal diameter of at least 5 mm.

Clinical indications Skin tests for venom allergy are indicated to confirm the presence of venom sensitization in patients who have had systemic reactions to insect stings (or repeated severe large local reactions) and are candidates for venom immunotherapy. Testing is also useful to distinguish among different types of venom (bee, wasp, etc);

Age limitation No age limitation.

Description of adverse/unintended reactions associated with the procedure Adverse events are very rare with venom skin tests. Local itching and induration is a normal positive response, and may take hours to subside. Anaphylactic reaction to venom skin tests is extremely rare. Unintended consequences of venom skin tests can occur when the tests are performed in individuals who have no clear history of anaphylaxis to a sting. This is because venom skin tests can be positive in 15–20 % of adults, and in more than 30 % of those who have been stung in the previous few months. A positive test in such individuals creates the perception of risk even when the history might indicate low risk (<3 %) of anaphylaxis. This is the case in people who have large local reactions to stings, in those with only cutaneous systemic reactions to stings, and in patients who have completed a 5 year course of venom immunotherapy.

1. Type and spectrum of adverse reactions
   - Local:
     Local adverse reactions are very uncommon but might cause delayed progressive swelling and induration of the test site, with itching and possibly pain.
   - Systemic:
     Systemic allergic reactions to venom skin tests are rare, and near-fatal or fatal reactions are exceedingly rare.
2. Prevalence and risk associated with the procedure:
   Early studies of venom skin tests included small numbers of subjects. They were focused on diagnostic accuracy, and reported no significant adverse effects. The only large study reporting on the safety of venom skin tests was that of Lockey et al [20]. In that survey of 3236 patients, 64 (2 %) had a systemic reaction during venom skin tests, 13 (0.4 %) of which were severe. Thirteen of 64 adverse reactions (20 %) were possibly vasovagal, and six other subjects (9 %) demonstrated no symptoms of immediate-type hypersensitivity. Thus, 45 (1.4 %) of the 3236 subjects tested had a systemic reaction that was considered to be a reaction of hypersensitivity, of which eight reactions (0.25 %) were severe.
3. Risk factors for adverse/unintended reactions:
   The risk of severe anaphylaxis is always increased in patients with a history of asthma. The severity of the previous reaction to a sting is not a risk factor for anaphylactic reaction to skin tests. In contrast to skin tests with inhalant allergens, the risk of anaphylaxis is not increased when intradermal tests are performed without initial prick/puncture tests. In fact, there are 2 studies of "accelerated" venom skin tests that reported no increased risk of adverse reaction [21, 22]. Guidelines and Practice Parameters in Europe and the United States do not recommend any precautions for venom skin tests in patients who are taking beta-blockers of ACEI medications.

Institutional/organizational safety recommendations Guidelines and Practice Parameters in Europe and in the United States do not express any concern about safety of venom skin tests, and do not recommend any specific precautions or safety measures [19, 18, 23].

WAO safety recommendations

1. Site:
   Both outpatient clinic and hospital setting
2. Personnel:
   Tests are performed by personnel who have undergone training and proficiency testing. A physician should be present.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Not required
5. Pretreatment:
   Not applicable
6. Duration of the supervised follow up after procedure:
   20 min
7. Contra-indications:
   See contraindications for SPT in previous section (inhalants)
8. Other considerations:
   The results of venom skin tests must be reviewed and interpreted by an experienced specialist in allergology, in the context of the clinical history of the patient and the natural history of the condition. The pitfalls of diagnostic allergy testing have been well-described [24].

Skin testing with drugs

Definition and short technical description Skin Prick- (SPT) and Intradermal Skin Tests (IDST) are the most useful modality for demonstrating an IgE-mediated mechanism underlying clinical symptoms [25], whereas epicutaneous patch testing (or SPT/IDST with delayed reading) is the logical first step in defining the relevant drug in delayed cell-mediated hypersensitivity to systemically administered drugs – and not only for contact dermatitis caused by topically applied drugs [26, 27]. However, depending on factors such as the clinical type of reaction, the drug suspected, the pathomechanism of the reaction, the availability of qualified test substances and the existence of a valid test protocol, an individual approach must be chosen for any specific situation, i.e. drug testing has to be performed in an individualized manner.

Generally it is advised to perform the tests 6 weeks to 6 months after the hypersensitivity reaction. SPT can be done with any soluble drug, for the IDST sterility is important. Patch tests can be performed with any form of commercial drugs. In general, for most of the drugs there is a lack of standardization of reagent concentrations. Only recently a guideline has been released listing all the published and recommended test concentrations for any drug reported [28].

Clinical indications Indications for SPT and IDST with drugs are immediate reactions manifested as erythematous eruptions/flushing, urticaria and angioedema, anaphylaxis, conjunctivitis, rhinitis and bronchospasm/asthma. The most common use of patch testing with drugs are maculopapular exanthemas, acute generalized exanthematous pustulosis (AGEP), and fixed drug eruptions [29, 30]. Other clinical entities where patch tests are being used are delayed-appearing urticaria, photosensitivity, drug reactions with eosinophilia and systemic symptoms (DRESS/DIHS), Abacavir hypersensitivity syndrome, Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN) [31].

Age limitation Skin testing with drugs can be performed at any age, although the experience in children is limited.

Description and prevalence of adverse/unintended reactions associated with the procedure Skin testing with drugs is a safe diagnostic approach, if performed according to the published guidelines [26, 28, 32, 33]. However tests may be associated with some risk of adverse local and also systemic reactions; a relapse of the previous reaction might be provoked with any skin test procedure.

1. Type and spectrum of adverse reactions:
   - Local:
     SPT, IDST and patch tests may cause local irritation resembling (false-) positive reactions. Skin necrosis and scarring might result from testing with toxic substances such as chemotherapeutic agents or when using non-physiological concentrations.
   - Systemic:
     Anaphylaxis after SPT with chymopapain, penicillin, tetanus toxoid, and other drugs, has been reported rarely, leading to the conclusion that SPT is a safe diagnostic procedure, although a theoretical and remote risk in principle remains [10].

   – IDST, being more sensitive than SPT, is more likely to induce systemic reactions. Urticaria, and rarely anaphylaxis have been described almost exclusively with β-lactams [reviewed in 25].
   – Systemic reactions associated with patch testing are extremely rare. In a retrospective study that evaluated 111 and 134 patients with a history of severe cutaneous adverse reactions to drugs no

severe side effects induced by the tests were reported [27, 30].

- Flare-up reactions:
  Flare-up reactions might be mediated by IDST and patch tests. A patch test-induced exfoliative dermatitis was observed in a patient with an adverse reaction to carbamazepine [34]. A relapse of a pruritic rash occurred following a prick test with pristinamycin [35]. The relapse of an AGEP has been provoked by patch testing with acetaminophen (paracetamol) while these tests remained negative [36]. Thus, patch tests, SPT, and IDST can induce a systemic reaction even though their results were negative [26].
- Fatalities:
  The few fatalities associated with skin tests, reported from 1895 to 1980, were associated with biologic products that are no longer used such as horse serum-derived tetanus or diphtheria toxins or pneumococcal antiserum [reviewed in 29]. A recent literature review on systemic reactions from skin testing concluded that the occurrence of systemic reactions with inhalant allergens has diminished over the last 30 years, whereas fresh food, hymenoptera venom and antibiotic SPT still carry some risk [10].
- Risk factors for adverse reactions:
  In general, patients with history of previous anaphylactic reactions, uncontrolled asthma or high degree of reactivity, small children or pregnant women,, may be considered at higher risk (i.e., they may react more readily and/or more severely to the minute test amounts applied [25].

Institutional/organizational safety recommendations
Several guidelines for performing drug skin tests have been published [26, 28, 32, 33].

WAO safety recommendations

1. Site:
   SPT and IDST with drugs should be performed in hospital settings of specialized centers. Since adverse reactions to drug patch testing are rare and rather not severe, tests can be applied at outpatient clinics.
2. Personnel:
   Trained technician or nurse under supervision of a physician. Personnel has to be prepared, trained and equipped for serious events, especially anaphylactic reactions.
3. Emergency equipment availability:
   Should be available on site (mandatory) for SPT and IDST. Not applicable for patch testing.
4. Emergency staff (ICU) availability:
   Should be available on site (mandatory) – only for patients who are tested for anaphylactic reactions, or for patients with a history of complicating conditions such as asthma, mastocytosis and severe cardiac disease.
   For all other patients should be available within 30 min
5. Pretreatment:
   Not applicable
6. Duration of the supervised follow up for safety after the procedure:
   Should remain in the centre for at least 20 min after completion of the procedure. After IDST in patients with previously diagnosed asthma (due to the suspected drug itself or as an underlying disease) supervised follow-up should be extended to 6 to 8 h [25].
7. Contraindications:
   Drug-induced autoimmune diseases, severe exfoliative skin reactions and severe vasculitis syndromes for SPT and IDST [25]. There are no absolute contraindications for patch testing with drugs.
   See also contraindications for SPT in previous section (inhalants)

Skin testing with occupational allergens

Definition and short technical description The techniques for skin test (ST) with occupational allergens are identical to ST with other (inhalant) allergens. As for other allergens, in routine the skin prick test (SPT) should be preferred over the intradermal skin test (IDST), because it causes less pain and there is a lower risk of systemic reactions. Since there is no clear definition of occupational allergens (also food and drugs are occupational allergens for some workers) for the purpose of this document we looked for potential adverse reactions after skin testing in occupational exposed workers. The following sensitizing substances most commonly cause occupational asthma and are used for skin testing: dust of cereal flours, enzymes, laboratory animals, farming (animals, cereals, hay, straw and storage mites), fish and seafood as well as low molecular substances such as isocyanates, platinum salts and acid anhydrides [37, 38]. Natural rubber latex (hereinafter referred to as latex) is discussed separately (see following section on skin testing with latex). Due to the fact that occupational allergies in comparison to sensitizations to ubiquitous allergens are rare, often no standardized SPT solutions are available. In these cases non-standardized patient-tailored allergen preparations have to be used. If the patient shows a positive reaction to such a SPT solution, control tests should be performed in a number of

healthy subjects in order to exclude an unspecific reaction.

Clinical indications For the diagnosis of occupational type I allergies, the common steps are a detailed case history, skin testing, in vitro diagnosis (mostly specific IgE antibodies), and specific inhalation challenge. The clinical indication for SPT with occupational allergens (including latex) is to demonstrate IgE-mediated sensitization to occupational allergens. However, in combination with work-related symptoms of the patient, SPT with occupational allergens is also relevant for compensation and further socioeconomic consequences.

Age limitation In general, STs with occupational allergens are performed only in working adults.

Description and prevalence of adverse/unintended reactions associated with the procedure Taking into account, that skin tests with occupational allergens are only performed in adults and that commercial ST solutions for occupational allergens (other than latex) usually contain only small amounts of antigens and proteins [39] ST and especially SPT with occupational allergens remains a safe diagnostic procedure.

1. Type and spectrum of adverse reactions
   - Local:
     If non-standardized patient-tailored allergen preparations are used, local adverse reactions might be caused by irritation or toxic reaction
   - Systemic:
     In general the risk of systemic reaction following ST with occupational allergens is low (lower with SPT than IDST). There exists one report about an anaphylactoid reaction (without cardiovascular symptoms) after a scratch test with iridium chloride in an occupational exposed process operator [40]. However, scratch tests have generally been abandoned because of non-standardized procedure.
   - Fatal:
     Out of 17 cases of anaphylaxis after SPT with various allergens listed by Liccardi [10] after Medline research (1980-2005) none referred to occupational exposure.
2. Risk factors for adverse reactions:
   Unknown

Institutional /organizational safety recommendations EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies [41]

WAO safety recommendations Although allergy ST is considered a safe procedure, it is not without risk of systemic reaction

1. Site:
   Both outpatient clinic and hospital setting
2. Personnel:
   Can be performed by trained nurse/technician under supervision of experienced physician
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Not required
5. Pretreatment:
   Not applicable
6. Duration of the supervised follow up after procedure:
   Should remain in the centre for at least 20 min after completion of the procedure
7. Contra-indications:
   Identical to ST with other (inhalant) allergens
8. Other considerations:
   None

Skin testing with latex

Definition and short technical description Natural rubber latex (NRL), commonly referred to as latex, is a vital natural resource that is used in the manufacturing of a wide variety of commercial products ranging from airplane tires to protective medical gloves. Ninety-nine percent of latex comes from one source: the sap-like fluid from the rubber tree *Hevea brasiliensis*. Sensitization to latex, which is a potent allergen, affects people who are frequently exposed to products made of latex such as health care and latex industry workers, patients with a history of multiple surgical procedures including children with spina bifida as well as specific food allergy patients.

Fourteen latex allergens have been identified and skin test (ST) extracts have to contain especially NRL allergens Hev b 1, 2, 3, 4, 6.01, 7.01, and 1 and recombinant Hev b 5 (rHev b 5). Skin prick test (SPT) should be performed, intradermal tests are not recommended (Cabañes et al. 2012) [42]. SPT extracts to determine latex allergy included commercial extracts, latex glove extracts and hevea leaves. Serial 10-fold dilutions of non-ammoniated latex (NAL, e.g. from Malaysian *Hevea brasiliensis* (clone 600) sap (Greer Laboratories)) or newly introduced ammoniated latex (AL, e.g. Bencard Laboratories, Mississauga, Ontario) allergens were employed in ST. Standardized extracts can provide a sensitivity of 93 % with a specificity of 100 % [42].

Also 'glove use tests' are performed. Considerable disparity exists between glove use protocols, with exposure times ranging from 15 min to 2 h. In general, the first

step involves placing a fingertip of the glove on a dampened finger; if the result is negative, the complete powdered glove is put on. A vinyl or nitrile glove is used on the other hand as a negative control. The result is considered positive if contact causes erythema, pruritus, blisters, or respiratory symptoms [42].

Clinical indications Latex is a common component of many medical supplies used in the hospital environment. Although latex is most often associated with disposable gloves, other items which may contain latex are breathing tubes, infusion sets, syringes, stethoscopes, catheters, dressings and bandages. Frequent users of latex products may develop a latex allergy. Allergic rhinitis and asthma mainly affect individuals exposed via inhalation, such as health care workers, lab workers, dentists, nurses, and physicians.

Patients at risk are also subjects with spina bifida and congenital genitourinary abnormalities who have undergone multiple procedures. While the incidence of latex allergy in the general population is 1 % to 2 %, in spina bifida (SB) patients, who are mostly children, incidence of latex allergy ranges from 20 % to 70 % [43]. As well, people who have certain food allergies, including banana, avocado, chestnut, apricot, kiwi, papaya, passion fruit, pineapple, peach, nectarine, plum, cherry, melon, fig, grape, potato, tomato and celery, may also have signs of a latex allergy due to cross-reactivity.

Diagnosis of latex allergy is based on clinical suspicion. A good clinical history taken by an experienced allergologist is very important. The history should record the presence or absence of other allergies, atopy, previous operations or medical procedures involving latex products, reactions induced by ingestion of fruits and whether the patient belongs to a risk group. The complementary diagnosis is based on STs and the determination of specific IgE [42].

Age limitation SPTs with latex are performed both in adults and children.

Description and prevalence of adverse reactions associated with the procedure Skin testing with latex allergen is associated with a significant risk of adverse systemic reactions

1. Type and spectrum of adverse reactions
   In most cases, subjects with adverse reactions after latex SPT showed a variety of different symptoms.
   - Local:
     There are no reports about isolated large local reactions after latex SPT. One health care worker in whom angioedema, hives and hypotension developed had no discernible wheal and flare reaction at the site of the SPT (Kelly et al. 1993) [44].
   - Systemic:
     Several reports of anaphylaxis during SPT for latex allergy have been published (Table 3). However, in these former cases mostly non-standardized SPT extracts prepared from powdered latex gloves or crude latex preparations directly from *Hevea brasiliensis* trees were used. In a study initiated with the goal to establish an FDA (Food and Drug

Table 3 Exemplary cases of systemic reactions during skin prick test (SPT) with latex

Number of cases reported	Type of ST	Culprit agent (type of latex)	Symptoms/fatalities	Reference	Comments (e.g. setting, age etc.)
1	SPT	Liquid latex material	Immediate flushing, tachycardia, urticaria, light-headedness	Spaner et al. 1989 [260]	34 year old female Operating room nurse
1	SPT	10 % aqueous dispersion	Cold, sweaty extremities, initial tachycardia, subsequent bradycardia, hypotension	Bonnekoh and Merk 1992 [261]	17 year old female dentist's assistance
1	SPT	100 HEP *Hevea brasiliensis*	Dizziness, difficulty with breathing, wheezing, tachypnoea	Nicolaou and Johnston 2002 [262]	39 year old female house wife
6	SPT	extemporaneous extracts (dilutions of 1:1000, 1:100, and 1:10) ($n = 5$), commercial SPT solution ($n = 1$)	Signs of anaphylaxis in different degrees	Nettis et al. 2001 [48]	6 female patients with age ranging from 26-51
9	SPT	1:100,000 dilution of latex glove extract	Systemic reactions	Kelly et al. 1993 [44]	9 of 107 patients: 85 children with spina bifida, 15 health care workers, 7 others
2 or 3[a]	SPT	50 % glycerine, 0.23 mg/mL total protein from gloves	Pruritus, flushing, urticaria, angioedema, asthma, cough chest tightness, wheezing, dyspnea, eye itching, nasal congestion	Valyasevi et al. 1999 [15]	2 or 3[a] patients from a clinic out of 1316

[a]in one patient reason of anaphylactic reaction was not clear because he was also positive to aeroallergens

Administration) – licensed extract for use in the United States an optimal diagnostic accuracy (SPT [IDST]: sensitivity 96 % [93 %], specificity 100 % [96 %]) without any systemic or large local reactions was obtained in 59 latex allergic adults with a non-ammoniated latex extract (SPT 100 μg/ml, IDST 1 μg/ml) [44]. More recently, the available standardized latex SPT reagents in Canada and Europe enable SPT with latex with a low risk of inducing systemic allergic reactions [45, 46]. However, so far there is no approved SPT solution for latex in the United States [47]. Not only SPT with latex per se is mentioned to be a putative risk factor for anaphylaxis during SPT but also a low age [9].

- Fatal:
  The authors did not find reports that patients died after latex SPT. However, in cases of anaphylactic reactions emergency pharmacologic intervention was necessary [48] and patients have been hospitalized for continued therapy [44].

2. Prevalence and risk factors for adverse reactions
   Young patients with spina bifida may be at higher risk of systemic reactions with latex SPT. As a rule, patients with a positive bronchial challenge test result presented the most severe reactions [48]. It has been suggested that in patients with a history of latex allergy with systemic symptoms in-vitro tests should be performed before SPT [44].

Institutional/organizational safety recommendations There are no published safety recommendations referring specifically to skin testing to latex allergens. The currently published position paper 'Latex Allergy' stated that standardized latex SPT extracts are considered safe, although isolated cases of anaphylaxis have been reported. Intradermal tests are not recommended [42]. The diagnostic algorithm for latex allergy which has been proposed by Hamilton et al. [46] may decrease the risk of adverse reactions during skin testing.

WAO safety recommendations Skin testing with latex allergens in highly sensitive patients is considered to be associated with some risk of systemic reactions.

1. Site:
   Both hospital and outpatient setting
2. Personnel:
   Should only be conducted by allergy specialists or equivalently trained medical nurse/technician
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should available within 30 min
5. Pretreatment:
   Not applicable
6. Duration of the supervised follow up after procedure:
   Time of supervised follow-up should be extended up to 40 min.
7. Contra-indications:
   Patients with a history of systemic reactions due to latex: no latex SPT or safety precautions essential
8. Other considerations:
   In patients with a history of latex allergy with systemic symptoms in-vitro tests should be performed before SPT. To reduce the likelihood of adverse reactions SPTs with latex allergen should be performed using different dilutions (beginning with higher dilutions). See also contraindications for SPT in previous section (inhalants)

Bronchial provocation tests (BPTs)

Bronchial provocation tests with allergen

Definition and technical description Inhalation or bronchial allergen challenge is a well-established and reproducible method to confirm sensitization to specific allergen in the bronchi. Allergen-induced reaction manifests as an early asthmatic response (EAR) and may or may not be followed by a more prolonged airway response (late asthmatic response; LAR).

Two methods can be used: continuous generation of an aerosol by a nebulizer and inhaled by the subject via a facemask or inhalation of a standardized dose of an aerosol by generating it intermittently. Any reproducible inhalation method can be used for either approach, the incremental allergen challenge usually employs 2-mins tidal breathing from a calibrated constant output nebulizer, while the single bolus method usually uses a counted number of deep breaths from a (breath-actuated, standard-dose) dosimeter [49, 50]. Both methods produce comparable airway responses.

The patient can also be challenged with allergen (e.g. wheat flour), with a use of a special/custom chamber, by the allergen as such especially in examinations for occupational asthma (e.g. baker's asthma) [51] or in environmental exposure chambers for clinical research purposes. Bronchial response to allergen is either early, late or both and is verified by measuring lung volumes (FEV1) by flow-volume spirometry or peak expiratory flow (PEF) values. The patient is closely followed for at least 6 to 8 h after the challenge. In some centers segmental bronchial provocation techniques through fibro-optic bronchoscope have been employed.

Clinical indications Allergen challenge is not a routine diagnostic procedure as patients are examined for their asthma or asthma suspicion and asthma diagnosis is obtained by other means. BPT with allergen is primarily a research tool in investigations on pathophysiology of asthma and on asthma controller therapy. However in some centers BPT with allergen is used to confirm sensitization and/or explain discrepancy between the clinical history, and the sensitization (skin tests and specific IgE).

Allergen provocation is also done to explore the causal relationship of allergen exposure at a workplace to patient's actual symptoms (occupational asthma) [51]. For example, in the Finnish clinical routine these tests are rarely performed and only in occupational settings.

Age limitation In clinical allergy practice inhalant allergen tests are seldom done to children or elderly, however no clear age limits have been established. Adults of working age are usually subjects of these tests, especially in occupational settings or research. There are centers where children are challenged with allergen (e.g. house dust mite) [52, 53].

Description of adverse/unintended reactions

1. Type and spectrum
   - Local:
     Bronchoconstriction is developing from few minutes to 3 h and patient usually experiences some cough, chest tightness and even wheezing. These symptoms are usually easily to control with bronchodilators. From 16 to 50 % of the patients, dyspnea may appear after 3 to 8 h as a LAR [54].
     Severe asthma attack resulting in prolonged exacerbation of asthma sometimes occur. Other accompanying local symptoms may include irritation of throat, trachea and bronchi, causing cough.
   - Systemic:
     Exceptionally, severe anaphylactic reactions caused by the allergen inhalation challenges can occur. Such reactions usually develop within few minutes and require epinephrine injection.
   - Fatal reactions:
     One case of death caused by rapid, severe bronchoconstriction and anaphylaxis have been reported during exposure to isocyanate 30 years ago [53].
2. Prevalence
   No systematic review is available on the occurrence of unexpectedly strong bronchial responses or anaphylactic reactions. For occupational allergen inhalation challenge it is considered that 12 % required repeated administration of an inhaled short-acting bronchodilator, while few (3 %, 95 % CI: 1–5 %) induced an asthmatic reaction that required additional oral or IV corticosteroids [55].
3. Risk factors
   Unstable asthma (FEV1 below 70 %, recent hospitalization for asthma requiring oral corticosteroids). The risk of moderate or severe reaction was increased when the subjects were challenged with a LMW agent and when they were using treatment with an inhaled corticosteroid [55].

Institutional/organizational safety recommendation Safety recommendations have been included in the state–of-the art documents published by the ERS/ATS, EAACI, or the French Society of Allergology [56].

WAO safety recommendations

1. Site:
   Hospital setting recommended. Outpatient clinic setting could be acceptable.
2. Personnel:
   The provocation test can be performed by a trained nurse/technician but only under the surveillance of a competent physician.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should be available within 30 min
5. Pretreatment:
   Is not necessary, but symptoms of positive reaction should be immediately relieved by the inhalation of short-acting b2-agonist or by nebulization (e.g. 2.5–5.0 mg of salbutamol). In case of more severe reactions oral or intravenous corticosteroids or epinephrine are administered.
6. Duration of the supervised follow up after procedure:
   The patient should be followed closely in a hospital setting for at least 7 h after provocation. The subject should never be left unattended during and following the challenge procedure and FEV1 should be closely monitored for at least 7 h post-challenge. After the last lung function measurement (usually at ≥ 7 h post-challenge), subjects should receive inhaled bronchodilators until the FEV1 returns within approx. 10 % from pre-allergen baseline. Only if this is achieved and the subject is clinically stable, subject can be sent home with the following precautions: secured transportation from research center to home address, provided with rescue medications, preferably not left alone at home, and emergency number(s) of on-call qualified physician who has been notified of the subject.

7. Contra-indications for BPT with allergen
   - Uncontrolled asthma and /or FEV1 < 70 % of predicted
   - Recent hospital admission or asthma exacerbation
   - Spirometry-induced bronchoconstriction (i.e., less than 2 baseline FEV1 measurements out of 8 attempts within 15 %);
   - Recent major surgery; severe disease of the heart, brain, digestive tract, liver, kidney
   - Active, recent or chronic infections; immunological disorder; cancer, history of anaphylaxis
   - Pregnancy
   - Use of systemic beta-blockers
8. Other considerations:
   Allergen preparations employed in the challenge should be as standardized as possible. Furthermore, to prevent sensitization and/ or bronchoconstriction in sensitized investigators, an exhaust hood and/or (HEPA) filters should be used during allergen nebulization.
   Other GCP-based prerequisites relate to data quality and integrity, consist of:

   - Adequate, well-ventilated challenge rooms with standardized humidity conditions within an irritant and smoke-free area,
   - Regularly calibrated and serviced equipment meeting ATS/ ERS criteria,
   - Standardized, validated SOPs,
   - Qualified laboratory and pharmacy, complying to locally required standards.

Bronchial provocation with lysine-aspirin

Definition and short technical description Bronchial challenge with a soluble form of aspirin (lysine – aspirin; L-ASA) is used to confirm a history of hypersensitivity reactions induced by aspirin or other NSAIDs in patients with an underlying chronic airway respiratory disease (asthma/rhinosinusitis/nasal polyps) and manifesting primarily as bronchial obstruction, dyspnea and nasal congestion/rhinorrhea [57]. Incremental concentrations of L-ASA are administered by a dosimeter-controlled jet-nebulizer in 30 min intervals and forced expiratory volume in 1 s is measured at 10, 20 and 30 min after each dose [58, 59]. The provocation is considered positive if at least 20 % fall in FEV1 as compared with post saline baseline value occurs [60]. Diagnostic inhalation challenges with other NSAIDs (indomethacin, sulpyrine, ketoprofen) have been also reported.

Clinical indications An inhalation provocation test with lysine -aspirin is used to confirm hypersensitivity to aspirin or other NSAIDs in patients with cross-reactive, respiratory type of hypersensitivity. It is an alternative to oral aspirin challenge test which is the diagnostic gold standard, but brings some risks of systemic reaction (see section on Oral Drug Provocation Test). Inhalation test with L-ASA is faster to perform than the oral test, but it is less sensitive and negative result of an inhalation test does not exclude NSAIDs-hypersensitivity. The diagnostic value of L-ASA BPT has been documented only in patients with a history of respiratory type of hypersensitivity to ASA/NSAIDs - called Aspirin Exacerbated Respiratory Disease (AERD) or NSAIDs Exacerbated Respiratory Disease (NERD) - and is considered to be specific, reproducible, and generally safe method for NERD confirmation [61].

Age limitation The test is usually performed in adults since NERD is rarely seen in children. However, a single study on L-ASA BPT challenges in children (aged 6–17 years) reported, similar to adults, general safety of this procedure [62]. Interestingly, in one child, urticarial symptoms were reported following L-ASA bronchial challenge.

Description of adverse/unintended reactions associated with the procedure

1. Type and spectrum of adverse reactions
   1. Local:
      Typical and expected symptoms include dyspnea and chest tightness accompanied with fall in FEV1 developing within 10–30 min after positive bronchial challenge. The symptoms can be easily relieved by inhaled/nebulized β2 agonist. In some patients an early prolonged reaction has been observed (fall in FEV1 developing with 2–3 h) [63], while in one study, several hours following lysine aspirin challenge the development of late bronchial symptoms was observed [64].
   2. Systemic:
      Bronchial reaction induced by inhalation of L-ASA may be accompanied by extrabronchial (nasal and/or cutaneous) symptoms in almost half of ASA-hypersensitive patients, and in some patients, inhalation of L-ASA results in development of isolated extrabronchial symptoms [65, 66]. Only a single case of severe, systemic reaction has been described in a patient with history of ibuprofen - induced dyspnea, but without typical asthma triad [67]. The reaction alter LysASA BPT started with facial flush, and generalized pruritus was followed by shortness of breath, cold sweating, and wheezing. Severe bronchoconstriction (75 % fall in FEV1) was associated with asphyxia and

hypotonia. The patient fully recovered after administration of epinephrine, oxygen, a short acting, bronchodilator by inhaler, methylprednisolone, and volume expander.

3. Fatal reaction:
   No fatal reaction has been reported

2. Prevalence and risk associated with the procedure:
   Although BPT with L-ASA is generally safe, it may be associated with systemic reaction, thus precautions during the procedure are necessary. The major risk is a significant bronchospasm, which however, can be easily relieved by appropriate treatment [68].
3. Risk factors for adverse reactions
   Low basal FEV1 (below 70 % of predicted), uncontrolled asthma, inappropriate increasing of the dose of inhaled Lys-aspirin.

Institutional/organizational safety recommendations General safety recommendations has been presented by the HANNA/ENDA guideline [57]

WAO safety recommendations The following WAO safety recommendations are proposed (Grade IV):

1. Site:
   4. Hospital or outpatient clinic setting
2. Personnel:
   - Physician should be responsible for supervising the L-ASA bronchial challenge procedure, which may be performed by a nurse.
3. Emergency equipment availability:
   - Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   - Should be available within 30 min
5. Pretreatment:
   - Is not necessary, but symptoms of positive reaction should be immediately relieved by the inhalation of short-acting b2-agonist or by nebulization (e.g. 2.5–5.0 mg of salbutamol). In case of more severe reactions oral or intravenous corticosteroids or epinephrine are administered.
6. Duration of the supervised follow up after procedure:
   - Patient should remain under observation in the office/hospital for at least 1 h after the completion of an aspirin inhalation challenge. The FEV1 value should have returned to within 10 % of the prechallenge baseline, before discharge from the hospital. The patient should be provided with a peak expiratory flow (PEF) meter and record the PEF values before leaving the hospital and every 2–3 h until late evening. In the case of any respiratory symptoms and a 20 % decline in PEF value, the patient should take short acting β2-mimetic and contact the center.
7. Contra-indications:
   - Uncontrolled asthma and/or FEV1 below 70 % of predicted
   - A history of very severe anaphylactic reactions precipitated by aspirin or other NSAIDs
   - Infection of respiratory tract within 4 weeks prior to the challenge
   - Recent major surgery, severe disease of the heart, brain, digestive tract, liver, kidney
   - Pregnancy
   - Use of systemic beta-blockers
8. Other considerations
   - Although associated with some risk of more severe reaction, Lys-ASA-BPT is generally considered safe, sensitive, specific and reliable diagnostics tool for confirming both AERD and NERD

Nonspecific bronchial provocations

Definitions and technical description During NS-BPT, a patient inhales under laboratory conditions increasing doses (concentrations) of a potentially bronchospasm-inducing agent or is exposed to forced hyperventilation during exercise. After inhalation of each dose FEV1 is measured. A challenge is completed when a significant fall in FEV1 occurs or a maximal cumulative dose (concentration) is administrated. The stimuli used for non-specific BPT can be classified as direct (methacholine, histamine, leukotrienes or prostaglandins), and indirect (e.g. exercise, eucapnic voluntary hyperpnea, hypertonic saline, adenosine monophosphate, and mannitol) depending if they act directly on a specific airway smooth muscle receptor or release mediators from inflammatory cells [69–71].

Clinical indications The test are used to confirm the presence or assess the degree of airway hyperresponsiveness, which is one of the main characteristics of asthma, and its measurement, using different methods, is important in establishing a correct diagnosis [72]. However, non-specific airway hyper responsiveness may be also present in other chronic respiratory conditions such as COPD, cystic fibrosis, or allergic rhinitis. These tests have been also used to monitor asthma treatment [73] and to monitor non-specific bronchial hyperresponsiveness before and after bronchial challenges with specific occupational and non-occupational agents [74].

Age limitations Since change in FEV1 is the primary outcome measure for these tests, the ability to perform reliable spirometric maneuvers is the major limitation. Therefore, the use of these testing methods is not recommended for those under the age of 6. There is no upper age limit to

perform NS- BPT. The use of impulse oscilometry, instead of spirometry, may expand the age groups able to perform reliable spirometric maneuvers as it requires passive co-operation instead of active participation [75].

Description of unintended/adverse reactions associated with the procedure

1. Type and spectrum of adverse reactions
   - Local:
     Cough is the most common side effect of these protocols [71]. Less common side effects include oropharyngeal pain and irritation, chest discomfort, and dizziness. There are isolated cases of angioedema and Vocal Cord Dysfunction reported in the literature. Clinical staff exposed to the bronchoprovocation agents are at increased risk of bronchospasm if they have asthma.
   - Systemic:
     No systemic reactions except for cough or gag have been reported.
   - Fatal:
     No fatal reactions following NS-BPT have been reported.

   The NS-BPT procedures are considered to be generally safe and adverse reactions are usually mild and fairly easy to control. Most patients recover spontaneously after the challenge test or after receiving a standard dose of a bronchodilator. Distressed
   patients respond very well to inhaled bronchodilators with or without oxygen supplementation.
2. Risk factors for adverse reactions/unintended reactions
   The risk of excessive reaction may be increased in individuals with low baseline lung function, if their asthma is not well controlled or during active respiratory infection.

Institutional/organizational safety recommendations
Both European and America guidelines propose safety measures and list contraindications for NS-BPTs [76–78].

WAO safety recommendations and contraindications

1. Site:
   Hospital or outpatient clinic setting – These procedures do not require hospital-based specialized centers and hospital admission is not necessary for the duration of the provocation [79].
2. Personnel:
   The procedure can be performed by trained technician/nurse who is familiar with the guidelines and knowledgeable about specific test procedures. Physicians who have expertise in the field should be readily available to manage acute asthmatic reactions or other complications.
3. Emergency equipment available:
   Should be available on site (recommended)
4. Emergency staff (ICU) available:
   Not required
5. Pre-treatment:
   No pre-treatment is necessary, however drugs which may potentially affect the reactions (see guidelines) should be withheld prior to the challenge [80].
6. Duration of supervised follow-up:
   Due to the characteristics of the agents used, late-phase reactions are not expected, except in rare cases after exercise tests. As a result, no special follow-up is needed after recovering from bronchospastic reactions.
7. Contraindications:
   - Severe airflow limitation (FEV1, < 50 % predicted or < 1.0 L) (absolute contraindication)
   - Moderate airflow limitation (FEV1 < 60 % predicted or <1.5 L(relative contraindication)
   - Uncontrolled asthma
   - Spirometry-induced bronchoconstriction (i.e., less than 2 baseline FEV1 measurements out of 8 attempts within 15 %);
   - Recent major surgery; severe disease of the heart, brain, digestive tract, liver, kidney
   - Active, recent or chronic infection
   - Pregnancy
   - Use of systemic beta-blockers
   - Current use of cholinesterase inhibitor medication (for myasthenia gravis) for methacholine challenges
8. Additional for exercise testing:
   The European Respiratory Society suggested [80]:
   - FEV1 greater than 75 % of the predicted normal value
   - The patient with unstable cardiac ischemia or malignant arrhythmias should not be tested.
   - Those with orthopedic limitation to exercise are unlikely to achieve exercise ventilation high enough to elicit airway narrowing.
   - For patients over 60 years of age, a 12-lead electrocardiogram (ECG) obtained within the past year should be available.
9. Other considerations:
   - Subjects should understand the procedure and be able to perform reliable spirometric maneuvers.

Nasal provocation tests

Nasal allergen provocation tests

Definition and short technical description Nasal allergen provocation test (NAPT) or nasal allergen challenge test is the method by which the nasal mucosa is

challenged by instillation of allergen into the nasal cavities. NAPT assesses the nasal response to the suspected triggering allergen.

There are several methods by which NAPT is performed. Some clinicians perform it by spraying the allergen solution as aerosols into the nasal cavity, while others apply a small allergen coated paper disk on the inferior turbinate. Nebulization or instillation by pipette/dropper are other forms of NAPT. Yet another form of allergen challenge is by using special challenge chambers with controlled environments and precise delivery of agents [81]. Therefore, there is no standardized uniform method for performing NAPT, and so also for the precise criteria for evaluating the positive response, and grading for the risk of adverse events [82, 83].

Clinical indications NAPT is performed to confirm the diagnosis of AR in the situation of discrepancy between the symptoms and the results of skin prick test (SPT) and/or serum specific immunoglobulin E (sIgE), to objectively assess disease severity and to monitor the response to pharmacologic treatment, for specific immunotherapy (SIT) in AR, to study the pathophysiological mechanisms of allergic inflammation, and to diagnose occupational rhinitis [84]. Nasal provocation tests are necessary for the diagnosis of local allergic rhinitis [85].

Age limitation NAPT can be done in both adults and children. Upper age limit depends on the presence of contraindicating disease conditions.

Description and prevalence of excessive/adverse reactions associated with the procedure

1. Type and spectrum of excessive/adverse reactions
   - Local:
     - The chance of side effects is influenced by the concentration of allergen and by the method of allergen application. The appropriate dose of allergen for provocation can be estimated based on the dose of SPT. The dose of allergen that elicits a positive response (3 mm) of SPT can be used for NAPT. The starting dose can be 1:1,000 then increased by either factor of 3 or 10 [86, 87] NAPT performed by spraying allergen or applying a small disk on the inferior turbinate carries a lower risk as compared to the methods using nebulization or instillation of allergen solution by a pipette/dropper.
     - Adverse reactions from NAPT can be divided into those upper airway reactions (mainly nasal) and lower airway reactions (bronchoconstriction). An excessive reaction of the upper airway due to NAPT is a severe nasal blockage or excessive nasal discharge. NAPT also carries a risk of a delayed reaction defined as the reappearance of nasal symptoms 3–12 h after NAPT [86, 88, 89]. Some researchers have reported that the immediate and late phase response of NAPT was 63 % and 37 %, respectively [90].
     - Lower airway adverse reaction to NAPT (bronchoconstriction) can occur when the allergen enters directly into the lower respiratory tract via the larynx. The chance of allergens directly entering the lower airways also depends on the method of NAPT used.
   - Systemic:

   No systemic reactions following NPTs have been reported
2. Risk factors for adverse reactions
   The possibility of excessive/adverse events of NAPT comes from either the use of excess allergen for NAPT or deposition of the allergen from nose/nasopharynx into the lower airways.

Institutional/organizational safety recommendations
Not available

WAO safety recommendations

1. Site:
   Outpatient clinic or hospital setting
2. Personnel:
   Technician/nurse with physician's supervision
3. Emergency equipment availability:
   Emergency equipment should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should be available within 30 min
5. Pretreatment:
   Not applicable
6. Duration of the supervised follow up after procedure:
   30 min
7. Contra-indications:
   - Intense nasal obstruction or septal perforation
   - Current nasal symptoms
   - Within 4 weeks after viral or bacterial infection.
   - Not well-controlled asthma, severe asthma or severe chronic obstructive pulmonary disease (COPD)
   - Cardiopulmonary disease where epinephrine is contraindicated
8. Other considerations:
   Proper cooperation of the patient when performing NAPT (especially when performing by allergen nebulization or pipetting/dropping) is mandatory. The patient should hold one's breath during allergen instillation to prevent the leaking of the allergen into the lower airways [91, 92].

Nasal aspirin provocation

Definition/description Nasal challenge with lysine aspirin or ketorolac (United States) can be used to diagnose aspirin exacerbated respiratory disease (AERD). Baseline symptoms and measurements of the upper and lower airway are made and then incremental doses of the aspirin or NSAID are applied internally to the nose, as drops or spray with close monitoring of symptoms and airway measurements. The timing of response differs from allergen challenge- so 45 min is allowed between application and measurement. An increase in nasal symptoms (obstruction, rhinorrhea, sneezing, itching) plus an objective decrease in the upper airway of >25 % minimal cross sectional area or volume 0–12 cm on acoustic rhinometry is a positive response [60, 93].

If negative after a total of 150 mg lysine aspirin an oral challenge should be undertaken.

Clinical indications Used to assess aspirin sensitivity in patients with rhinitis and/or polypoid rhinosinusitis, and/or asthma.

Age limitation Usually done in adults since AERD is uncommon in children. Upper age limit depends on health status, particularly spirometry- see below. Avoid in pregnancy.

Description of adverse/unintended reactions associated with the procedure

1. Local:
   Since topical aspirin is applied to possibly sensitive tissue the reaction usually involves the upper airway first with predominant symptoms being nasal obstruction, rhinorrhoea, sometimes sneezing, itching. The lower airway may become involved, with asthma symptoms, but this is less frequent than with oral challenge and rarely severe. Laryngospasm reported following ketorolac challenge. About 5–10 % of subjects experience mild gastric irritation.
2. Systemic:
   Very rarely skin reactions such as urticaria and angioedema can occur, again less commonly than with oral challenge where some 25 % are affected.
3. Fatal:
   No fatalities have been reported using nasal aspirin challenge
4. Risk:
   Using nasal lysine aspirin the challenge dose can be very accurately controlled. 6 % of 131 subjects developed asthma symptoms, only 1.5 % showed a significant >20 % decrease in FEV1. Skin reactions occurred in 5.3 %, mainly urticaria, one patient developed facial angioedema [94]. Compared with the standard oral aspirin challenge and desensitization, intranasal ketorolac and modified aspirin challenge significantly attenuated the mean percentage decrease in FEV(1) values (8.5 % vs 13.4 %; $P = .01$) and decreased the percentage of extrapulmonary reactions (23 % vs 45 %; $P = .002$), particularly laryngospasm (7 % vs19%; $P = .02$) and gastrointestinal reactions (12 % vs 33 %; $P = .001$). This protocol was significantly shorter, lasting an average of 1.9 vs 2.6 days ($P = <.001$). In fact, 83 % of the patients completed the new protocol in less than 48 h compared with only 20 % in the oral challenge control group ($P < .001$) [93].
5. Risk factors for excessive reactions:
   - Laryngospasm to aspirin-challenge inadvisable
   - Failure to monitor nasal airway objectively
   - Dose miscalculation

Institutional/organizational safety recommendations EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity [60].

WAO safety recommendations

1. Site:
   Both hospital and outpatient clinic setting
2. Personnel:
   Technician/nurse supervised by physician
3. Emergency equipment availability:
   Should be available on site (suggested).
4. Emergency staff (ICU) availability:
   Available within 30 min
5. Pretreatment:
   Pre-treatment with all usual asthma therapy on day of study permitted; nasal therapy stopped one week beforehand.
6. Duration of the supervised follow up after procedure:
   30 min
7. Contra-indications:
   History of anaphylactic to NSAIDs Other contraindications as listed in previous section (NPT)
8. Other considerations:
   - Informed consent is mandatory.
   - In very severe aspirin hypersensitivity, for safety reasons start with lowest dose.
   - Use of acoustic rhinometry to monitor nasal airway is recommended (Nasal Inspiratory PeakFlow is less sensitive) [95].

Nasal endoscopy

Definition and short technical description

Nasal endoscopy is the gold standard and the most valuable tool in the clinic to afford the diagnosis (presence, severity, etiology, and follow-up) of the majority of rhinologic pathologies [96–98].

Nasal endoscopy is performed by a flexible or rigid scope attached to a light source by glass fiber. For diagnostic examination, a scope with an optic angle from 0–45° is used with a caliber of 2,5–4 mm. Other optics (45–70°, 4 mm) are mostly used in surgery. Nasal endoscopy may eventually be preceded by local administration of anaesthetic and/or decongestive drugs. First, the bottom of the nose through the nasopharynx is to be inspected with an evaluation of the nasal septum, the lower turbinate and meatus, the choanae and the nasopharyx. Afterwards, the scope follows the edge of the middle turbinate towards the *rostrum sphenoidale*, with examination of the middle and upper turbinates, the mucus drainage from the sinuses, possible accessory ostia from the maxillary sinus, and the aperture of the sphenoidal sinus. At last, to get a view of the ostiomeatal complex, the ethmoidal bulla, the access to the frontal sinus, and the olfactory cleft must be attempted.

Clinical indications

The following reasons for nasal endoscopy can be considered in allergy practice:

- The physical examination of the nasal cavities and paranasal sinuses. The different structures of the nose can be evaluated: nasal septum, upper, middle and lower turbinates and meati, ostiomeatal complex, cavum, nasopharynx, and olfactory cleft. Even oropharynx and larynx can also be examined using flexible endoscopy. Although subjective in nature (physician interpretation) it can provide an objective evaluation of nasal signed (i.e. nasal congestion, rhinorrhea/postnasal drip). Despite being more difficult to perform, rigid nasal endoscopy usually provides a better examination view than flexible endoscopy and anterior rhinoscopy [99].
- To assess severity, and to follow-up (after medical or surgical treatment) of nasal and sinusal diseases [97, 98, 100, 101]:
- For differential diagnosis of sinus diseases e.g.
  - Structural abnormalities: septal deviation, turbinate hypertrophy, choanal atresia, or adenoid hypertrophy.
  - Nasal vasculitis, granulomatosis, or bleeding diseases.
  - Benign tumors and malignancies (unilateral versus bilateral)
- To obtain biopsies (i.e. nasal mucosa, nasal polyps, tumors) and microbiological samples for both disease diagnosis and translational research [99].

Age limitation (children, adults, elderly)

Nasal endoscopy is possible in all ages, including children [99, 102]. The only relative limitation is the lack of patient's collaboration.

Description of adverse/unintended reactions associated with the procedure

1. Type and spectrum of adverse reactions:
   Nasal endoscopy adverse reactions are mainly local. When exploring the nasal cavities a very mild discomfort with sensation of foreign body, nasal itching, and sneezing is quite common. Contact of endoscope head with the nasal mucosa, mainly linked to some unexpected patient head sudden movement, can induce some burning or pain sensation and rarely minor epistaxis. Although possible, vasovagal reactions are very uncommon. When exploring the throat with flexible scopes, a nausea sensation can also be induced. There is no data in the literature reporting prevalence and risks associated with the nasal examination using nasal endoscopy.
2. Risk factors for unintended reactions:
   Non-compliant patients, mainly children, and patients with significant nasal septum deviations and/or risk of epistaxis constitute the relative risk factors for these adverse reaction.

Institutional/organizational safety recommendations

Both European (European Rhinologic Society [ERS], European Academy of Allergology and Clinical Immunology [EAACI]) and American (American Academy of Otolaryngology, Head and Neck Surgery [AAOHNS], American Academy of Allergy Asthma and Immunology [AAAAI]) scientific societies have produced a number of position papers which include the efficacy, safety, and main technical recommendations for the use of nasal endoscopy in daily practice, clinical trials, and sinonasal and skull base surgery [99, 103, 104].

WAO safety recommendations

1. Site:
   Both hospital and outpatient clinics
2. Personnel:
   Nasal endoscopic, either rigid or flexible, should be performed by a well-trained physician, either otorhinolaringologist or not (allergologist, pneumonologist, pediatrician, or even general practitioner).
3. Emergency equipment availability:
   Not required
   Comment: For potential minor epistaxis, a wet gauze or merocel pack as well as silver nitrate sticks for cauterization of potential minor nasal bleedings should be available. For unusual but potential vasovagal adverse reactions, a reclining chair or a litter should be available in the clinic.
4. Emergency staff (ICU) availability:
   Not required

5. Pretreatment:
   In general, no pretreatment is mandatory. If examination is difficult to perform, bothersome and/or painful, local anesthesia (lidocaine, cocaine) may be used [105]. A nasal decongestant may also be useful mainly in the presence of nasal deviation, turbinate hypertrophy or swollen mucosa. Both local nasal anesthesia and nasal decongestion may help the physician to have a better view of nasal cavities, turbinates, middle meatus, and nasopharynx as well as to make the patient feel more comfortable during the endoscopic examination.
6. Duration of the supervised follow-up after nasal endoscopy:
   After nasal endoscopy, patients do not need a special follow-up supervision. Only in the case of minor complications (nasal bleeding, vasovagal reaction) the patient may remain in observation in the clinic as needed (usually less than one hour).
7. Contraindications:
   There are no major contraindications for nasal endoscopy. Minor contraindications may be severe nasal hyperreactivity (can be solved by using local anesthesia), non-collaborative patients, and predisposition to nasal bleeding (nasal vasculitis or granulomatosis, Rendu-Osler syndrome).
8. Other considerations:
   The presence of nasal deviations, turbinate and adenoid hypertrophy and chronic rhinosinusitis with or without nasal polyps are usually considered as exclusion criteria in clinical trials investigating the effect of medications in allergic rhinitis. Since posterior nasal deviation and small size nasal polyps are not easily visualized using anterior rhinoscopy, a number of patients with this concomitant problems (10–15 % of general population has CRS and 4 % nasal polyps) may be wrongly included in such clinical trials.

Food provocation tests

Definition and short technical description

The oral food challenge (OFC) test involves having a patient ingest a food gradually, in incrementally increasing doses, under medical supervision to determine if there is allergy or tolerance [105–109]. The food may be prepared and presented in the manner in which it is typically consumed, or its taste and texture may be masked by mixing it with other foods. When the food is presented in its natural form, the test is considered an "open" feeding. An open OFC is commonly used in clinical practice [110], but may introduce bias. The food is masked for single-blind or double-blind, placebo-controlled OFCs, the latter format being considered the least prone to patient and observer bias and is therefore considered the "gold standard" [107, 111]. Testing is performed when the patient is in good general health and without flares of atopic disease, has eliminated the food from their diet, is not using medications that interfere with interpretation of the test (for example antihistamines), or medications interfering with gastric digestion and threshold levels such as anti-ulcer drugs [112]. Patients are observed throughout the OFC, and the feeding proceeds unless the clinician diagnoses an allergic response. If a typical serving size of the food is ingested without symptoms, tolerance is diagnosed. This may require an open feeding of a larger amount following a masked feeding. If a reaction is elicited, treatments may be administered to reverse the allergic reaction, and the conclusion is that the patient is allergic. The time required for feeding the test substances (test food and placebo), and observing for reactions, varies depending upon specific protocol and the anticipated outcomes, e.g., immediate or delayed reactions, but usually takes several hours.

Clinical indications

An OFC is indicated to confirm that an allergic or other adverse reaction to a food exists [105–108, 113]. The test is recommended as a diagnostic procedure because, in contrast to a positive allergy skin or serum sIgE test that indicates sensitization but is not solely indicative of allergy, the OFC may verify or exclude clinical allergy [111, 114, 115]. An OFC is typically administered when other tests, including the medical history, skin testing and/or serum tests are inconclusive, and there is motivation to add the food to the diet or clarify the existence of the allergy [105]. Typical circumstances warranting an OFC include: suspicion of an allergy because of a possible allergic reaction, but having inconclusive supporting tests; no exposure to the food but having positive tests, or evaluating if an allergy has resolved when other tests remain inconclusive. The OFC test can detect immediate or delayed allergic and even non-immunologic reactions. An OFC may also be indicated for research purposes, or to determine an individual's threshold of reactivity [107]. When making the decision to perform an OFC, the clinician should also consider the: risk of reaction (based on history and prior tests), potential severity of a reaction (may relate to the food tested, history, presence of asthma in the patient, test results), patient or family preferences, nutritional importance of the food, social aspects of being able to advance the diet, and emotional consequences should the food not be tolerated.

Age limitation (children, adults, elderly)

The test may be conducted at any age. The test can induce anaphylaxis; therefore, the physician should be confident in recognizing and treating anaphylaxis in the

age group being tested, and understand the health issues that may increase adverse reaction risks or present contraindications in the age group tested (e.g., heart disease, obstructive lung disease, pregnancy, etc.) [107, 108].

Description of adverse/unintended reactions associated with the procedure

1. Type and spectrum of adverse reactions
   A physician-supervised OFC may induce allergic reactions that could range from mild to severe, including anaphylaxis. Food-induced anaphylaxis can be fatal [116]. Although severe reactions have been documented [117, 118], fatal reactions during supervised OFCs have not been reported. Nonetheless, fatal reactions can occur. Symptoms in the event of a reaction, which are expected and should be anticipated, most commonly affect the skin, followed by gastrointestinal and respiratory symptoms [118, 119]. Cardiovascular symptoms are uncommon but must be anticipated, especially in adults. Delayed and biphasic reactions are possible, but also uncommon [120].
2. Prevalence and risk associated with the procedure
   The likelihood of having a reaction and the severity of a reaction is not accurately predictable by current tests [105–107, 109, 121, 122]. Data regarding risk assessment is limited by biases introduced by patient selection and other factors (foods tested, age, clinical approach, definition of a positive test, etc.). Variability in patient selection likely accounts for the large range of OFC outcomes; for example studies in children report reaction rates of 19–48 % [111, 118, 120, 123]. The emotional impact, for example increasing anxiety, is also not predictable. Reactions are most often managed with antihistamines, but epinephrine, including more than one dose, may be needed [120]. Epinephrine is administered, in general, for under 10 % of challenges (overall challenges, including those without reactions) [111, 120, 124]. Additional treatments may be required (e.g., oxygen, intravenous fluids, bronchodilators, etc.).
3. Risk factors for adverse/unintended reactions
   It is presumed that risk factors for reactions include increasingly positive test results, the food tested, personal sensitivity and target organ reactivity (e.g., asthma) [105, 123]. Clinical decisions about stopping an OFC, for example continuing dosing if a reaction has not clearly occurred, but is suspected, may also introduce increased risk that must be weighed against benefits of confirming the allergy [105, 106]. Dosing amount, and frequency between doses, may play a role in outcomes, but this has not been systematically studied [105, 107, 125]. Reactions, including severe ones, may occur on the first dose of an OFC [119, 121]. Very slow and gradual dosing does not necessarily reduce the risk of allergic reaction severity [107, 125]. Using capsules to mask the food allergen is not generally recommended. The use of capsules may result in more severe or uncontrolled reactions because they may release allergen in an unpredictable fashion and also oral symptoms are bypassed [126].

Institutional/organizational safety recommendations

A number of safety recommendations have been promulgated by various organizations, expert panels and authors [105, 107, 113, 114].

WAO safety recommendations

1. Site:
   Both hospital and outpatient clinic setting
2. Personnel:
   Trained personnel, including a physician, with experience in the procedure and skill
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should be available on site (in less than 5 min) or within 30 min depending on the risk assessment
5. Pretreatment (if any):
   Not applicable
6. Duration of the supervised follow up after procedure:
   Observation periods should be determined based on clinical circumstances, but generally a 1–2 h observation is suggested for patients who tolerate the full food dose during the OFC and at least 4 h when a significant reaction occurs; discharge instructions should include the possibility of late reactions (patients understand how to identify and treat);
7. Contra-indications:
   The test should not be performed when the food has recently caused a life-threatening reaction or if the patient has a chronic medical condition that would pose a health threat in the event of anaphylaxis (angina, cardiac disease, pregnancy, severe chronic lung disease, use of beta-blockers, etc.).
8. Other considerations:
   Recording a peak flow or spirometry may be considered and intravenous access should be secured if anaphylaxis or severe reactions such as enterocolitis [127] are likely, or if emergency intravenous access would be deemed difficult. Monitoring of the blood pressure may be necessary.

It is important to select a starting dose that is likely below the patient's reaction threshold. Upon positive food challenge the patient should be advised in regard to dietary restriction through trained personnel (e.g., dietician) and equipped with emergency medications (e.g., self-injectable epinephrine) for allergic reactions upon accidental exposure. Patients should be encouraged to eat the food on a regular basis after a negative test.

Oral drug provocation test

Definition and short technical description

Oral drug provocation test (DPT) is the controlled administration of a drug to diagnose immune-mediated (allergy) and non-immune-mediated drug hypersensitivity reactions. Incremental doses of the drug are administered with the aim of inducing symptoms emulating those reported by the patient but at a very low scale and in a safe and controlled manner. DPT should be performed placebo-controlled, single blinded, and, in situations where psychological factors may be present, even double-blind. The rational approach is provided in different reviews [128–130] and protocols are published for several drugs but these have not been standardized [57, 61, 129, 131–135].

Clinical indications

DPT are usually performed if other less dangerous testing methods are not available or do not allow a firm conclusion, and the outcome is of clinical relevance to the patient. DPT may be carried out in the following situations:

- To confirm the presence of hypersensitivity in a patient with equivocal history
- To exclude hypersensitivity, when clinical history suggests it may not be the culprit drug, when the reaction does not appear to be drug hypersensitivity reaction, and when skin tests or in-vitro tests are not available.
- To assess cross-reactivity to related drugs (e.g. an alternative betalactam antibiotic or another COX-1 inhibitor in hypersensitivity to NSAID)
- To certify tolerance to an alternative drug

Age limitation

There is no data on the age limit for DPT. In general, the procedure is limited by the ability to objectively assess the supposed hypersensitivity reaction that may be elicited and the risk to the patient when the reaction is provoked.

Description of adverse/unintended reactions associated with the procedure

The intention of the DPT is to evoke a hypersensitivity reaction, however the magnitude of the reaction should be limited to the minimum. Thus the major task is to minimize the potential risk of development of generalized and/or severe reaction

1. Type and spectrum of adverse/unintended reactions
   Adverse reaction is anticipated to be similar in manifestations to those occurring in the hypersensitivity reaction, with a similar time kinetic but usually milder and of shorter duration. In practice it may vary from mild, local and transient to generalized and severe and in some instances potentially fatal.
2. Prevalence and risk associated with the procedure
   The prevalence and risk of adverse reaction is dependent on the correct assessment of causality of the hypersensitivity reaction, risk-benefit evaluation of the patient undergoing DPT, assessment of the general health of the patient on day of procedure, and compliance with the technical requirements of DPT [122, 124].
3. Risk factors for adverse/unintended reactions
   Patients with severe co-morbidities such as uncontrolled asthma, cardiac, hepatic, renal or other organ specific or systemic diseases which can be worsened or activated if the hypersensitivity reaction is provoked would be at higher risk. In such instances, DPT is considered only if the drug under suspicion is essential for the patient.

Institutional/organizational safety recommendations

The most comprehensive safety guideline on DPT is from the European Network for Drug Allergy (ENDA) [57]. Other guidelines are on the general aspect of the diagnosis and management of drug allergy [18, 62, 136–138].

WAO safety recommendations

General statement All DPT must be preceded by an individual risk-benefit assessment [139].

1. Site:
   - Both hospital and outpatient clinic setting
   - DPT can be done in the clinic setting if previous reaction was mild [140]. Patients with more severe reactions should be hospitalized for DPT [128].
2. Personnel:
   DPT should only be carried out by a trained nurse/technician under the direct supervision of the allergist.
3. Emergency equipment availability

Should be available on site (mandatory)

4. Emergency staff (ICU) availability
   Should be available on site (within 5 min) or within less than 30 min reach depending on the risk assessment.
5. Pretreatment
   - There should not be any pre-treatment that may mask early signs of a reaction.
   - H1-antihistamines should be discontinued (duration depending on the half-life of the preparation) before the procedure. Corticosteroids, anti-leukotrienes and tricyclic antidepressants may modify response to the challenge and should be reviewed. Medications that may cause problems if emergency treatment becomes essential e.g. ß-blocking agents have to be reviewed and decision made on whether to stop the drug prior to DPT [141].
6. Duration of the supervised follow up after procedure
   The duration of supervised follow-up after procedure is dependent on the expected time latency between drug ingestion and reaction onset based on the previous hypersensitivity episode. In general, immediate-type reactions need a short observation period, whereas delayed-type reactions in the history may necessitate similarly long observation periods after procedure. If a mild reaction has occurred during DPT, observation after stabilization is recommended for at least 2 h. After severe reactions, hospitalization is mandatory because of the possibility of biphasic episodes that can be lethal if not recognized early and treated adequately [142]. It is recommended that before going home, all patients are given an action plan that stresses when to seek medical attention and a number to call in case of emergency.
7. Contraindications:
   DPT should not be carried out when the hypersensitivity reaction is serious and potentially life-threatening including: anaphylaxis, drug hypersensitivity syndromes/drug reaction with eosinophilia and systemic symptoms, acute generalised exanthematous pustulosis, exfoliative dermatitis, erythema multiforme major/Stevens-Johnson Syndrome or toxic epidermal necrolysis, generalised bullous eruptions, vasculitis and other drug-induced autoimmune disease, or specific major organ involvement such as cytopenia. Pregnancy is considered a contraindication for DPT unless the drug is essential during pregnancy or delivery [128, 137, 143].
8. Other considerations:
   - On the morning of the DPT, the patient should have had only a light breakfast and morning medications taken or omitted (as instructed by the attending allergist). The patient's health status should be good, without any sign of allergy or viral infection. Blood pressure, pulse rate, peak flow meter reading (in asthmatics or when bronchospasm is anticipated), and intravenous cannula inserted (if the initial reaction was suggestive of a systemic reaction/anaphylaxis).
   - Prior to each incremental provocation dose, blood pressure, pulse rate, peak flow meter reading (in asthmatics or if bronchospasm anticipated), and any new symptom/sign must be clearly recorded.

Insect sting challenge

Definition and short technical description

Sting challenge (SC) is the ultimate standard for the diagnosis of insect venom allergy [144, 145]. During this procedure patient is deliberately stung by a living insect of the culprit species. Before SC the respective insect needs to be entomologically classified.

In general, a blinded, placebo-controlled procedure is not possible and incremental doses of culprit venom cannot be applied, making the SC test less controllable compared to other challenge tests in allergic patients. A thorough patient work-up and the evaluation of contra-indications are, therefore, of eminent importance.

Clinical indications

Depending on the individual risk profile and culprit, insect venom immunotherapy (VIT) may not be effective in 5–20 % of the patients. SC aims to identify those individuals on maintenance VIT to assess effectiveness and who are not protected after 3–5 years of VIT. Although the standard management of insect venom hypersensitivity does not include SC in the United States [25, 146], the results of SC tests may help physicians to decide on whether VIT should be performed with a higher venom dose and are an invaluable research tool. If standard VIT is not effective (systemic allergic reaction at SC despite VIT), a higher maintenance venom dose will be used (usually 200 μg venom). Later on, VIT effectiveness may be re-evaluated by a subsequent, second SC (after the higher maintenance dose has been reached).

Additionally, results of SC may improve patient quality of life if it can be demonstrated that one does not develop an allergic reaction to a sting of the culprit insect (i.e., less anxiety about future sting reactions).

Age limitation

There is no age limit for SC. However, in patients who are not capable of understanding the procedure, and who cannot give their informed consent SC cannot be done.

Description of unintended/excessive reactions associated with the procedure

1. Type and spectrum of unintended/excessive reactions at sting challenge:
   Adverse reactions include pain at sting site, and local reactions which may be large. In case of VIT failure, systemic allergic reactions may occur varying between minor and very severe, and affecting respiratory and/or cardio-circulatory function.
2. Prevalence and risk associated with the procedure:
   Pain at sting site, and a minor local reaction (wheal, erythema, and swelling) is undesirable but is viewed as a normal sting reaction. Large local reactions with a diameter of more than 10 cm, and/or local reactions of a duration of up to several days are very rare. According to pooled data of observational or randomized studies systemic allergic reactions may occur in 18.0 % (range 0–59 %) of bee honeybee venom allergic patients and in 4.3 % (range 0–12.3 %) of yellow jacket (vespid) venom allergic patients [146]. The vast majority of systemic reactions are mild to moderate; however, cases of severe systemic allergic reactions and in the absence of an early efficient emergency therapy, even a fatal anaphylactic reaction have been described.
3. Risk factors for unintended/excessive reactions:
   - Systemic reactions need to be accepted in order to identify treatment failure [147]. The general risk for the patient in terms of a life-threatening reaction is significantly lower in a medical setting when adequate treatment of symptoms is started immediately after first onset of symptoms, than at field sting. In patients on VIT, several factors determine the overall risk for a systemic allergic reaction at SC (thereby indicating VIT failure). Patients allergic to honeybee venom are at a higher risk for systemic allergic reactions than patients allergic to vespid venom [148–151]. Systemic allergic reactions during SC are also more likely in patients who are on ACE (angiotensin converting enzyme)-inhibitor therapy [53–150, 152, 153]. The rate of systemic allergic reactions at SC depends on the venom doses applied during VIT with higher therapeutic venom doses (individual or cumulative) decreasing this rate [148, 151, 154]. The risk-lowering effect of a higher therapeutic venom dose is not specific for the type of Hymenoptera venom used for VIT. Thus, during SC, the magnitude of risk reduction is the same irrespective whether the patient has received a double VIT (standard dose of two different venoms), or a double dose of the same venom [151]. Duration of VIT is inversely correlated with the risk for a systemic allergic reaction during SC [151]. Severe systemic allergic reactions which have been observed before SC during the build-up or maintenance phase of VIT are also associated with an increased risk for a systemic allergic reaction during SC [151, 154–156]. In addition, certain underlying diseases (mastocytosis) increase the risk for systemic reactions at SC [151, 157].
   - Factors, which influence the severity grade of a sting reaction, have not been systematically investigated. However, mastocytosis is a clear risk factor for very severe sting reactions [157]. Finally, the severity of systemic allergic reaction will increase if the patient presents with severe co-morbidities such as asthma or cardiovascular diseases.

Institutional/organizational safety recommendations

Guidance on how to perform SC has been described, and a guideline was published by the Interest Group on Insect Venom Allergy of the EAACI [144, 158].

WAO safety recommendations

The patient must be screened for any contraindication to SC; risks, benefits and alternatives of the procedure shall be discussed with the patient, and written informed consent for the procedure must be obtained. If the patient is unstable (in case of an organ dysfunction), or if the patient requires a medication possibly disposing him to a higher risk, SC shall be postponed until conditions have been improved.

Drugs, which might ease symptoms of an allergic reaction (thereby evoking falsely negative results at SC) should be discontinued before SC. These drugs may include corticosteroids, H1-antihistamines, or anti-IgE antibodies. The respective half-life of the medication has to be considered when planning a SC. If an ACE-inhibitor therapy is indispensable it should not be discontinued just because a SC is planned. ß-blocking agents should be stopped prior to SC if possible.

Before SC, treatment protocols (indicating venom doses of at least 100 µg, and adherence to injection intervals) should be requested from patients who have been treated elsewhere. Patients should remain fasted for at least six hours before SC, and should not be on a medication potentially interfering with anaesthesia. Chronic diseases like asthma or arterial hypertension should be stable and the patient should not suffer from any relevant acute disease. Blood pressure, pulse rate, and, in asthmatics, pulmonary peak flow or FEV_1 should be measured. Before SC, a peripheral intravenous

cannula with a large bore should be inserted into all patients.

1. Site:
   - Both hospital or outpatient clinic setting
   - After SC, some patients will require a subsequent in-hospital surveillance or treatment. Therefore, SC should be performed at a site which is sufficiently close to a hospital specialized on emergency treatment.
2. Personnel:
   The insect can be put onto the patients' skin and can be motivated to sting by a trained nurse or by other assistance personnel. SC shall be done under direct supervision of an allergist.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should be available within 30 min
5. Pretreatment:
   No specific pretreatment is necessary.
   Cardiovascular or bronchial diseases, which might represent a specific risk in the context of SC, should be treated to reach a stable situation.
6. Duration of the supervised follow up after procedure:
   Monitoring of all subjective or objective signs and symptoms is required during and after SC. After SC the patient should be monitored for at least two hours or longer, depending on the patient's history and on the outcome of SC. After severe systemic allergic reactions, hospitalization is mandatory until complete recovery (minimum duration 24 h).
7. Contraindications:
   SC should not be done in patients who already had a systemic allergic reaction after a field sting while still being in the maintenance phase of VIT. In patients with repeated side effects during the maintenance phase of VIT, SC should not be performed unless a tolerance of VIT has been reached. Severe or poorly controlled cardiovascular/respiratory diseases (FEV_1-value ≤ 70 %) as well as pregnancy are contraindications for SC.
   If medications which might lower the risk for systemic allergic reactions at SC cannot be safely withdrawn, they shall be continued. However, results of subsequent SCs must be interpreted with caution since there is an increased chance for falsely negative results, and the reaction to a later field sting may differ from that observed after SC.
8. Other considerations:
   For patients with mastocytosis and other risk factors an alleviated maintenance dose should be given from the start.

Therapeutic procedures

Subcutaneous Allergen Specific Immunotherapy (SCIT)

Definition and technical description of the procedure (SCIT)

Since the beginning of the twentieth century, allergenic vaccines were administered subcutaneously (SCIT). The favorable clinical results obtained in the early empirical attempts, rapidly lead to a widespread use of SCIT, which remained for decades the only form of allergen immunotherapy. Some clinicians occasionally attempted to use routes different from the subcutaneous one [159–161], but the alternatives to SCIT remained of very limited interest for many years [162].

SIT is started by increasing subcutaneous injections of allergen up to a maintenance dose. Several protocols of SCIT administration (mainly regarding the modality to achieve the maintenance dose) have subsequently been proposed and used in clinical practice, such as the "rush", ultra-rush or the "cluster" [163, 164].

After reaching the maintenance dose, the interval between injections is usually increased to monthly, and continued for 3 to 5 years. For hymenoptera venom allergy, the interval between maintenance doses can be delayed to every 4 months [165], and performed life-long especially in patients with significant risk factors such as mastocytosis or previous severe sting reactions.

Indications to SCIT

Allergen-specific immunotherapy (SIT) is a "biological response modifier" that affects the immune response towards allergens at different levels. For this reason, SIT is currently considered a cornerstone of the management of allergic respiratory diseases (allergic rhinitis/asthma) and of Hymenoptera venom allergy.

Age limitation

Usually SCIT is considered in children 5 years and older.

Description of adverse/unintended reactions associated with SCIT

1. Type and spectrum of adverse events (AEs)
   After the earliest descriptions of AEs due to SCIT [165] these are generally classified according to a system introduced in Europe since the 1990s [166] (largely based on the Mueller's classification for hymenoptera venom reactions) [167] and up-dated by the World Allergy Organization and other organizations [168, 169]. Nonetheless, other classifications have been repeatedly proposed (for review see 174), always distinguishing between local and systemic reactions, grading systemic reactions according to their severity and the number of organs involved, and distinguishing between early (<30 min) and delayed reactions [162, 163, 168, 170]. Local reactions are

limited to the site of injection and include itching, swelling, pain or induration.
Systemic reactions include rhinoconjunctivitis, bronchospasm, urticaria/angioedema, generalized itching, abdominal cramps sometimes ending as respiratory failure or shock. Other reactions are considered nonspecific (headache, tiredness, general malaise). Also vaso-vagal reactions due to the injection (nausea, vomiting, bradycardia, hypotension, sweating) may occur. This again proposes the distinction between "generic" systemic adverse events and overt anaphylaxis [171], since the definition of anaphylaxis itself has been repeatedly changed during the last years. In general the involvement of more than one organ/system strongly suggests anaphylaxis, as well as a clinically relevant drop in blood pressure, loss of consciousness or respiratory compromise [171, 172]. The more recent classification of systemic AEs, has been proposed by the World Allergy Organization [172]. The majority of the AEs are immediate (i.e. within 30 min) and therefore are presumed to be due to specific IgE, but delayed reactions may also occur.

2. Prevalence of adverse reactions and risk associated with procedure
The fact that the injection of allergens in atopic subjects could cause AEs including severe reactions and even death, had been recognized and published since the early 1980s [173–175]. The rate of systemic reactions with SCIT largely depends on the administration schedule, the type of allergen and the survey method (e.g. controlled trial VS questionnaire-based surveys). When evaluating the data from literature, it has to be noticed that the practice of SCIT largely differs between USA and Europe. In USA, allergen mixtures are commonly used, and the extracts are at higher concentrations [176], whereas in Europe, the usual attitude is to vaccinate with few (1 to 3) allergens. On the other hand, the improved manufacturing procedures and quality of extracts, the improved standardization of allergen extracts, and the divulgation/education efforts [163, 164, 168], have probably contributed to the decline of severe/fatal reactions.
The majority of data on the safety of SCIT come from the USA surveys that have been regularly conducted over the past 40 years. According to past and more recent surveys the occurrence of fatal adverse events is less than 1 per 2,500,000 injections [177–181], although the occurrence of fatal or near fatal events has progressively declined over the years [179, 182–184]. The occurrence of systemic AEs with SCIT is approximately 0.05–0.6 % of doses administered. On the other hand, no large population-based surveys have been conducted among the European Countries, and the data from clinical trials are largely incomplete [185]. To date, the largest safety survey on SCIT was conducted in Italy [186], which analyzed over 1,700 patients, showing a 3.3 % of systemic reaction rate with no fatalities.
3. Risk factors for adverse reactions
Based on the available data derived from the large USA surveys as well as European data reports, severe and uncontrolled asthma seems to represent the most prominent risk factor for severe side effects [177–179]. Other factors indicated in the official position papers have to be considered as relative contraindications and should be considered individually [168]. This is especially true for children below the age of 5, where severe AEs are more difficult to recognize and to treat [168]. Another well recognized risk factor is human error, including wrong dosing administration, injection into vessels, and lack of emergency measures immediately available [187]. Although in the past it was reported that large local reactions are more frequently described in those patients who experience systemic reactions, it is now well accepted that on an individual basis large local reactions are poor predictors of future systemic reactions [188].
Finally, it has been sometimes suggested that SIT can induce autoimmune diseases. Recently published data, however, demonstrate no increase in autoimmune disease, and thus recommendations state that it should not be considered a contraindication to the treatment [189]. Recent data have also shown no need to avoid its use in well controlled HIV infection [190, 191]. Pregnancy seems not to be a significant risk factor for SIT [191, 192]. Although a hypothetical risk can exist, based on pathophysiologic considerations, there is no evidence that the use of betablockers (especially the cardioselective ones) or angiotensin inhibitors enhance the risk of adverse events in patients taking SCIT [193, 194].

Institutional/organizational safety recommendations

EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy (2006 Alvarez-Cuesta E) [163]

Allergen immunotherapy: a practice parameter third update (2011 Cox L et al) [164]

Sublingual immunotherapy: World Allergy Organization position paper 2013 update [195]

WAO safety recommendations for SCIT

These WAO recommendations refer to subcutaneous form immunotherapy (SCIT) only. Sublingual immunotherapy (SLIT) has not been included in this document since the vaccine (drops or tablets) is NOT administered

in a medically supervised setting. Although the first dose of allergen vaccine may be administered at the allergist's office, it has never been a formal requirement.

1. Site:
   SCIT treatment may be started and continued in an outpatient setting
2. Personnel:
   Only trained allergist may initiate and supervise SCIT. The injections can be made by a nurse under physician supervision.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   Should be available on site (in less than 5 min) or within 30 min, depending on the risk assessment
5. Pretreatment:
   The use of premedication with oral antihistamines/oral antileukotrienes still remains a matter of debate. On one hand it has been claimed that premedication may delay or mask systemic reactions. On the other hand, it has been reported that premedication could reduce the frequency and severity of AEs. The strength of recommendation on this matter is still weak, thus its employment largely remains in the hands of the physician.
6. Precautions and duration of the supervised follow up after procedure:
   Although some of the adverse events of SCIT can be avoided, others occur unpredictably and without explanation. Immunotherapy should be administered with a 26- to 27-gauge syringe, and the injection should be given subcutaneously in the lateral/posterior portion of the arm. The skin should be pinched and lifted off of the muscles to avoid intramuscular or intravenous injection and the skin should be wiped with disinfectant before giving the injection.
   It is well known that some fundamental precautions can be taken to reduce the risk of severe/fatal AEs. First, the correct administration (i.e., patient's name, batch, and allergen) has to be verified and recorded. As recommended in all guidelines it is essential that the patient have a careful examination and medical history taken [168–172, 174–187]. These include the objective assessment of current respiratory symptoms/signs (e.g. asthma/rhinitis), the evaluation of previous systemic reactions to SCIT (immediate or delayed), and the presence of any concomitant acute respiratory illness. When feasible, a Peak Expiratory Flow evaluation should be done, considering a value of less than 70 % of best predicted a warning signal [162, 163, 167]. After each injection the patient should be observed for at least 30 min.
7. Contraindications:
   Pregnancy has been always prudently suggested as a potential contraindication to SIT, and to SCIT in general, although no evidence was present in the literature. A recent survey demonstrated that the use of SIT in pregnancy, when clearly indicated, does not increase the risk of perinatal or foetal adverse events [191, 192]. The suggestion of not starting SCIT during pregnancy, and not stopping an already ongoing SIT remains valid, based on common sense.
8. Other safety considerations:
   Safety of maintenance of SCIT may be improved by monitoring of symptoms, appropriate adjustment of vaccine dosing etc.

Venom immunotherapy

Definition and technical description of the procedure (VIT)

Venom immunotherapy (VIT) is so far the only effective treatment that prevents anaphylaxis and improves quality of life in patients with venom allergy [23, 196–198]. Several protocols of VIT have been described and used in clinical practice: Conventional protocols are started with weekly injections of increasing venom doses from 0.01 to 100 μg over 2 months. In rush protocols the increase to the maintenance dose is reached by daily increasing doses of venom for 2 to 3 days. In ultrarush protocols the increase to a total dose of 100 μg is reached by injections every 30 min in 3.5 h (Table 1) [196]. After reaching maintenance dose the interval of injections is increased from weekly to monthly, and 6–8 weeks from the second year. The recommended duration of VIT is 3 to 5 years, in patients with risk factors like mastocytosis or previous severe sting reactions, VIT may be continued indefinitely or as long as the risk of accidental stings remains. The maintenance dose of 100 μg protects over 95 % of wasp and ant venom allergic and 80–90 % of bee venom allergic patients from systemic allergic reaction (SAR) when re-stung [148, 196, 197]. In case of a SAR to a re-sting during VIT an increase of the maintenance dose to 200 μg protects most of these patients from further SAR [196].

Indications for VIT

US guidelines recommend VIT in all patients with a history of SAR and positive diagnostic tests – skin tests and/or venom specific serum IgE. Excepted are children with only cutaneous reactions [23]. European guidelines [196] do not recommend VIT also in adults with only cutaneous reactions, unless there are special risk factors or a severe reduction of QOL [198].

Age limitation

VIT may be given to children including those of pre-school age [23, 196], although the balance between discomforts versus benefits of treatment should be considered on an individual basis. In general, it is best to wait until a child is old enough to understand and accept the treatment. Elderly patients have an increased risk of very severe SAR to accidental stings with lasting morbidity, e.g. myocardial infarction, cerebral infarction or even fatal outcome [199, 200], which may be prevented by VIT. Although older age and comorbidities also increase the risk of reactions to VIT itself, these are usually milder and easier to manage than a field sting. Therefore, there is no upper age limit for VIT.

Adverse reactions associated with VIT

1. Type and spectrum of adverse reactions
   Local reactions at the injection site are common, and may be large (>5 cm in diameter) or last more than 24 h. Immediate-type adverse reactions are common during VIT. The majority of these are mild skin-only reactions, but some may be severe.
2. Prevalence of adverse reactions and risk associated with procedure
   The reported proportions of patients experiencing one or more significant reactions requiring medical intervention are 10–20 % for bee and *Myrmecia* ant VIT, and 5 % for Vespula VIT [23, 148]. Fatal reactions to VIT have not been reported. Rush and ultrarush protocols (Table 1) protect most patients more rapidly but may increase the number of SAR side effects [196, 201].
3. Risk factors for adverse reactions
   In addition to the species of venom used, risk factors for SAR due to VIT include older age, coexisting cardiovascular or pulmonary disease, antihypertensive medications, elevated baseline serum tryptase and mastocytosis [200–204]. Intercurrent illnesses (e.g. fever, infection) may also increase the risk of an adverse reaction. Dialysed aqueous venom and Aluminium hydroxide depot extracts have somewhat lower risks of SAR [205, 206].

Institutional/organizational safety recommendations

The most recent international guidelines addressing the issue of VIT safety are: The guidelines of the EAACI [195]. The practice parameter update 2011 of the AAAAI [23] and the WAO anaphylaxis guidelines 2013 [207].

WAO safety recommendations

1. Site:
   - Both hospital and outpatient clinic settings
   - VIT treatment may be started and continued either at a hospital or in the office
2. Personnel: technician/nurse/physician
   The treating physician should recommend and supervise VIT. The injections can be made by a nurse under physician supervision.
3. Emergency equipment availability
   Should be available on site (mandatory)
4. Emergency staff (ICU) availability
   Should be available on site (in less than 5 min) or within 30 min, depending on the risk assessment and the immunotherapy protocol used.
5. Pretreatment
   Pre-treatment with oral antihistamines during the dose build-up phase reduces the risk of SAR during VIT, and does not impact on the overall efficacy of VIT [208].
6. Precautions and duration of the supervised follow up after procedure
   - xPrior to each VIT injection, the patient should be asked about: (i) reactions or unexpected symptoms following the last visit or injection, and; (ii) any new health problems including newly prescribed medications. Blood pressure and pulse rate should be routinely measured before every injection is given. Issues identified may lead to modifications as follows:
     - Reactions on previous visits or injections consider reduced VIT dose
     - Intercurrent illness consider delaying treatment
     - Newly prescribed antihypertensive medications consider temporarily withholding antihypertensive medications for 24–48 h prior to each visit for VIT.
     - Poorly controlled blood pressure or new onset (or worsening) of possible cardiac or lung disease (e.g. angina, asthma) consider pausing VIT until further investigations and/or stabilisation of condition.
   - After each injection the patient should be observed for at least 30 min.
   - If there is no SAR the next injection can be given or the patient can be discharged after 30 min of observation.
7. Contraindications
   Contraindications for VIT are concomitant active neoplastic and auto-immune diseases [194]. VIT should not be started during pregnancy but can be continued if well tolerated before pregnancy.
8. Other safety considerations
   - Safety of maintenance VIT may be improved by careful monitoring of symptoms and appropriate adjustment of vaccine dosing.
   - After an SAR the next injection dose should be decreased.

- Discharge may be considered 1 h after complete regression of all symptoms, but after a severe reaction (hypotension or hypoxemia), observation for a longer period should be considered.
- If there are repeated SAR due to VIT, pre-treatment with oral antihistamines on the evening before and 1 h before the following VIT injections should be considered [208].
- Other options are to switch to a conventional protocol using dialysed aqueous or Aluminium hydroxide depot preparations [205, 206], and pretreatment with Omalizumab [209].

Oral Immunotherapy for Food Allergy (OIT)

Definition and short technical description

Oral immunotherapy is a promising concept for the treatment of food allergy. The majority of clinical trials focused on peanut, cow's milk, and hen's egg allergy [210, 211]. Meta-analysis revealed a substantially lower risk of reactions to the relevant food allergen in those receiving OIT [211]. There are several protocols for OIT used throughout the world [212]. In general, OIT starts with oral administration of very low doses of food protein, e.g. 2 mg of peanut protein [213], which is given on a daily basis. The doses are progressively increased over time. Regular dose increments, e.g. biweekly, are performed mostly under medical supervision [212]. When a defined target dose is reached, this maintenance dose, e.g. 800 mg peanut protein [213] is continuously administered on a daily basis and continued for several years. However, it is important to note that, in fact, maintenance dose ranged among various centers from 400 to 8000 mg of peanut protein [214]. Moreover, to date there is no recommended duration for OIT as long-term studies are still missing [211].

Clinical indications

OIT is a promising treatment approach, but it is associated with risk of adverse reactions, including anaphylaxis; it is therefore not currently recommended for routine clinical use [215]. Patients with peanut or tree nut allergy might especially benefit from OIT, as natural tolerance is rare. In addition, patients with persistent cow's milk or hen's egg allergy will be candidates. The objective of OIT is to achieve first a clinical desensitization, which means the tolerance to a certain amount of the allergen with an ongoing therapy, and later a long-term tolerance, which means the permanent loss of reactivity also after stopping OIT [212].

Age limitations

OIT could be performed at all ages; however, most OIT trials have been performed in children [210, 211].

Description of adverse reactions associated with the procedure

1. Type and spectrum of adverse reactions
   The most common adverse reactions are local, e.g., oral pruritus, or gastrointestinal, e.g., abdominal pain. More severe adverse reactions affect the respiratory tract, e.g., wheezing or multisystem reactions
   [210–212]. The development of allergic eosinophilic esophagitis has been described [216].
2. Prevalence of adverse reactions and risk associated with the procedure.
   Currently OIT is only recommended in controlled clinical studies until the short- and long-term safety profile is better known and understood [215].
3. Risk factor for adverse reactions
   Augmentation factors, e.g., infection, menses and exercise seem to be risk factors for adverse reactions.

Institutional / organizational safety recommendations

EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy [215].

WAO safety recommendations

1. Site:
   - Both hospital and outpatient clinic settings; however, OIT is currently not recommended for routine clinical use [214, 215].
2. Personnel: technician / nurse / physicians:
   - Physicians experienced with food allergy and specific immunotherapy should recommend and supervise OIT. During the build-up phase food should be administered by a nurse under physician's supervision.
3. Emergency equipment availability:
   - Should be available on site (mandatory)
4. Emergency staff (ICU) availability:
   - Should be available on site (in less than 5 min) or within 30 min, depending on the risk assessment.
5. Pretreatment:
   - Antihistamines or omalizumab can be considered
6. Precautions and duration of the supervised follow-up after procedure:
   - During the build-up phase, doses are progressively increased over time, e.g. biweekly, under medical supervision [212, 213]. After each dose the patient should be observed for at least 2 h. The same dose is given at home daily until the next increase. In the maintenance phase the tolerated dose is given daily at home [212, 213].
7. Contraindications:
   - Commonly, OIT is not performed in patients with unstable asthma or in pregnancy.

8. Other safety considerations:
   - Doses should be adjusted during infection as this has been described as an important augmentation factor.

Drug desensitization

Definition and short technical description

The term desensitization is used for procedures inducing clinical tolerance or tolerization to drugs eliciting hypersensitivity reactions [217, 218]. Rapid desensitization protocols are used for type I IgE/mast cell-mediated allergic reactions and slow desensitization protocols are used for type IV delayed drug hypersensitivity reactions [218] and other hypersensitivity reactions such as aspirin exacerbated respiratory disease (AERD) [138].

Desensitization procedures are based on protocols in which suboptimal doses of the drug allergens are reintroduced, starting at 1/100 to 1/1000 the target dose or lower for patients presenting severe reactions, and increasing at fixed time intervals by doubling or higher increments until reaching the target dose. These protocols introduce the sensitizing medication in few hours up to 6–8 h. Desensitization protocols allow allergic patients to receive their first line therapeutic agents to treat infections, cancer or chronic inflammatory diseases. The induction of clinical tolerance is temporary and largely depends on the half-life of the medication. The desensitization state persists from few hours, in the case of antibiotics administered every 6 to 8 h to several days in the case of aspirin. It is generally accepted that once more than two half-lives of the medication have spanned the patient is no longer desensitized and will need redesensitization. Successful desensitization can be achieved in patients with IgE/mast cell mediated hypersensitivity reactions (allergy to beta lactams or other antibiotics) [219] and platinium salts [220, 221] who present symptoms including urticaria, angioedema, wheezing, laryngeal edema, nausea, vomiting, diarrhea or hypotension. Anaphylaxis in which tryptase levels are found elevated in serum is not a contraindication for rapid desensitization. Other hypersensitivity reactions (patients with aspirin exacerbated respiratory disease [222], non-beta lactam antibiotics [223], sulfonamides [224] and other chemotherapeutics including monoclonal antibodies [221]) have been successfully desensitized with different and slow protocols, some of them involving several days. Patients with chronic urticaria exacerbated by aspirin and other NSAIDs (named NSAIDs Exacerbated Cutaneous Disease-NECD) may be refractory to desensitizations [225] with few successful cases [226]. There are few standardized protocols for delayed reactions and caution has to be taken to avoid desensitization in patients with severe cutaneous or systemic reactions. Only patients with non-severe delayed reactions are candidates for slow desensitizations [218].

Clinical indications for drug desensitization

Rapid and slow drug desensitizations are indicated:

1. If the drug is considered first line therapy (e.g. patients with platin-sensitive recurrent ovarian cancer, cystic fibrosis patients with antibiotics allergy, patients with NSAIDS intolerance in need of dual antiplatelet therapy).
2. If the drug is more effective than the alternatives.
3. If non-cross reacting therapeutic agents are unavailable.
4. The drug administrated after desensitization has a unique therapeutic effect (aspirin in patients with NSAIDs – exacerbated respiratory disease complicated with nasal polyps).

Age limitation

Most published protocols assess clinical efficacy of desensitization in adult populations. There are several published desensitization protocols for children (desensitization protocols to antibiotics [227] and chemotherapy [228]). The success rate of adult and pediatric desensitization protocols is similar with a range from 50 to 100 %.

Adverse/unintended reactions associated with the drug desensitization

In 30 to 50 % of all desensitization procedures mild symptoms occur and there are no reported deaths resulting from a desensitization protocol. Anti-histamines are used commonly as pre-medications and some protocols are modified once patients have presented reactions to subsequent desensitizations [228].

1. Type and spectrum of adverse reactions
   Most of the desensitization protocols are well tolerated by the majority of patients. However, reintroduction of a drug to an allergic patient carries high risk including anaphylaxis.
   Reactions during rapid desensitization protocols can occur in minutes and can range from flushing and urticaria to hypotension and oxygen desaturation. During aspirin desensitization in patients with Aspirin Exacerbated Respiratory Disease (AERD) the tolerant state is achieved by repeating the provoking dose of aspirin so that aspirin sensitivity has to be demonstrated during the procedure. Thus, except for so called “silent desensitization” adverse reaction (respiratory or cutaneous) are intentionally evoked during the procedure, but the magnitude is controlled and limited by rapid administration of

reliever drugs. These reactions can appear at every step of desensitization protocols. In patients desensitized to aspirin, breakthrough reactions usually occur after the oral dose of 45–60 mg of aspirin [229] but it can be seen at higher doses. Protocols for rapid desensitization to aspirin in patients with cutaneous symptoms differ from the AERD protocols [225, 230] in which the prevalence of adverse reactions is only up to 19 %.
During intravenous rapid desensitization protocols most of the adverse reactions are seen when the drug is infused at the maximal concentration and during the last step of the protocol [221].

2. Prevalence and risk associated with the procedure:
Side effects may complicate 12 to 52 % of desensitizations to antibiotics and from 4–33 % of desensitizations to chemotherapeutics and 29 % desensitizations to monoclonal antibodies [220, 221]. In repeated desensitizations the rate of adverse reactions decreases to less than 10 % with over 6–10 desensitizations [221] and the spectrum of reactions ranges from cutaneous reactions [228] to anaphylactic shock [220].
3. Risk factors for adverse/unintended reactions
The severity of the initial hypersensitivity reaction is the most important risk factor, but other factors such as the time course of the HS reaction (in patients with delayed HSR it is most reasonable to hospitalize patients for longer time), the concomitant use of other medications such as beta-blockers and ACE inhibitors and the severity of the underlying disease need to be taken into consideration.
For patients desensitized to chemotherapy and monoclonal antibodies the presence of atopy, a previous severe reaction and the presence of severe cardiovascular disease are risk factor for severe reactions. In addition patients on beta blockers and on ACE inhibitors are at risk for severe hypotension and cardiovascular collapse during desensitization.
Risk factors for severe or moderate reaction during aspirin desensitization include: age: 30–40, duration of AERD less 10 years, FEV1 < 80 %, uncontrolled asthma, previous asthma –related ED visits and lack of antileukotriene pretreatment [229].

Institutional/organizational safety recommendations
Not available

WAO safety recommendations
1. Site:
   - Both hospital and outpatient clinic settings
   - Comment:
     – All high risk desensitizations should be done in the intensive care unit. High risk desensitizations are those performed on patients with initial grade 3 anaphylactic reactions associated with hypotension and/or oxygen desaturation, patients with severe/unstable cardiovascular diseases and/or on beta blockers and patients with FEV1 < 70 %.
     – Once high risk patients have presented a successful desensitization in the intensive care unit repeated desensitizations can be done in the outpatient setting provided resuscitation medications including epinephrine, oxygen and intubation materials are available.
     – Patients with hypersensitivity reactions involving the skin and/or two organs without changes in vital signs can be desensitized for the first time in the outpatient setting with trained staff and emergency equipment available on site (see below).
     – In patients requiring repeated desensitizations (desensitizations to chemotherapy, monoclonal antibodies), after an initial successful desensitization, subsequent procedures can be performed in outpatient settings.
     – For patients with delayed drug hypersensitivity slow desensitization protocols can also be done as outpatient procedures based on the severity of the initial reaction and the disease being treated (allopurinol desensitization for gout)
2. Personnel:
   Desensitization should be supervised by well-trained, experienced allergists and nurses. One on one nursing should be available for each desensitized patient and an allergist should be available on site at less than 3 min of the desensitization procedure.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff availability
   Should be available on the site
5. Pretreatment
   Pretreatment with systemic steroids is not recommended unless required by current guidelines for cancer treatment (dexamethasone for taxanes administration) [231]. Pretreatment with H1 and H2 antihistamines is recommended for rapid desensitization for chemotherapy, monoclonals and antibiotics but no controlled studies have been done comparing outcomes of desensitizations with and without pre-medications [221, 232]. Whitaker et al. [233] indicated that pretreatment with antihistamines alone or with glucocorticosteroids did not reduce the risk of reactions in desensitized patients. Leukotriene receptor blockade with montelukast and prostaglandin inhibition with aspirin have provided excellent

protection against severe reactions in patients desensitized to chemotherapy and monoclonals [234, 235]. In early trials with paclitaxel and docetaxel approximately 30 % of patients presented acute infusion reaction and pretreatment with antihistamines and glucocorticosteroids and slower infusion rate reduced the rate of adverse reactions to 10 % [236]. Based on these results many current chemotherapy regimens include pretreatment with corticosteroids, antihistamines and proton-pump inhibitors.
Although some authors [217] do not recommend pretreatment with antihistamines as it may mask early signs of hypersensitivity reaction [138], current studies in populations of desensitized patients without pre-medications are lacking and no recommendations can be made.
Leukotriene receptor antagonists may alleviate symptoms of breakthrough reactions in aspirin hypersensitive patients [237] by shifting reaction from bronchial to naso-ocular symptoms and these pre-medications are strongly recommended at the present time for all patients desensitized to aspirin.

6. Precautions and duration of the supervised follow up after procedure
The severity of the initial hypersensitivity reaction is the most important risk factor, but other factors such as the time course of the HS reaction (in patients with delayed HSR it is most reasonable to hospitalize patients for longer time), the concomitant use of other medications such as beta-blockers and ACE inhibitors and the severity of the underlying disease need to be taken into consideration.
Patients should be in stable condition (FEV1 > 70 % in patients with asthma) before the start of the desensitization. In patients with cystic fibrosis the baseline FEV1 may be substantially lower and risk assessment should be done but low FEV1 is not considered a formal contraindication for desensitization.
Duration of the supervision depends on the initial hypersensitivity reactions. In case of immediate reactions, rapid desensitized patients to chemotherapy, monoclonal and antibiotics are supervised for 30 min in the hospital for acute post desensitization reactions and then for the next 24–48 h for delayed post desensitization reactions.
7. Contra-indications
The contra-indications to drug desensitization may be absolute or relative, when the risk /benefit evaluation is performed.
   - Absolute contraindications
     - Previous severe/life threatening cutaneous drug induced disease (SJS/TEN, DHS/DIHS/DRESS, AGEP)
     - Cutaneous and systemic vasculitis
     - Drug induced autoimmune disorders
     - Drug induced organ involvement (hepatitis, nephritis, cytopenias, pneumonitis)
     - Immune complex disorders (serum sickness disease)
   - Relative contraindications
     - Treatment with beta blockers and ACE inhibitors
     - Unstable underlying disease (asthma, coronary heart disease)

   Maculo-papular rashes are not contraindications for desensitization and slow protocols are generally successful.
8. Other considerations:
Treatment of chronic disease should be continued, but drugs that can influence the course of reaction like beta blockers and ACE inhibitors should be discontinued at least 24 h prior to desensitization to avoid prolonged and intractable hypotension during anaphylaxis induced by the desensitization procedure.

Treatment with Anti-IgE and other biologicals

Definition and short technical description

Omalizumab is a humanized monoclonal antibody directed against human IgE. It prevents binding of soluble IgE to the IgE receptor. This is currently the only monoclonal antibody directed against IgE which is licensed.

Indication

Omalizumab is indicated for adults and adolescent and children (6 years of age and above) with moderate to severe persistent asthma, who have a positive skin test or in-vitro reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids [238–244]. Although new clinical data suggest that there are also other patient populations with asthma or other allergy or atopy related conditions, who would benefit from with Omalizumab, the clinical indication is currently limited to the above described patient group. More recently omalizumab has been approved for use in chronic idiopathic urticaria [245].

Age limitations

Anti-IgE is indicated for adults and adolescence (12 years of age and above).

Description of adverse reactions

1. Anaphylaxis

The frequency of anaphylaxis attributed to Omalizumab is estimated to be at least 0.2 % of patients, based on an estimated exposure of about 57,300 patients from June 2003 through December 2006. Anaphylaxis has occurred

as early as after the first dose of Omalizumab, but also occurs beyond 1 year after beginning of regularly scheduled treatment [246]. Omalizumab has received a box warning label by the FDA [247–249].

2. Malignancy

Although malignant neoplasms were observed in 20 of 4,127 (0.5 %) Omalizumab-treated patients compared to 5 of 2,236 (0.2 %) control patients in clinical studies, the direct relationship between this treatment and the development of malignancies is completely unclear. The observed malignancies in Omalizumab-treated patients included a variety of different types, including breast, non-melanoma skin, prostate, melanoma, and others [238]. However, the impact of longer exposure to Omalizumab, or the use in patients at higher risk for malignancies, is not known and the application of omalizumab in patients with preexisting malignancies contradicted [239].

3. Eosinophilic conditions

In rare cases, patients with asthma on therapy with Omalizumab presented a serious systemic eosinophilia, sometimes presenting with clinical features of vasculitis, consistent with Churg-Strauss syndrome.

4. Fever, arthralgia, rush

Some patients have experienced a constellation of signs and symptoms including arthritis, arthralgia, rush, fever and lymphadenopathy with an onset 1 to 5 days after the first of subsequent injections.

5. Parasitic infection

It is not clear if treatment with omalizumab may be associated with increased morbidity attributable to parasitic infections [240].

6. Immunogenicity

Omalizumab does not seem to be associated with development of immunogenicity [243].

Institutional/organizational safety recommendations

Not available

WAO safety recommendations

1. Setting:

Both hospital and outpatient clinic setting

2. Personnel:

All personnel, supervising the patient during and after the injection, should be trained to handle anaphylactic reactions.

3. Emergency equipment availability

Should be available on site (mandatory)

4. Emergency staff availability

Should be available on site (in less than 5 min)

5. Pretreatment

No pretreatment

6. Precautions and duration of the supervised follow up after procedure

Following immediate reaction and intervention in the rare case that anaphylaxis occurs, the patient should be further followed up in an appropriate emergency setting.

Consider in-office waiting time – 2 h for the first injection of omalizumab and then 30 min after each subsequent dose. Patient should have an epinephrine autoinjector available [250].

7. Contraindications

The use of Omalizumab is contraindicated in the patients with a history of severe hypersensitivity reactions to Omalizumab or any of the preparation's ingredients. Furthermore Omalizumab should not be used to treat acute bronchospasm or status asthmaticus.

Treatment with products from human plasma

Definition and short technical description

Products made from human plasma are increasingly being used also in the field of allergy and asthma treatment. They include preparations of human immunoglobulins [251, 252], used for subcutaneous and intravenous administration. More recently, also other plasma components have been isolated and made available in commercial preparations, which can be used for various other conditions. A prominent example is the C1 esterase inhibitor [253]. C1 esterase inhibitor is manufactured from human plasma, purified by a combination of filtration and chromatographic procedures. Several precautions have been implemented to reduce the risk of viral transmission, since this factor, as well as

immunoglobulin preparations in general, are being derived from a large pool of donors.

Indication

Indications for the use of human immunoglobulin preparations are the treatment of primary humoral immunodeficiencies, chronic immune thrombocytopenic purpura, and others [251, 252, 254, 255].

C1 esterase inhibitor is indicated for routine prophylaxis against angioedema attacks in patients with Hereditary Angioedema (HAE) [254].

Age limitations

Immunoglobulins are available for all ages, but some C1 inhibitor preparations have limited recommendations in children.

Description of adverse reactions

All adverse reactions are related to direct or indirect effects and mechanisms, known to occur by human immunoglobulins and plasma preparations [256]. These are particularly:

- Hypersensitivity reactions
- Renal dysfunction and renal failure
- Thrombotic events
- Hyperproteinemia, increased serum viscosity, and hyponatraemia
- Aseptic meningitis syndrome (AMS)
- Hemolysis
- Transfusion related acute lung injury
- Volume overload
- Transmissible infectious agents
- Interference with laboratory tests, due to the passively transferred antibodies in immunoglobulin preparations

The following risk factors have been identified for the development of thrombosis:

- Advanced age, prolonged immobilization, hypercoagulable conditions, history of venous or arterial thrombosis, use of estrogens, indwelling vascular catheters, hyperviscosity and cardiovascular risk factors.
- Patients predisposed to renal dysfunction, including those with any degree of pre-existing renal insufficiency, diabetes mellitus, age > 65, volume depletion, sepsis, paraproteinaemia, or patients receiving no nephrotoxic drugs.
- The only serious adverse reaction observed in clinical studies with C1 esterase inhibitor was cerebrovascular accident. The most common adverse reactions observed have been headache, nausea, rash and vomiting.

Institutional safety recommendations

Not available.

WAO safety recommendations

1. Site:
   Both hospital and outpatients clinic settings
2. Personnel:
   All personnel in direct contact to the patient must be experienced in handling hypersensitivity reactions.
3. Emergency equipment availability:
   Should be available on site (mandatory)
4. Emergency staff availability:
   Should be available on site (in less than 5 min)
5. Pretreatment:
   Pretreatment regimen including analgesics, antihistamines, and/or anti-inflammatory medications, steroids, hydration may be indicated in some patients to avoid or diminish common adverse effects
6. Precautions during the supervised follow up after procedure:
   Based on the individual risk of the patient (see above), special precautions have to be taken before administering these preparations. They may include, but are not limited to: Periodic monitoring of renal function and urine output, assessment of blood viscosity, analysis of signs and symptoms of hemolysis, and the presence of anti-neutrophil antibodies and anti-HLA antibodies in both, the product and patient serum. These should be obtained in case of an increased index of suspicion.
7. Contraindications:
   Immunoglobulin preparations are contraindicated in patients who have a history of anaphylactic or severe systemic hypersensitivity reactions to the administration of human immunoglobulin. Administration is also contraindicated in IgA-deficient patients with antibodies to IgA and a history of hypersensitivity. Anaphylaxis has been reported with the intravenous use of immunoglobulin preparations and is theoretically possible following subcutaneous administration.
   The C1 esterase inhibitor is contraindicated in patients who have manifested life-threatening immediate hypersensitivity reactions, including anaphylaxis to the product.
8. Other considerations:
   In case of hypersensitivity reactions stop infusion of injection immediately. Have epinephrine immediately available for treatment.

Management of emergencies in allergy practice

All medical staff involved in either diagnostic or therapeutic allergy procedures should be trained in the recognition and management of allergic emergencies including anaphylaxis.

Recommended equipment and medications

The following equipment is necessary at the allergy office performing diagnostic allergy procedures:

Equipment

1. Trolley for patient to lie flat if needed
2. Oxygen and suction equipment, including tubing, masks etc.
3. Airway management equipment according to skill level (basic essentials are oral and nasopharyngeal airways, bag-valve-mask for ventilation)
4. Intravenous access cannulae (20-16G) and giving sets; needles and syringes.
5. Manual blood pressure cuff
6. Nebulizer mask (for inhaled/nebulized epinephrine)
   Note: In a hospital setting it is recommended that there also be immediate access to ECG, pulse oximetry and non-invasive blood pressure monitoring equipment, advanced airway management devices for intubation and cricothyrotomy, and an intravenous infusion pump.

Drugs and fluids

1. Epinephrine (2 packs of 5 ampoules of 1 mg/1 ml)
2. Corticosteroid for intravenous injection
3. Antihistamines for oral or intravenous use
4. Two 1 L bags of normal saline
   Note: In a hospital setting, it is recommended that there also be immediate access to (a) smaller bags of saline for setting up an epinephrine infusion according to local hospital protocol and (b) a second line vasoconstrictor (metaraminol or vasopressin)

Management of adverse reactions

Algorithms for management of allergic emergencies have been described by several state- of- the-art documents available [206, 207, 257–259]. Management of excessive or emerging adverse reaction should be prompt, but must be preceded by assessment of the situation and should involve careful clinical assessment of a patient. Any intervention should be tailored to the type and severity of symptoms and vital signs.

1. Local/Mild reactions
   - Allergy skin testing or allergen injection during immunotherapy which are associated with development of local redness, edema and pain can be relieve by local application of cold compresses followed by oral antihistamine. For mild allergy symptoms, such as hay fever or hives, an oral antihistamine may be sufficient. For stuffy nose, decongestant can be given and for itchy, watery eyes, allergy eye drops may be sufficient.
   - Difficulty breathing or wheezing related to e.g. inhaled allergen or oral food challenge should be assessed by measurement of respiratory function (spirometry) and could be relieved by inhalation of 2 puffs of albuterol or other beta2-agonist from an MDI
   - If other symptoms like swollen lips, tongue, tightness in the throat, hoarseness or trouble speaking occur they should be consider as potential developing laryngeal edema and injection of adrenaline should be considered. Similarly, symptoms such as nausea, abdominal pain, vomiting, tachycardia, anxiety or dizziness may herald development of anaphylaxis and should be treated accordingly.
   - The patient even with mild symptoms related to the procedure should be observed continuously and any worsening of symptoms should be assessed as potential signs of anaphylaxis.
2. Severe allergic reactions
   If criteria for a diagnosis of anaphylaxis are met (that is, involvement of two or more organ systems, or the onset of cardiovascular collapse/ hypotension, an appropriate treatment protocol (Table 4) should be initiated. The reaction (although rarely) may not respond to a single intramuscular dose of epinephrine, thus the supervising doctor should be prepared to escalate treatment. Reactions limited to the skin may settle without treatment and/or be managed symptomatically with oral antihistamines. Parenteral antihistamines should generally be avoided as there is no proof of benefit and they may themselves trigger the onset of hypotension. The efficacy of steroids is unknown and so their use is not recommended as a routine.

Summary and recommendations

Diagnosis of allergic disorders may require intentional exposure of patients to potentially allergenic or irritating substances and sometimes involves deliberate induction of allergic symptoms to offending compounds during provocation tests. Intentional application to a sensitized patient of potentially dangerous substances (allergy vaccines) is also a part of routine management of allergic diseases. Unwanted, excessive or even dangerous reactions associated with these procedures can be minimized or even avoided if the procedure is performed in appropriate manner and setting, medical personnel are aware of its potential risk and are prepared to appropriately handle the situation.

Table 4 Standard protocol for anaphylaxis management

1. Initial steps

- Call for assistance
- Give epinephrine 1:1000 at a dose of 0.01 mg/kg IM in the lateral thigh (maximum 0.5 mg)
- Lie patient flat with legs elevated unless this causes increased respiratory distress, in which case the patient may prefer to sit up. However, return to supine position if there is any deterioration in conscious state
- Airway management (according to skills and equipment) if required
- Document a simple systolic BP by palpation (radial/ brachial pulse) and then deflate the cuff to just below systolic pressure as a tourniquet and gain IV access. If equipment is available, start physiological monitoring (ECG, oxygen saturations, 5 minutely noninvasive BP) and give oxygen if severe respiratory distress and/or hypotension.

If the patient is hypotensive, also:

b. Give IV normal saline bolus 20 mL/kg

c. Gain additional wide bore IV access (14G or 16G in adults) and prepare to give additional fluid and/or adrenaline infusion if the patient does not respond to initial management

For upper airway obstruction/stridor, also:

d. Continuous nebulization of epinephrine (5 mL of 1mg/ml)

2. If there is inadequate response, an immediate life-threatening situation or deterioration

- Repeat IM epinephrine injection every 3–5 min as needed or start an IV epinephrine infusion as per hospital guidelines/protocol. Monitor BP closely. Nausea, vomiting, shaking, tachycardia or arrhythmias in the setting of normal or raised BP is likely to represent adrenaline toxicity rather than worsening anaphylaxis

If the patient remains hypotensive, also:

- Further N/saline fluid boluses (up to 50 mL/kg in total) may be required in the first 20 min
- In the hospital setting, consider adding a selective vasoconstrictor (see Table 1).

When indicated at any time, prepare to initiate cardiopulmonary resuscitation (CPR) including standard IV adrenaline dosing if the patient goes into cardiac arrest. Prolonged CPR is indicated because the arrest is usually sudden (no preceding hypoxia) and potentially reversible

3. Disposition

- Consider to use systemic corticosteroids to prevent potential late phase reaction

Severe reactions should be monitored for a minimum 4 h after the last dose of adrenaline

Following review of available literature the group of WAO allergy experts, representing various continents and areas of allergy expertise, reports on risk associated with diagnostic and therapeutic procedures in allergy practice. Based on known/expected risk and taking into account existing allergy guidelines/recommendations a set of safety requirements for performing allergy procedures have been proposed. The consensus on safety requirements for performing specific procedures recommends appropriate qualifications of personnel, optimal setting where the procedure should be performed, necessary availability of safety equipment, access to specialized emergency service and required time of medical supervision. The group proposes also general recommendations which should be followed in allergy practice, regardless of the type of diagnostic/therapeutic procedure.

The general recommendations include:

1. Procedures for the diagnosis and treatment of allergic diseases should be performed by medical personnel (physician/nurse/technician) fully aware of risks associated with the procedure and trained in the recognition and management of allergic emergencies, including anaphylaxis.
2. Some procedures can be performed by trained nurse/technician, but always under close supervision of the allergist.
3. Although most procedures can be done in both outpatient and hospital settings availability of appropriate rescue service should be secured.
4. Basic emergency equipment and rescue medications should be available on site during each allergy procedure.
5. Depending on the type of procedure emergency staff (ICU) should be available on site or should be reached within a specified time.
6. Before a procedure is initiated, contra-indications should be considered and risk/benefit ratio for each procedure should be assessed.
7. The patient should receive full information on the purpose and potential adverse effects associated with each procedure and for some procedures should be asked to sign an informed consent.
8. If anaphylaxis or severe reactions are likely, intravenous access should be secured before the procedure is started.
9. Continuous monitoring of patient by authorized personnel during is the procedure necessary to secure safety of performed procedure.
10. After the procedure is completed the patient should remain under close supervision for a specified period of time.
11. Before the patient is released, she or he should be provided with appropriate instruction in how to handle potential adverse symptoms and what to do in case of an emergency.
12. Medical personnel who have asthma or had a prior reaction to a testing agent should take precautions to minimize exposure (adequate ventilation, exhalation filters, hoods or closed chambers) or avoid performing these tests.

Guidelines for the use and interpretation of diagnostic methods in adult food allergy

Abstract

Food allergy has an increasing prevalence in the general population and in Italy concerns 8 % of people with allergies. The spectrum of its clinical manifestations ranges from mild symptoms up to potentially fatal anaphylactic shock. A number of patients can be diagnosed easily by the use of first- and second-level procedures (history, skin tests and allergen specific IgE). Patients with complex presentation, such as multiple sensitizations and pollen-food syndromes, frequently require a third-level approach including molecular diagnostics, which enables the design of a component-resolved sensitization profile for each patient. The use of such techniques involves specialists' and experts' skills on the issue to appropriately meet the diagnostic and therapeutic needs of patients. Particularly, educational programs for allergists on the use and interpretation of molecular diagnostics are needed.

Keywords: Food allergy diagnosis, Skin prick test, Molecular allergens, Molecular-based diagnosis, Challenge test, Basophil activation test

Background

Food Allergy (FA) is an increasingly recognized problem in relation to its prevalence in the general population. In Italy, it corresponds to 8 % of all patients with allergies [1–3] and the broad spectrum of its clinical manifestations, ranging from mild symptoms up to potentially fatal anaphylactic shock (Table 1). FA significantly affects the quality of life of patients and their families [4]. In adults, FA may persist from childhood or may develop at an older age. In the latter case, once established, FA is maintained throughout life, while paediatric FA frequently disappears during adolescence. FA may be responsible for signs and symptoms that occur shortly after consumption of the culprit food (from a few minutes to a few hours). The earlier they arise, the more serious they are. At times, symptoms appear after physical exercise (food dependent exercise induced anaphylaxis, FDEIA) and the ingestion (about 3 h before) of a specific food, which is safely eaten in the absence of exercise [5].

FA most commonly affects the skin (atopic dermatitis, urticaria, angioedema, eczema and various skin rashes) [6, 7]. Frequently, gastrointestinal manifestations are associated with cutaneous symptoms. The gut is rarely the only organ affected by food allergy. Symptoms range from dyspepsia and meteorism to colic, diarrhoea (rarely constipation), vomiting, gastroesophageal reflux, up to the most complex malabsorption syndromes, generally due to the infiltration of inflammatory cells in the gastrointestinal mucosa [8–10]. In some cases, mainly in pollen-allergic patients sensitive to molecules homologous to those contained in specific foods, symptoms appear in the form of itching and burning of the oral mucosa, papules or vesicles in the mouth, swelling of the lips and difficulty in swallowing, being defined as oral allergy syndrome [11]. Rhinitis, conjunctivitis, asthma and laryngeal edema are all possible FA manifestations independent from sensitization to inhalant allergens [12].

Each year 4–5/100,000 patients experience an anaphylactic shock, with a cumulative risk equal to 0.5–2 % [13].

Table 1 Main food allergy symptoms

Organs and systems	Clinical manifestations
Respiratory	Oculorhinitis
	Bronchial asthma
	Oedema of the glottis
Skin and subcutaneous tissue	Erythematous rash
	Itching without rash
	Urticaria-angioedema
	Atopic dermatitis
	Eczema
Gastro-enteric	Oral Allergy Syndrome
	Abdominal pains
	Vomiting
	Diarrhoea
Cardiovascular system	Hypotension
	Cardiac arrest
	Anaphylactic shock

Foods are the main cause of anaphylactic shock for children and young adults, whereas for older people, insect stings are mainly responsible. This syndrome is due to the involvement of the cardiovascular system with a drop in blood pressure due to vasodilation and leakage of fluids from the circulation, with systemic consequences [14]. The term anaphylaxis (without shock) is referred to a reaction involving multiple organs, usually the skin, gastrointestinal tract and respiratory system.

There is no consensus on allergy due to food contaminant and additives. Clinicians sometimes report the disappearance of the characteristic symptoms of food allergy after an additive-free diet, despite the fact that there is no scientific evidence on their actual role in causing symptoms [15–17]. In any case, reactions are not mediated by an immunological mechanism and are classified as non-allergic hypersensitivity reactions. There is a possibility that food reactions also stem from some non-protein food component or from other mechanisms, for example, cell-mediated mechanisms. These include reactions to orally ingested nickel, the so-called Systemic Nickel Allergy Syndrome, which is characterized by the appearance of gastrointestinal symptoms (typically meteorism, colic and diarrhoea) and skin manifestations (eczema, urticaria and angioedema) in sites without nickel contact in patients with nickel contact dermatitis, and responds positively to a low-nickel diet [18].

Diagnostic efforts are directed to the identification of the food(s) involved in triggering and/or maintaining the symptoms. This can be achieved by using all available diagnostic methods applied in an appropriate sequence, avoiding non-standardized ones.

The purpose of this document is to define guidelines for the use and interpretation of scientifically validated and recognized diagnostic methods for food allergy.

Basic concepts of FA

Primary forms of FA are due to a sensitization process caused by ingestion. In the secondary forms, the patient is sensitized by inhalation to allergens containing molecules homologous to those contained in certain foods, whose ingestion may cause symptoms usually in the oral cavity, in the frame of an oral allergy syndrome [19]. Several molecules with different characteristics act as food allergens. Some of them are stable, enduring heating, cooking, storage and digestion (linear epitopes), while others are less stable (conformational epitopes) losing their allergenicity in cooking and preservation [20]. The patient with FA can be sensitized to both labile and stable components. The stability/lability to physical agents (heat, gastric pH, enzymes like protease, pepsin and so on) is a requisite for an allergen to interact with the IgE antibody. Thus, a component sensitive to heat will be virtually absent in a cooked food, while a determinant resistant to heat, pH and peptidase (for example, Lipid Transfer proteins–LTP) will reach the bowel practically intact despite cooking and passage through the gastric and pancreatic digestion [21]. A particular situation arises with the use of antacid drugs that do not allow (or partially allow) the denaturation of acid sensitive proteins, thus resulting in unpredictable symptoms [22]. Other substances can act as "co-factors", increasing the likelihood of anaphylaxis from food allergens. They include alcohol, non-steroidal anti-inflammatory drugs (NSAIDs), hormonal influences, bacterial or viral or parasitic infections [23] and chemicals [24–26].

The large variety of clinical manifestations (Table 1) and the complexity of allergens often make the diagnosis of FA difficult. A component-specific profile, other than extract-specific, should be used for an optimal definition of the sensitization. In addition, in the specific field of food allergy, it is crucial to discriminate between cross-reactions and co-sensitization, particularly for members of the plant kingdom ("pollen food allergy"), and to more accurately estimate the risk of severe reactions. The sensitization to cross-reactive molecules is relatively rare in childhood but tends to appear during adolescence and remains stable in adults. The recent adoption of individual allergenic molecules (Molecular-Based Diagnosis, MBD) in diagnosis allows for the definition of a more precise IgE profile for the patient, e.g. adding prognostic information related to the possible risk profile of the reaction. Understanding the fine relationships between the results of in vivo and in vitro tests and the

patient's clinical picture is the key for any further clinical decisions.

Diagnostic methods for food allergy

A correct diagnosis of FA requires an established diagnostic procedure. The first step is always the patient's history aimed at identifying the suspected relevant food(s) and the relationship between the ingestion of a specific food and the occurrence of symptoms. Then, the dependence of clinical manifestations from an immune mediated reaction must be assessed. This can be done by both in vivo and in vitro tests.

The standardized diagnostic methods are classified into first, second and third level.

First-level methods

Medical history

Medical history is essential in every field of medicine because it allows one to obtain all the information and data that can help to move towards the diagnosis of a certain disease. It comprises the physiological, family (investigating all possible genetic risk factors or any family predisposition) and the past and current medical history. The latter investigates the disorder for which the patient consulted a doctor. In suspected FA, repeated clinical manifestations related to the ingestion of given meals are highly indicative. Medical history should be addressed to clarify (a) the presence or absence of similar symptoms in other people when they consumed the same food(s); (b) the ingested food(s) in the 2–4 h before the onset of symptoms; (c) the allergens that may contaminate food preparation (for example, casein, latex, ovomucoid); (d) the cooking and storage of food; (e) the presence of triggering factors; (f) the existence of other allergies (such as respiratory or skin allergy) or other diseases [27]. A correct diagnostic approach also requires a complete physical examination. When too much time has elapsed from the appearance of symptoms, it could be difficult to identify the offending food, in particular, if the allergen is not easily identifiable or "hidden". Medical history can be re-evaluated starting from the results of in vitro and in vivo tests, which could demonstrate sensitizations to foods that were initially not considered [28, 29].

Skin tests

Skin prick test (SPT) The SPT is a well-standardized, simple, cheap and low-risk diagnostic test. It should be the first step performed and both inhalant and food allergens should be tested. Table 2 shows a panel of food allergens to be tested, supplemented, where appropriate, by foods chosen according to patient's history and dietary diaries, and Table 3 shows the technical procedure to be used. The SPTs to foods have a low specificity with a low positive predictive value. Thus, a positive result, unless confirmed by the clinical data, does not allow for a definitive diagnosis of FA [28–30]. In children, cut-off values for the SPT reaction diameter for certain food allergen (milk: 8 mm, egg: 7 mm, peanut: 8 mm) have been identified but are not universally acknowledged. However, oral food challenges were always positive (100 % specificity) in children with cutaneous reactions of this diameter or above [31, 32]. In general, SPT have an excellent sensitivity with high negative predictive value (>90 %), thus a negative result generally rules out the possibility of an IgE-mediated sensitization. However, this is true only for foods containing stable proteins, such as casein from cow's milk, egg ovomucoid, albumin and peanut vicilins, which are well represented in the extract. The SPT performed with allergenic extracts containing thermolabile molecules, such as pathogenesis-related-10 (PR-10) proteins have a low negative predictive value. For these allergens, the prick + prick (P + P) procedures with fresh foods can be useful.

The major limitations of allergen extracts for SPT are represented by (a) the content, because each extract is a heterogeneous mixture composed of major and minor allergenic proteins, and other biologically inactive components such as non-allergenic proteins, glycoproteins and carbohydrates, (b) the production process, because some allergens may undergo partial degradation during the extraction, (c) the cross-reactions, as different biological sources may contain cross-reactive allergens.

An in vivo MBD approach (available in vitro for many molecules, shown in Table 4) is also possible with extracts containing high concentrations of LTP (a gastro- and thermo-stable protein from *Rosaceae*) and palm profilins (Pho d 2, an ubiquitous gastro- and heat labile plant protein). Their use, to complete the diagnostics performed with extracts from whole sources, allow for a

Table 2 Food panel for Prick test

Egg	Peach
Peanut	Apple
Beta-lactoglobulin	Cod
Banana	Hazelnut
Carrot	Walnut
Casein	Fish
Bean	Pea
Wheat flour	Chicken
Shrimp	Tomato
Lactalbumin	Rice
Pork	Celery
Corn	Soybeans
Almond	Egg yolk

Table 3 Technical procedure for SPT

Apply one drop for each allergen extract to be tested, maintaining a minimum distance of 3 cm between drops on the volar part of the patient's forearm (5 cm from wrist and 3 cm from the antecubital fossa)
Apply pressure, through a sterile, disposable lancet, to each single allergen, pricking to a depth of 1 mm for each drop, perpendicular to the skin's surface
Hold for about 3 s with moderate pressure without moving the hand or turning to avoid bleeding
Carefully remove the allergen solution with blotting paper
The same procedure is to be followed to test histamine (10 mg/ml) as a positive control and physiological glycerine as a negative control
Reading of the results: after 15 min from the performance of the test
Interpretation of the test: a positive result is defined by the appearance of a wheal of at least 3 mm in average diameter. Responses to histamine and the negative control should be carefully considered. The latter verifies that the patient does not suffer from dermographism and the former demonstrates a "normal response" to histamine (with no negative interference from drugs or other conditions, such as hypo-reactivity of the skin)

Table 4 Native or recombinant molecules available for SPT

Molecule	Source
Lactalbumin	Cow's milk
Beta-lactoglobulin	Cow's milk
Casein	Cow's milk
Ovalbumin	Egg white
Ovomucoid	Egg white
LTP (Pru p 3)	Peach
Profilin (Pho d 2)	Palm tree
PR10 (Mal d 1)	Apple (not available in Italy)

more precise assessment of the ingestion risk of the suspected food [33].

Prick + prick (P + P) P + P is performed with fresh food, in particular vegetables, when the commercial extract is negative (or unavailable) but the clinical history is suggestive. When the food is solid, the technique involves firstly puncturing the fresh food (some allergens are located just under the skin of the fruit) and then the patient's skin with a lancet according to the SPT standard procedure [34]. When the food is liquid, the technique is the same as in SPT.

P + P has a good diagnostic reliability [35] with high predictive negative values. In the case of a positive result, it must be always taken into account that some foods are rich in histamine and lectins and can produce false positives. Obviously, the use of skin P + P with fresh food is not entirely risk-free and highly sensitive subjects may suffer systemic adverse reactions [36].

Atopy patch test (APT)

The APT is performed through the same technique used for common patch testing to identify the responsible hapten in contact dermatitis, and is aimed at assessing the delayed cell-mediated hypersensitivity to foods that may especially occur in children with atopic dermatitis or gastrointestinal reactions to foods. In 2010, the APT was considered an emerging test, like BAT and MBD [37], but subsequent studies did not confirm its diagnostic role to be as important as the other two techniques.

Second-level methods

In vitro assays for total serum IgE (tIgE) and specific IgE (sIgE) to foods

Like SPT and P + P, in vitro tests only certify a sensitization and the interpretation of results is the allergist's task. Thus, measuring tIgE may be useful in grading allergy conditions, but only when used in combination with other tests. Indeed, tIgE alone has no predictive value in relation to the diagnosis of FA. The assay of sIgE for food extracts is a second-level test in the view of costs. Thus, it should be requested only after skin tests. However, it may still be exceptionally considered a first-level test in those conditions in which SPT cannot be performed (e.g. very young paediatric patients, concomitant antihistamine therapy or skin alterations, risk of systemic reactions). Importantly, an in vivo test is able to detect the biological effects (revealed by wheal, redness, itching, etc.) caused by the presence of sIgE bound to skin mast cells, while the serum test only detects the presence of circulating IgE specific to a particular allergen. It is therefore possible that the results of the two tests are different [38].

Nowadays, quantitative methods with extracts have levels of sensitivity (and negative predictive values) comparable to APT, with high specificity and positive predictive value [37]. The test is suitable to detect the IgE specific for a given allergen, in a quantitative way, in a range between 0.10 and 100 kU/L. As for the SPT reaction diameter, specific IgE levels exceeding a certain value ("diagnostic cut-off") showed a predictive value of 95 % for a symptomatic allergy [32, 39] (Table 5). Thus, in the presence of a compatible clinical history, sIgE can confirm the diagnosis of FA without requiring further challenge tests. However, the predictive values vary from one study to another. The results are influenced by many variables such as the patient's age, duration of food allergen avoidance at the time of testing, selection of patients

Table 5 Sensitivity, specificity, positive (PPV) and negative (NPV) predictive value of tests for the detection of specific IgE in vitro for the most common food allergens

Allergen	Sensitivity (%)	Specificity (%)	PPV (%)	VPN (%)	Diagnostic cut-off (kUA/l)
Egg	61	95	98	38	6
Milk	57	94	95	53	15
Peanut	57	100	100	36	14
Codfish	63	91	56	93	3
Soybean	44	94	73	82	30
Wheat	61	92	74	87	26

and clinical disorders, and have been validated on non-European test subjects. It is also important to stress that the values of specific IgE <0.10 kU/L does not exclude the possibility of an IgE-mediated allergic reaction and that the confirmation of a negative test, in the case of strong clinical suspicion, can only be achieved with negative SPTs and negative challenge tests.

In vitro MBD

Diagnostics based on allergenic extracts allow for the identification of the allergen source (e.g. fish, egg, milk, etc.) but not the molecular component to which a patient is sensitized, which can be studied instead through in vitro MBD and therefore used to improve the result of a sIgE test [20]. In vitro MBD uses molecular allergens isolated from a given allergen source (purified or native allergens) or produced by recombinant DNA technology (recombinant allergens) (Tables 6, 7). This approach improves the description of the IgE repertoire against food allergens or their molecular components and explains cross-reactions and their role in FA.

The MBD approach should be used to distinguish patients with genuine sensitization towards a food (with high risk of accidental ingestion) from those with co-sensitization, i.e. sensitization to ubiquitous proteins present in pollen (which act as primary sensitizing) and also common in food (with a much lower risk of adverse reaction). Again, it is possible to identify patients characterized by sensitization to food independently by a sensitization to aeroallergens (primary sensitization) and patients with a "pollen-food syndrome", where the first sensitization occurs via inhalation and the great homology between the allergen of the "first sensitizer" and some food allergens is responsible for the patient's symptoms presenting as an oral allergy syndrome [40, 41].

Identifying cross-reactions is a further benefit of MBD. The allergist is able to understand whether a single, a few closely related or several widely different food allergen sources should be considered in a dietary approach. The allergist will also be able to assess the risk of a given FA identifying, by in vitro MBD, patients sensitized to "relatively harmless" or potentially very dangerous components [20] that need the prescription of life-saving drugs such as auto-injectable adrenaline together with a strict allergen avoidance. The use of MBD requires allergists to acquire new skills.

Primarily, they need to learn the new allergen nomenclature [42, 43]. International classification ranks the allergenic source first by its scientific name, from which it takes the first three letters of the generic name and the first letter of the species (or two letters when confusion is possible): e.g. apple is scientifically called "*Malus domestica*": therefore Mal d indicates the allergen source. Adding a number (1, 2, 3 etc.) indicating the chronological order of the identification allows for the classification any allergenic molecules: e.g. for apple the identified molecules are named Mal d 1, Mal d2, Mal d 3, and Mal d 4.

It is also important to know the molecular allergenic content of foods. Some molecules are specific for a given food, allowing the identification of the primary sensitizer, others share common epitopes (antibody binding sites) and the same IgE can induce an immune response to allergenic molecules with similar structures from different allergen sources [33]. In the example of apple, Mal d 3 is an LTP molecule homologous to the LTP of peach, nuts, apricot, cherry, etc. and an exclusion diet should prohibit all these foods, but only according to the patient's history [44]. Indeed, Mal d 1 is highly homologous to the birch pollen allergen Bet v 1 and characteristically induces an oral allergic reaction [45].

The molecular structure and physiochemical properties of allergens are major determinants of their clinical relevance. For example, LTPs are particularly resistant to high temperature and enzymatic degradation, so cooking and digestive processes are unable to deactivate their allergenic capacity. For this reason, LTP exposure through the gastrointestinal tract may induce sensitization in predisposed individuals and may trigger severe reactions in sensitized patients [46]. The specific patient's sensitization profile is relevant in terms of risk assessment. In

Table 6 Major food allergens and components available for molecular diagnostics using ImmunoCAP (or ImmunoCAP ISAC)

Allergens (or allergen source)	Protein family
Cupin superfamily	
Vicilins	Ara h 1 (peanut)
Legumins	Ara h 3 (peanut), Cor 9 (hazelnut)
Prolamin superfamily	
2S albumin	Ber e 1 (brazil nut), Ara h 2 (peanut), Gly m 6
Lipid transfer protein (LTP)	Pru p 3 (peach), Cor 8 (hazelnut), Art v 3 (*Composite*) Jug r 3 (walnut)
Cereal prolamines	Tri 19 (wheat) Tri a 14
Pathogenesis-related (PR) proteins	
PR10: intracellular proteins	Pru p 1 (peach), Api g 1 (celery), Gly m 4 (soy)
PR3: chitinase Class 1	Hev b 11, Hev b 2.6 (latex, banana, avocado)
Profilins	Pru p 4 (peach) (Bet v 2, Phl p 12, Hev b 8)
Cross-reactive carbohydrate determinants	MUXF3 (celery, tomato)
Tropomyosins	Pen a 1 (shrimp)
Calcium binding proteins	
Parvalbumin	Gad c 1 (codfish)
Milk proteins	Bos d 4 (α-albumin), Bos d 5 (β-lactoglobulin), Bos d 8 (casein), Bos d lactoferrin (lactoferrin)
Egg protein	Gal d 1 (ovomucoide)
	Gal d 2 (ovalbumin)
	Gal d 3 (conalbumin)
	Gal d 4 (lysozyme)

Table 7 Families of protein carbohydrate molecules mainly involved in food allergy

Molecules associated with allergy to food source (or source allergen)
PR-10 proteins (homologous to Bet v 1)
Non-specific lipid transfer proteins (nsLTP)
Profilins
Storage proteins
Thaumatin-like-proteins (TLP)
Cross-reactive carbohydrate determinants (CCD)
Molecules associated with allergy to food of animal origin
Tropomyosins
Parvalbumins
Caseins
Lipocalin, Family of lysozyme, Family Transferrins, Ovomucoids

fact, the simultaneous sensitization to peach LTP Pru p 3, Pru p 1 and Pru p 4 in the same patient seems to exert a protective role in comparison with Pru p 3 sensitization alone, as it is associated with less severe symptoms [47]. Similarly, it has been recently shown that in peach-allergic patients with tomato hypersensitivity, sensitization to rPru p3 seems to be a surrogate biochemical marker for a severe tomato allergy, whereas the presence of anti-rPru p 1 IgE may be an indicator of a mild tomato allergy [48].

Profilins are pan-allergens (present in many plant species not botanically related) protease sensitive and less heat sensitive that mainly induce an oral allergy syndrome, while severe reactions are rare [49].

Therefore, the allergist approaching the MBD should know the chemical, physical and immunological characteristics of all allergenic families, their biodegradability, cooking/heat resistance/sensibility etc. The stability/lability of a molecule (along with the clinical history) helps the clinician to evaluate the risk of systemic versus local reactions. Stable allergens are generally associated with severe systemic reactions, whereas labile allergens are associated with low/mild reactions and cooked food is often tolerated.

Moreover, it is essential to know to which family the various molecules belong and their structural similarity within the family (generally characterized by a greater than 50–70 % sequence homology).

In the above-mentioned example of apple, MBD can distinguish between fruit allergy due to LTP sensitization and a pollen-related apple allergy. Sensitization to Mal d 3 (an LTP protein) indicates a fruit allergy where peach is often the primary sensitizer [50, 51]. Sensitization to Mal d 1 (a PR-10 protein) is seen in birch-pollen allergic patients and is caused by cross-reactivity with the main birch allergen Bet v 1 [52, 53]. The presence of IgE antibodies to profilin (e.g. Mal d 4, homologous of Phl p 12) is indicative of an apple allergy related to a grass-pollen sensitization [53, 54].

Patients with IgE antibodies to Mal d 2 and 3 (LTP stable proteins) are at higher risk of developing systemic reactions. IgE antibodies to Mal d 1 and/or profilin and not to Mal d 2 and 3 suggest that predominantly local oral symptoms may occur. Apple-allergic patients sensitized to Mal d 3 may tolerate peeled apples. Apple-allergic patients sensitized to Mal d 1 and/or profilin (that are labile proteins) may often tolerate cooked apples.

MBD is a complex area, but as it provides new and relevant information for the allergist, it will soon become a standard tool for the diagnosis of FA. Educational programs for allergists on the use and interpretation of MBD are needed [55].

In vitro MBD is defined as single or multi-plexed IgE assay microarray. By the single-plexed diagnostics the choice of the components to be tested is relies on the allergist's judgment, based on the patient's sIgE profile. In poly-sensitized patients, a complete recognition of the IgE profile might require a large number of assays. In these cases, it may be reasonable to use the multi-plexed allergen microarray (AMA) that allows for the detection of specific reactivity to over 100 allergen components. The most popular form (the ImmunoSorbent Allergen Chip—ISAC) currently contains inhalants, foods, latex and insect venom. Despite AMA not being a quantitative assay, the correlation between the results of microarrays and the results of sIgE tests are largely super-imposable. Thus AMA is suitable in both paediatric and adult serum samples when the number of molecular components to be tested using single-plexed methods is too high to be cost-convenient or when the need for extensive research of sensitization is required [20]. This is particularly true in highly complex patients presenting symptoms of a cross-sensitization to inhalant and food and clinical evidence of food allergy. AMA is a powerful in vitro test that requires specific expertise but provides a very large amount of information to the allergist.

MBD diagnostics, especially microarray, are expensive compared with traditional tests, unless a single test is considered. Economic considerations may influence the decision of using a single or multiplex approach in individual patients. Using the microarray diagnostics allows for the performance of a broad-spectrum analysis of a patient's IgE profile with a small blood sample. It may reveal unanticipated sensitivities, possibly to potentially harmful molecules, making the interpretation of such sensitization difficult in the case of a clinically silent history, but giving the allergist the chance to investigate other hypersensitivities and to alert the patient towards possible risks. This clearly demonstrates that in vitro diagnostics, including MB, should be evaluated within the framework of a patient's clinical history, since allergen sensitization does not necessarily imply clinical responsiveness.

Third-level methods

Oral provocation test (OPT)

OPTs are the most reliable tests in the diagnosis of clinically relevant IgE associated food allergies once allergen specific IgE has been detected. The OPT remains the "gold standard" to establish or exclude the liability of a particular food in causing an adverse reaction [56–61]. The actual value of this method is its functional result. Indeed, only foods causing a clinical evidence of allergy are considered positive. When first and second-level methods have been unable to indicate the food that is responsible for the symptoms, the clinical relevance of a detected sensitization may also be investigated by a targeted elimination diet to perform before the OPT [28]. Furthermore, if multiple triggers are suspected, the elimination diet can help in selecting food to be tested through the OPT, which remains the most important diagnostic tool in food allergy diagnosis. Once the diagnostic work-up has been concluded, the elimination diet of the culprit food/s usually represents the treatment for known food allergies, as well as educating the patient about proper food preparations and the risks of occult exposure [28, 29]. Ongoing investigations are currently evaluating the role of food immunotherapy as a potential FA therapy, to be performed by highly skilled specialists in appropriate settings [29, 62].

The OPT is a third-level procedure that should be carried out when previous diagnostic levels were unable to give sufficient information for the clinical diagnosis [60]. During the patient follow-up, OPT is useful in detecting an acquired tolerance for the specific food. The functional identification of the causative food allows one to avoid its assumption as well as the establishment of unnecessary rigid diets. Due to the potential risk of severe adverse events, the test has to be performed in a hospital setting with personnel trained in resuscitation procedures and the availability of emergency drugs.

The indications of OPT [61, 63–65] are: (a) to identify the food responsible for acute reactions, or to monitor the unexpected tolerance in case of a history of allergy; (b) to determine the offending food in chronic conditions such as atopic dermatitis or eosinophilic esophagitis; (c) to expand the diet in subjects with multiple dietary restrictions; (d) to establish the degree of tolerance to cross-reactive foods and to establish possible acquisition of a spontaneous tolerance to food.

The contraindications are: (a) previous severe anaphylactic reactions (especially recent); (b) level of specific IgE exceeding the cut-off for which there is a high probability

that the oral test is positive; (c) reactivity to individual molecules identified with the MBD that indicate a possible severe reaction; (d) reactions occurred during the performance of the SPT and (e) a progressive systemic disease, in particular when the patient is taking medications that could mitigate (antihistamines, corticosteroids) or amplify (β-blockers, ACE inhibitors, NSAIDs, etc.) the reaction [63, 64].

The test consists of gradually increasing doses of the appropriately diluted food, starting from the lowest dose and checking the presence of relevant symptoms. The test can be performed in three different settings [63]:

Open OPT is used for immediate reactions when the risk of severe reaction is reduced. It can be performed on an outpatient basis with a simplified protocol of administration and an observation time of about 2 h. It can be strongly influenced by the age and by the subject's psychological behaviour. If negative, the food can be reintroduced into the diet. In the case of suspected positive reaction, it should be checked in a double-blind OPT setting.

Single-blind placebo OPT (SBP-OPT) it consists of two sessions, one with placebo and one with the suspected food. When a strong psychological component is suspected, the placebo should be tested first. The patient undergoing the SPB-OPT is informed that the food may or may not be present in the administered dose. If the answer is negative or positive symptoms are observed, it is not necessary to continue the investigation. Repeated sessions with placebo or suspected food are useful for the confirmation of vague symptoms. In the case of positivity with placebo, a DBP-OPT will be necessary. In the case of a negative result, the tolerated food must be ingested in its natural form 2 h after or on the day after the test. The tolerance should be checked with follow-up.

Double–blind placebo controlled test (DBPCT) the gold standard. The foods to be tested are prepared by professional personnel not involved in the clinical examination. Placebo and food must have a very similar look and taste. Only when the test is completed can the doctor and patient know the pattern of administration and discuss the results [66].

Fourth-level methods

Basophil activation test (BAT)

The BAT can be used in the study of IgE (and non-IgE) mediated allergic reactions [67, 68]. The rationale of this test is the change in the phenotype of activated basophils after in vitro incubation of the patient's whole blood with the allergen. The BAT is a useful complementary tool to the in vitro diagnosis of FA caused by milk, egg, peanut and wheat [69, 70] when IgE may be involved. Interestingly, a recent study of 20 peanut-allergic children showed that when basophils were stimulated with decreasing doses of allergens until threshold sensitivity was reached, 19 were negative to peanut but 17 were positive to rAra h 8, suggesting that the children sensitized to Ara h 8 but not peanut storage proteins may be at risk of systemic allergic reactions, especially when eating large amounts of peanuts [70].

Reactions unrelated to IgE may also be assessed by BAT, as evidenced for wine and beef [71, 72]. Recently it was also used in the decision-making process for the reintroduction of milk in children allergic to casein [73]. Today, BAT is the only assay that mimics, in the test tube, what happens in vivo. After an extensive validation, BAT should distinguish sensitization from a clinical allergy. The method still suffers some critical issues that can make it a routine test only in specialized laboratories (Fig. 1).

Complementary alternative tests

Frequently, patients undergo complementary/alternative tests after a negative response to a common diagnostic work-up or when non specific symptoms predominate (e.g. migraine, abdominal discomfort, chronic urticaria or other skin abnormalities, chronic fatigue, weight gain or lack of success in weight loss diets), which are erroneously classified as "food allergy" [74]. It represents a common diagnostic label suggested by physicians without specific expertise in the field of FA mechanisms and food-related disorders [75, 76].

The most common (not validated) alternative diagnostic techniques are:

In vivo:

- Electrodermal tests: they measure the change in the skin's electrical conductance once the subject has been exposed to an allergenic substance through specific devices [75].
- Kinesiology: it registers the decreased strength of muscular contraction related to contact with an allergen [75].
- Provocation/neutralization testing: it identifies the onset of "untoward effects" provoked by the administration (intradermal or sublingual) of allergenic substances [76]. The same technique is used as a therapeutic tool.

In vitro:

- Leukocytotoxic tests: they detect the shape/volume abnormalities of peripheral leukocytes when an allergen in a solid and not measurable phase comes into contact with them [77].

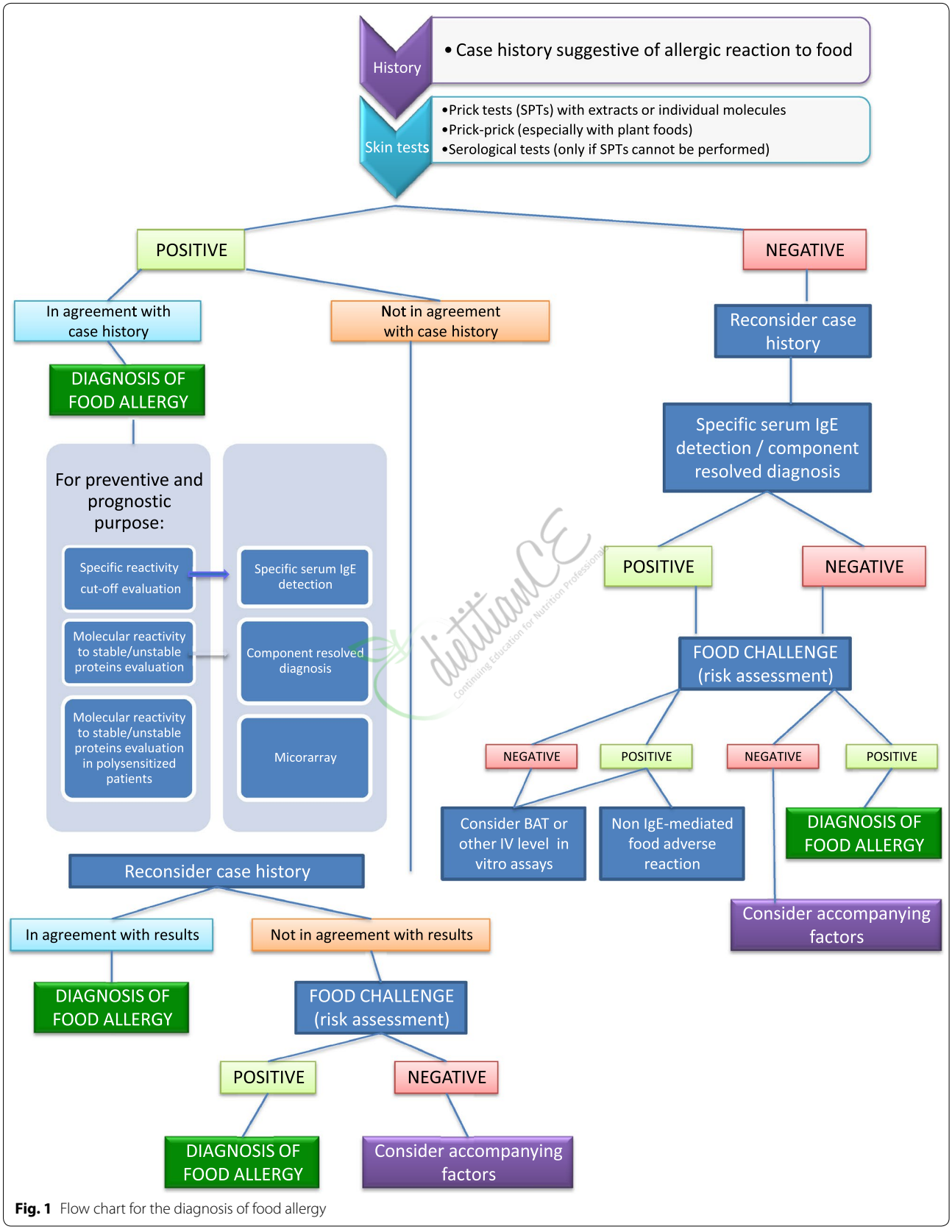

Fig. 1 Flow chart for the diagnosis of food allergy

A direct comparison between such tests and gold standard methods has so far failed in all cases to demonstrate their validity [78]. Their use is strongly discouraged.

Conclusion

The diagnosis of FA is an integrated procedure that can be carried out in different steps (Fig. 1). Some patients can be diagnosed easily by the use of first- and second-level tests, while complex patients, with poly-sensitization and pollen-food syndromes, frequently require a third-level approach. In recent years, the diagnostic assays for FA have been significantly expanded and standardized tools and procedures are now available to the allergist.

Currently, demanding issues are related to FA diagnosis: (1) Identified food(s) should be excluded from the diet; (2) the patient must be properly informed about the relative risk of ingesting the sensitizing foods, even inadvertently as hidden foods in different preparations; (3) the allergist should explain all preventive and curative measures to be taken in case of allergic reactions, including potential medical urgency. In particular, the patient must be informed of the possibility that certain concurrent conditions could favour the onset of FA. This involves a great deal of renewed research specialists and experts on the subject to be able to respond appropriately to the diagnostic and therapeutic needs of patients.

The soft computing-based approach to investigate allergic diseases: a systematic review

Abstract

Background: Early recognition of inflammatory markers and their relation to asthma, adverse drug reactions, allergic rhinitis, atopic dermatitis and other allergic diseases is an important goal in allergy. The vast majority of studies in the literature are based on classic statistical methods; however, developments in computational techniques such as soft computing-based approaches hold new promise in this field.

Objective: The aim of this manuscript is to systematically review the main soft computing-based techniques such as artificial neural networks, support vector machines, bayesian networks and fuzzy logic to investigate their performances in the field of allergic diseases.

Methods: The review was conducted following PRISMA guidelines and the protocol was registered within PROSPERO database (CRD42016038894). The research was performed on PubMed and ScienceDirect, covering the period starting from September 1, 1990 through April 19, 2016.

Results: The review included 27 studies related to allergic diseases and soft computing performances. We observed promising results with an overall accuracy of 86.5%, mainly focused on asthmatic disease. The review reveals that soft computing-based approaches are suitable for big data analysis and can be very powerful, especially when dealing with uncertainty and poorly characterized parameters. Furthermore, they can provide valuable support in case of lack of data and entangled cause–effect relationships, which make it difficult to assess the evolution of disease.

Conclusions: Although most works deal with asthma, we believe the soft computing approach could be a real breakthrough and foster new insights into other allergic diseases as well.

Keywords: Allergy, Artificial intelligence, Artificial neural networks, Asthma, Fuzzy logic

Background

Recent advances in healthcare innovation have challenged us to think about the pioneering potential of big data coming from the digital world to invade the medical field. Big data introduces the exciting technological ability to digitize human beings in order to achieve a real personalization of medicine. Soft computing (SC) methods possess the extraordinary ability to exploit meaningful relationships of digital big data, making them suitable for the diagnosis, treatment and prediction of the outcome in many clinical scenarios. In the field of allergy these methods may be extremely useful to obtain important data and information on the characteristics and the management of many allergic diseases. Existing literature on the relationship between SC models and allergic diseases will be presented and discussed in this paper, highlighting the novel perspectives of this pioneering approach.

Soft computing methods

SC is a branch of computer science introduced in the early 1990s [1]. It includes a collection of techniques that resemble biological processes more closely than

Table 1 Main soft computing uses in medicine

Main applications
Classification and prediction of disease categories
Diagnosis and prognosis
Medical decision-making processes
Physiological signal analysis
Epidemiological studies
Genetic association studies
Pharmacokinetics
Imaging
Geo-spatial distribution of diseases

traditional methodologies. SC deals with approximate reasoning, imprecision and uncertainty in order to achieve robustness and low-cost solutions for complex data analysis. This approach could excel in modern medicine, where the analysis and application of a large amount of knowledge are necessary to solve complex clinical problems, which in most cases are not deterministic. Table 1 lists the most important fields of application of SC methodologies in medicine.

SC models encompass automatic computing procedures, without human intervention, and are able to learn a task from a series of training examples. Moreover, they aim to generate sufficient output simple enough to be easily understood by the humans. Differently, classic statistical approaches are generally characterized by having an explicit model of probability, with the assumption that in most cases they require the intervention of an expert with regard to variable selection, transformation and overall structuring of the problem [2]. The general approach of SC modeling data analysis typically consists of four stages as shown in Fig. 1: (i) collection and encoding of clinical data in an electronic form suitable for further processing; (ii) data processing with techniques of feature extraction and dimension reduction (i.e., principal component analysis), selecting the most predictive parameters; (iii) pattern modeling selecting an SC model; (iv) extraction of knowledge by evaluating accuracy, sensitivity and specificity.

In the third step of Fig. 1, the most common SC models considered in this systematic review are shown: artificial neural networks (ANN), support vector machines (SVM), bayesian networks (BN), and fuzzy logic (FL).

Artificial Neural Networks

The ANN is a flexible non-linear model inspired by the brain's interconnections. ANNs possess an adaptable knowledge that is distributed over many neurons and synaptic connections. They are generally based on interconnected nodes (neurons), processing units able to compute input, activation and output functions. Each

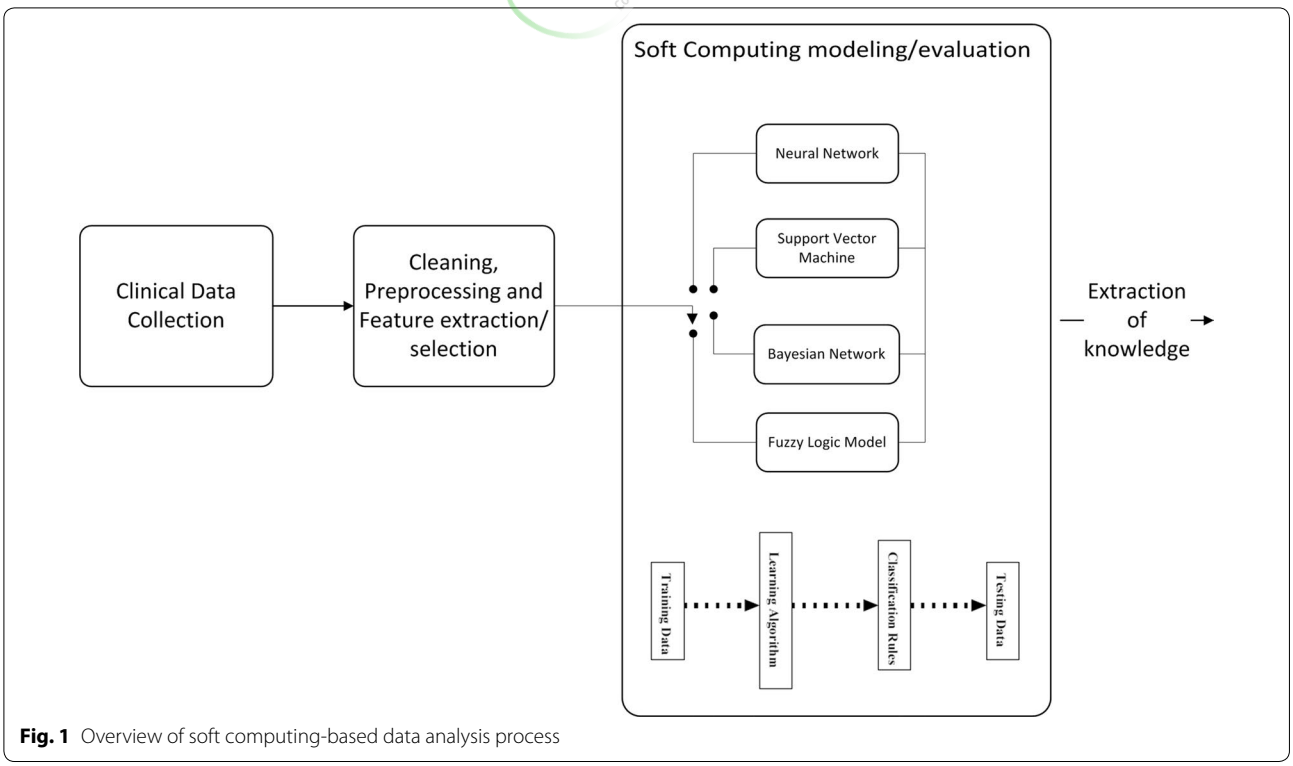

Fig. 1 Overview of soft computing-based data analysis process

connection (synapse strength) is provided by a weight adapted during the learning phase. The most common example of ANN is the multi-layer perceptron (MLP) [3]. The topology of the network is composed by interconnected nodes arranged in multiple layers. In the first layer each node corresponds to each input variable. The layers in the middle (hidden layers) represent the core of the non-linear model while the number of hidden nodes represents the complexity of the network. The relationships among variables are built using a sufficient number of training data and represented as functions using methods such as maximum likelihood estimation, maximum a posteriori or back propagation. The utility of ANN models lies in the fact that they can be used to infer a function from observations (training data). This is particularly useful in applications where the complexity of the data or tasks makes designing such a function by hand impractical. An interesting example of MLP, as shown in Fig. 2, was proposed by Hirsch et al. in 2001 to analyze an enormous amount of surveys to screen a population for asthma [4]. The trained neural network received as input 6825 screening questionnaires and was able to predict a final diagnosis of asthma with an accuracy of 74%.

The support vector machine

The SVM [5] is one of the most common machine learning models able to map the N input variables with a kernel function in an N-dimensional space (N-hyperplanes). The model is based on an algorithm able to find the best separating hyperplanes (maximum-margin hyperplane). Typically, SVM models are used for classification and regression analysis.

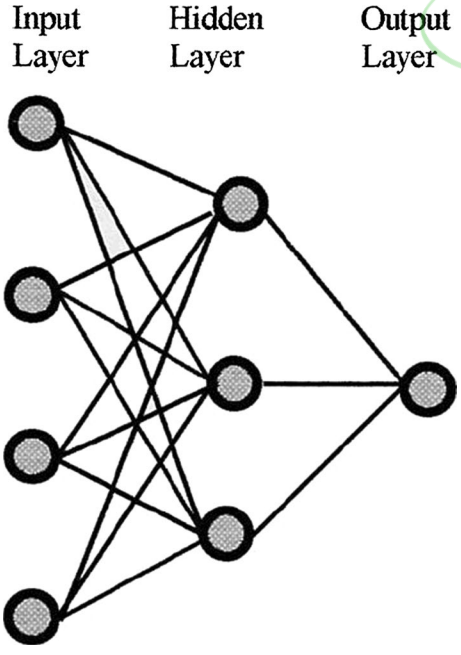

Fig. 2 The topology of multi-layer perceptron neural network to screen a population according to individual likelihood of asthma [4]. It is composed by interconnected nodes structured in three main layers. The input nodes represents questionnaire responses and the single output node represents probability of asthma

The Bayesian networks

The BN are suitable for providing a graphical representation of variables and their complex relationships. BN have the advantage of creating predictive models directly from data. The topology is an acyclic graph in which a set of nodes represents the variables, while the edges between nodes represent the probabilistic relationships between variables. More specifically, a node with an incoming arrow is conditioned by the node from which the arrow originates. Despite traditional regression approaches, BN are more flexible and accurate in small samples if we incorporate correct prior information and advantageous in handling missing data, which is prevalent in the clinical field. Moreover, they are not limited to representing the dependencies of a single outcome variable on predictor variables. Figure 3 reports an example of a BN model for studying asthma severity of Prosperi et al. [6]. The model reported in a tree topology was able to explain the main dependencies between severity and variables such as body mass index (BMI), forced expiratory flow (FEF), inhaled corti-costeroids (ICS), long-acting β_2-agonists (LABA) after a stepwise search in the whole original variable space.

The fuzzy logic model

While ANN, SVM and BN are important examples of SC models based on mathematical structures underlying learning, the FL approach [7] is based on integration of structured human knowledge into workable algorithms. Input and output of FL model are defined, converted to linguistic parameters (fuzzification) and the relationship among variables is generated through a set of rules (inference rules) defined by the experts. Finally, the output is represented by the aggregation of obtained results of input modules, converted into a numerical value (defuzzification) and classified. The FL approach is an alternative to the classic statistical methods where every proposition must either be "true" or "false". Instead, fuzzy logic asserts that things can be simultaneously "true" and "not true", with a certain membership degree to each class. FL techniques are used to deal with uncertainty and can be very powerful when there are poorly characterized parameters. In Fig. 4 an example of FL model provided by Zolnoori et al. to predict the level of asthma controls is reported [8]. The system is composed of 14 variables organized in five modules related to respiratory symptoms severity

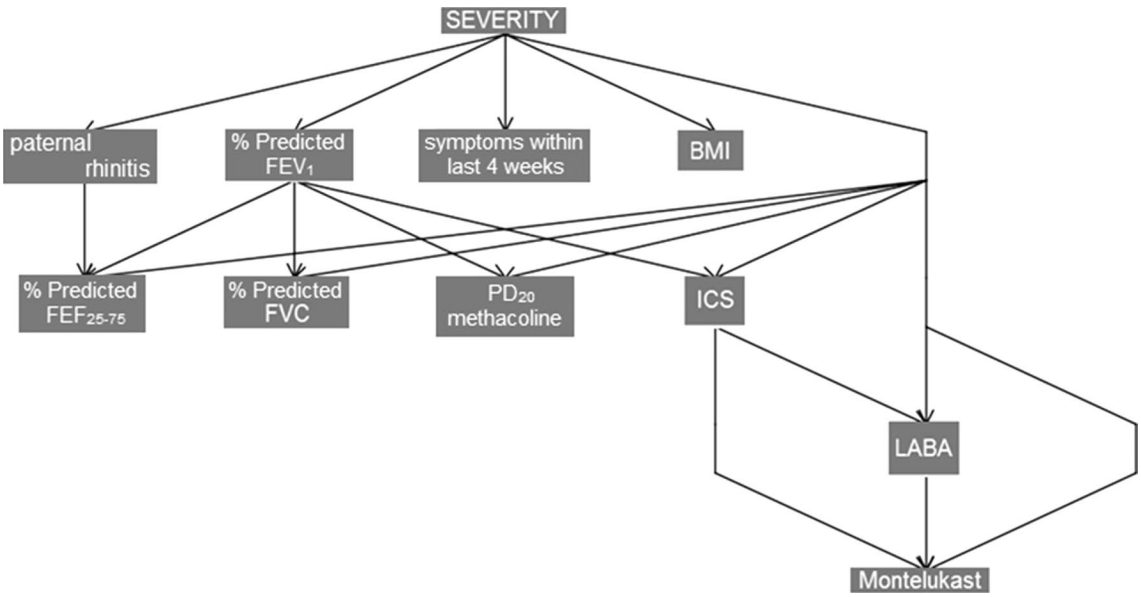

Fig. 3 Bayesian networks model evidences conditional dependencies between severity as diagnosed by the physician and the variable space selected by a stepwise search [6]. *BMI* body mass index, *FEF* forced expiratory flow, *ICS* inhaled corti-costeroids, *LABA* long-acting β_2-agonists

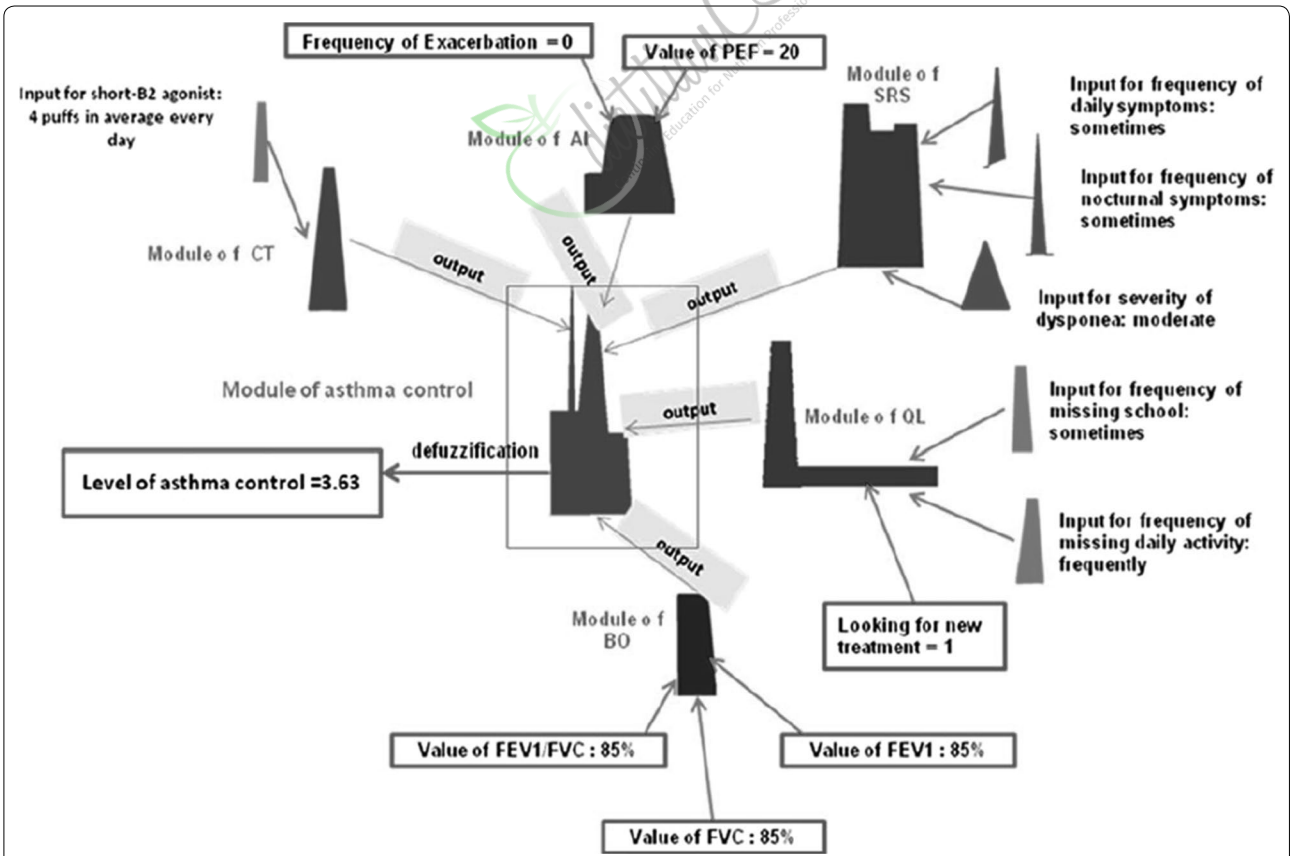

Fig. 4 Schematic view of fuzzy logic model able to combine input variables related to severity of respiratory symptoms (SRS), quality of life (QL), current medical treatment (CT), instability of asthma (AI), bronchial obstruction (BO) to infer the level of asthma control (AC) [8]

(SRS), bronchial obstruction (BO), asthma instability (AI), current treatment (CT), and quality of life (QL). All these variables are represented with fuzzy rules defined by experts and then aggregated in a fuzzy network. The output of the system is given by the degree of asthma control classified in five categories: excellent (0–1), good (1–3), fair (3–5), poor (5–7), and very poor (7–10).

Methods

Literature search

The research was performed on PubMed and ScienceDirect, covering the period starting from September 1, 1990 through April 19, 2016. We explored studies dealing with the most frequently adopted SC models (ANN, SVM, BN, FL) and allergic diseases. Research in PubMed was performed using medical subject headings (MeSH®) to report the most common SC methodologies employed to study the most frequent allergic diseases included under the Mesh term "hypersensitivity". The keywords used to search were based on the following logical linguistic pattern: ("Hypersensitivity"[Mesh]) AND ("Neural Networks [Computer]"[Mesh]) OR ("Support Vector Machines"[Mesh]) OR ("Bayes Theorem"[Mesh]) OR ("Fuzzy Logic"[Mesh]). Instead, the electronic search strategy on ScienceDirect was performed with the following queries: ("asthma" or "adverse drug reactions" or "allergic rhinitis" or "atopic dermatitis" or "allergic conjunctivitis") and ("artificial neural networks" or "support vector machine" or "Bayesian network" or "fuzzy logic").

Inclusion and exclusion criteria

The research was limited to clinical cross-sectional studies and case–control studies of articles published in peer-reviewed journals. Case–study reports, genetic association studies, cost-effectiveness healthcare studies, pollen/climate changes and classification of respiratory sounds were discarded from the review.

Study selection

The research was conducted independently by two authors, who evaluated whether the information of each reference was relevant or not. Each disagreement between the two reviewers was resolved by discussion until a consensus was reached. If the abstract did not include enough information to evaluate inclusion or exclusion, the full text of publication was reviewed if available. Otherwise, the paper was excluded. The selected papers were sorted by relevance and grouped for each allergic disease (Table 3). In this report, we first review recent findings for SC model-related allergic diseases (summarized in Table 2), evaluating the accuracy, sensitivity and specificity of SC models. We then critically discuss the potential strength and future implications for research in this field.

Results

We identified 10,643 references from citation database queries, respectively 10,486 from ScienceDirect and 157 from PubMed. The systematic review, whose details are shown in Fig. 5, revealed 27 papers dealing with clinical trials related to allergic diseases and SC models in the above-mentioned period.

In the present systematic review, the selected papers were grouped according to the specific type of allergy (Table 3): 18 works on asthma detection and diagnosis, six on adverse drug reactions (ADR), one on allergic rhinitis, one on allergic conjunctivitis and one on atopic dermatitis.

Studies on asthma

Findings from clinical studies about asthma suggest that SC models are mainly suitable for classification of exacerbations, severity, recognition of asthmatic patients vs. controls and for asthma level control. Predicting the correct category of exacerbation severity is challenging in order to assess the appropriate hospitalization of the patient [9]. Sanders et al. [10] first proposed an SC model to detect asthma exacerbation in patients from the pediatric emergency department. They employed a BN model analyzing variables related to past diagnoses, allergies, family history, medications, social history and vital signs (temperature, respiratory rate, and oxygen saturation). The output of the network was the probability of a patient being admitted to the emergency department with asthma exacerbation and being eligible for treatment using asthma-care guidelines (GINA). The implemented model was able to identify guideline-eligible patients with an accuracy of 96%. Dexheimer et al. [11], using the same database as Sanders et al. [10], compared BN and other SC methodologies such as ANN and Gaussian processes, for identifying asthma exacerbations. Here, the accuracies achieved were 96, 95.6 and 94%, respectively, with no significant differences. In a recent prospective study, Farion et al. [12] compared different SC models using tenfold cross-validation and BN achieved the best performances. In a second phase of work they compared BN results with predictions derived by the pediatric respiratory assessment measure (PRAM) score [10] and with those made by physicians, obtaining high comparable results. In another study, Finkelstein et al. [13] integrated the SC methodologies in a decision support home telemonitoring platform to predict asthma exacerbations. The study dataset was based on daily self-reports administered on 26 adult asthmatic patients at home. All the collected data were analyzed with a BN classifier and an SVM able to predict asthma exacerbation with an accuracy of 80%. Zolnoori et al. [14] developed an intelligent clinical decision support system

Table 2 Studies dealing with SC models and allergic diseases

Authors	Application	Subjects	Description	Input features	SC model	Findings
Prosperi et al. [6]	To predict asthma severity	383 children with asthma (age 6–18 years)	Use of unsupervised statistical learning techniques, such as exploratory factor analysis (EFA), hierarchical clustering (HC) to identify asthma phenotypes	Lung function, inflammatory and allergy markers, family history, environmental exposures, body mass index, age of asthma onset and medications	BN	Significant recognition of asthma severity
Farion et al. [12]	To predict asthma exacerbation	322 Phase 1: 240 children (age 1–17 years) Phase 2: 82 children (age 1–17 years)	Phase 1: selection of the most accurate machine learning model with WEKA tool Phase 2: comparison of performance of BN with PRAM score and Physicians	42 attributes corresponding to the patient's history, current asthma exacerbation, primary assessment and a selected secondary assessment	BN	Phase 1: Accuracy = 68% Phase 2: Accuracy = 70.7%
Finkelstein et al. [13]	To predict asthma exacerbation	26 adult asthmatic patients	Use of a modern telemonitoring system at home	Daily self-reports	SVM	Accuracy = 80% Sensitivity = 84% Specificity = 80%
Sanders et al. [10]	To predict asthma exacerbation	4023 patients (age 2–18 years)	Use of a SC model to identify patients eligible for asthma care guidelines	Past diagnoses, allergies, family history, medications, social history and vital signs (temperature, respiratory rate, and oxygen saturation)	BN	Accuracy = 96% Sensitivity = 90% Specificity = 88.3%
Dexheimer et al. [11]	Same of Sanders et al. [10]	4023 patients (age 2–18 years)	Comparison of machine learning models to best identify patients eligible for an asthma care guideline	Same of Sanders et al. [10]	ANN,BN, Gaussian processes (GP)	BN accuracy = 96% GP accuracy = 95.6% ANN accuracy = 94%
Pifferi et al. [20]	To classify asthma control levels	77 patients (age 7.5–17 years)	Assessment of spirometry and fractional exhaled nitric oxide (FeNO) measurements to classify asthma control according to GINA guidelines	1st model: values of spirometry; 2nd model: values of FeNO; 3rd model: values of spirometry and FeNO	ANN	3rd model achieved best performances of classification Accuracy = 86.4%
Pifferi et al. [17]	To classify asthmatic vs control	123 90 asthmatic children (age 9–16 years) and 33 controls (age 12–13 years),	Pattern recognition analysis of the exhaled breath temperature curve	The rate of temperature increase and the mean plateau value	ANN	Accuracy = 5% Sensitivity = 77.2% Specificity = 99%
Jaing et al. [21]	To classify how children manage their asthma	305 children (age 5–14 years)	Each participant was given 10 asthma-based problems and asked to manage them	Each management decision and its order	ANN	Significant classification of five major classes representing different approaches to solving an acute asthma case

Table 2 continued

Authors	Application	Subjects	Description	Input features	SC model	Findings
Kharroubi et al. [24]	To classify health state of patient with asthma	307 subjects	Estimation of a preference-based index for asthma (five-dimensional asthma quality of life utility index)	99 features about health statuses	BN	BN model is more appropriate than conventionally used parametric random-effects model
Hirsch et al. [4]	To classify asthmatic vs control	6825 adults (age $\geq$ 16)	Respiratory questionnaires were analyzed by experts and compared with results provided by neural network	12 answers provided by the respiratory questionnaire (wheezing, chest tightness, shortness of breath, night cough), family history of asthma and associated conditions of hay fever or eczema	ANN	Accuracy = 74%
Chatzimichail et al. [19]	To classify asthmatic vs control	112 children (age 7–14 years)	Three step analysis:1-feature selection with Principal Component Analysis, 2-pattern classification, 3-performance evaluation	46 prognostic factors including data on asthma, allergic diseases, and lifestyle factors	SVM	Accuracy = 95.54% Sensitivity = 95.45% Specificity = 95.59%
Goulart et al. [32]	To classify allergic conjunctivitis vs control	102 48 with allergic conjunctivitis and 54 controls (age 3–14 years)	Allergic conjunctivitis questionnaires were analyzed by experts and compared with results provided by neural network	7 items selected from a questionnaire of 15 answers	ANN	Accuracy = 100%
Takahashi et al. [33]	To classify atopic dermatitis vs control	4610 answers, 2714 infants (12 months old) and 1896 children (2 years old)	To analyze the predictive accuracy of the predictive model for effect of atopic dermatitis in infancy, from the data of the epidemiological survey	Family history (father, mother, siblings, grandfather, grand-mother), food restriction, food allergy, age, food restriction of mother, egg introduced time, cow's milk introduced time	ANN	Accuracy = 96.4% Sensitivity = 88.6% Specificity = 99.5%
Christopher et al. [31]	To classify allergic rhinitis vs control	872 patients of all age groups	Allergic rhinitis reports of intradermal skin tests were analyzed by experts and compared with results provided by neural network	Patient's history and 40 clinically relevant allergens	ANN	Accuracy = 88.31% Sensitivity = 88.3% Specificity = 88.2%
De Matas et al. [23]	To predict the clinical effect of salbutamol	23 12 healthy volunteers and 11 mild asthmatics	In vivo and in vitro data of human subjects were analyzed using SC modeling	Demographic data and urinary levels of salbutamol and metabolite	ANN	Accuracy = 83.5%

Table 2 continued

Authors	Application	Subjects	Description	Input features	SC model	Findings
De Matas et al. [22]	To predict the clinical effect of salbutamol	18 mild-moderate asthmatic patients	SC modeling to predict the bronchodilator response at 10 (T10) and 20 (T20) min after receiving each of the 4 doses for each of the 3 different particle sizes	Aerodynamic particle size (APS), body surface area (BSA), age, pre-treatment forced expiratory volume in one-second (FEV1), forced vital capacity, cumulative emitted drug dose and bronchodilator reversibility	ANN	Accuracy = 88%
Gandhi et al. [25]	To predict hypersensitivity reaction	2458 reports concerning thrombotic events selected from AERS (adverse event reporting system) database	Retrospective analysis focused on thrombotic events associated with C1 esterase inhibitor products	Adverse events; demographic and administrative information; drug/biologic information; report sources; patient outcomes; drug therapy start and end dates; indications for use/diagnosis	BN	Potential signals of C1 esterase inhibitor product—associated thrombotic events among patients with hereditary angioedema were identified
Naranjo et al. [28]	To predict the posterior probability of a drug (BARDI tool)	51 patients	BARDI tool, calculates the posterior probability of a drug being the cause based on epidemiologic and case data	Reactions after receiving aromatic anticonvulsants	BN	Accuracy = 93% Sensitivity = 94% Specificity = 50%
Lanctot et al. [29]	To predict the posterior probability of a drug (BARDI tool)	27 cases of skin reactions	BARDI, combined with the LTA, a biochemical test that determines the percent of cell death because of toxic metabolites of a drug	Skin reactions associated with sulfonamide therapy	BN	Accuracy = 96% Sensitivity = 79% Specificity = 38%
Lanctot et al. [30]	To predict the posterior probability of a drug (BARDI tool)	106 challenging cases	BARDI, compared with the Adverse Drug Reaction Probability Scale (APS)	Drug- and nondrug-induced adverse events	BN	BN model discriminate better than ADR drug from nondrug-induced cases.
Kadoyama et al. [26]	To predict hypersensitivity reaction caused by anticancer agents	1,644,220 reported cases from 2004 to 2009 (AERS database)	SC model to detect important pattern related to anticancer agents-associated adverse events	Adverse events; demographic and administrative information; drug/biologic information; report sources; patient outcomes; drug therapy start and end dates; indications for use/diagnosis	BN	Potential signals were detected for paclitaxel-associated mild, severe, and lethal hypersensitivity reactions, and docetaxel-associated lethal reactions
Sakaeda et al. [27]	To predict hypersensitivity reactions caused by platinum agents	1,644,220 reported cases from 2004 to 2009 (AERS database)	The BN analysis aims to search for previously unknown patterns and automatically detect important signals, i.e., platinum agent-associat d adverse events, from such a large database	Adverse events; demographic and administrative information; drug/biologic information; report sources; patient outcomes; drug therapy start and end dates	BN	Significant association between the platinum agent-and mild, severe, and lethal hypersensitivity reactions

Table 2 continued

Authors	Application	Subjects	Description	Input features	SC model	Findings
Lurie et al. [16]	To classify asthma severity	113 patients (age 42.9 ± 16.3 years)	Implementation of a fuzzy model able to combine patients' and doctors' asthma perceptions	Doctor assessment, variables self-assessed by patients (dyspnea, perceived treatment efficacy, asthma-related quality of life questionnaire (AQLQ)), patients' sociodemographic characteristics, and asthma characteristics	FL	Accuracy = 73%
Zolnoori et al. [8]	To classify asthma control level	42 asthmatic patients	Implementation of a fuzzy model able to estimate the level of asthma control and help physicians to manage their patients more effectively	Respiratory symptom severity, bronchial obstruction, asthma instability, current treatment and quality of life	FL	Accuracy = 100%
Zolnoori et al. [14]	To classify asthma exacerbation	25 patients	Implementation of a fuzzy model able to estimate the level of asthma exacerbation and help physicians to manage their patients more effectively	Status of breathless, status of wheeze, status of alertness, status of respiratory rate, status of talk, status of pulse/min heart rate, value of PEF after initial bronchodilator, value of $paCO_2$, value of SaO_2%	FL	Accuracy = 100%
Zolnoori et al. [15]	To classify asthma severity	28 patients	Implementation of a fuzzy model able to estimate the four categories of asthma severity and help physicians to manage their patients more effectively	Bronchial obstruction, response to drug, skin prick test, severity of respiratory symptoms, instability of asthma, IgE value, quality of life	FL	Accuracy = 100%
Zolnoori et al. [18]	To classify asthmatic vs control	278 139 asthmatic patients and 139 non-asthmatic patients (age range 6–18)	Implementation of a fuzzy model to help physicians to manage their patients more effectively	Medical history, environmental factors, allergic rhinitis, genetic factors, consequences of asthma on lung tissues, response to laboratory tests and response to short-term medicine	FL	Sensitivity = 88% Specificity = 100%

SC soft computing, *ANN* artificial neural networks, *SVM* support vector machines, *BN* Bayesian networks, *FL* Fuzzy logic, *PRAM* pediatric respiratory assessment measure. *GINA* global initiative for asthma

(CDSS) based on the FL model to assess the level of asthma exacerbation. Input variables included the status of breathlessness, status of wheeze, status of alertness, status of respiratory rate, status of talk, status of pulse/min heart rate, value of PEF after initial bronchodilator, value of $paCO_2$, value of SaO_2%. The model was able to classify patients in four categories of asthma exacerbation including mild, moderate, severe and respiratory arrest imminent (RAI), achieving an accuracy of 100% (Cohen's coefficient $k = 1$). The studies analyzed so far suggested the high accuracy of an SC method to detect the correct level of asthma exacerbation. Within this review we also examined the role of SC methodologies in classifying the severity of asthma. Zolnoori et al. [15], in a second study, evaluated asthma severity by implementing a fuzzy rule expert system composed by seven input modules. Analyzed variables included bronchial obstruction, response to drugs, skin prick test, severity of respiratory symptoms, instability of asthma, IgE antibodies value and quality of life. This work evidenced a complete correspondence between model's and physician's evaluation (mild intermittent, mild persistent, moderate persistent, severe persistent) with and accuracy of 100% (Cohen's coefficient $k = 1$). In another study, Laure et al. [16] proposed the fuzzy approach to model the patient's perception of asthma severity. The model included variables self-assessed by patients (dyspnea, perceived treatment efficacy, asthma-related quality of life questionnaire (AQLQ), patients' socio-demographic characteristics, and asthma characteristics. The output of the model was compared with doctor assessment of asthma severity according to (GINA) guidelines. The study highlighted a clear tendency of the patient to underestimate asthma severity compared to the doctor assessment. This finding suggest that assessment of asthma severity should consider both patients' and doctors' perceptions of the disease and should include an AQLQ measure. Prosperi et al. [6] adopted BN to analyze non-linear relationships among variables and identified prognostic factors of asthma severity. Input variables included lung function, inflammatory and allergy markers, family history, environmental exposures, body mass index, age of asthma onset and medications. SC methodologies were found to help investigators to identify complex patterns and structures in the data, despite needing a thoughtful selection of input features and an appropriate data labeling in the case of identification of real asthma subgroups. Other studies examined the capabilities of SC methodologies to distinguish asthmatic patients from controls. Pifferi et al. [17] tested an ANN by extracting input features from the exhaled breath temperature curve (i.e., the rate of temperature increase and the mean plateau value). The model was tested in 90 asthmatic children and 33 healthy age-matched controls. ANN was able to recognize asthmatic children and controls with an accuracy of 99.3 and 70.3% respectively. In another study, an FL model was developed, and a sensitivity of 88% and specificity of 100% was obtained for a cutoff value of 0.7 of ROC curve [18].

These studies show the utility of an intelligent patient-based SC system to support asthma diagnosis, especially in developing countries, because of limitations on access to medical specialists and laboratory facilities. This review revealed that the use of SC methodologies could also have an important impact on analyzing huge amounts of screening questionnaires and to predict allergic diseases. In 2001 Hirsch et al. [4], proposed the use of ANN to screen a population for asthma, using the responses to a respiratory questionnaire. A random sample of 180 from 6825 respondents to a community survey underwent clinical review. Each survey was labeled according to likelihood of asthma, combining three independent expert opinions. The ANN was trained using the 12 questionnaire responses as input and the probability labels as outputs. Using the known probability labels from the training set, it was possible to derive the expected proportion of true asthmatic patients. In 2013 Chatzimichail et al. [19] proposed an intelligent approach based on SVM for asthma prediction in symptomatic preschool children based on questionnaire analysis. In this study 112 patients ranging from 7 to 14 years of age were analyzed. The performances of the SVM were evaluated by using the tenfold cross-validation approach and achieved an accuracy of 95.54%. Some studies emphasized the use of SC methodologies to objectivize the categorization of asthma control levels. Pifferi et al. [20] developed three ANNs (multi-layer perceptron) able to classify children with allergies according to three classes provided by GINA assessment (controlled, partially controlled and uncontrolled asthma) using only the input value of two simple measurements, namely spirometry and fractional exhaled nitric oxide (FeNO). Among the three tested models, only the input combination of values of spirometry and FeNO was able to provide high accuracy. More specifically, the model was able to recognize 100% of children with uncontrolled asthma, 74% with partially controlled asthma and 99% with totally controlled asthma. The same work presented a cross-sectional study of 77 children with allergic asthma. In this case the selected ANN model prospectively identified correctly 100% of uncontrolled, 79.5% of partially controlled and 79.6% of the controlled children. In another work, based on an FL model to predict the asthma control level, the clinical features of spirometry were combined with another four input classes of variables provided by the patients: respiratory symptom severity, asthma instability, current

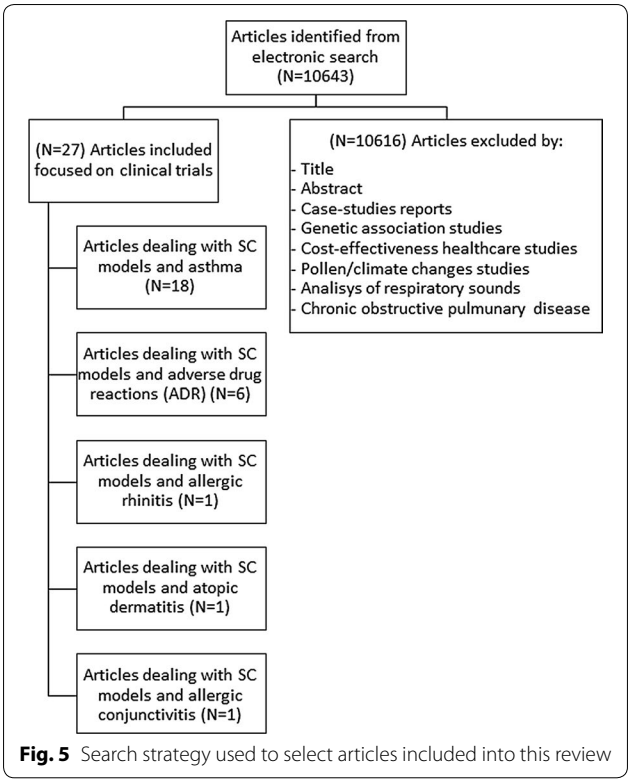

Fig. 5 Search strategy used to select articles included into this review

treatment and quality of life. The model was able to discriminate five categories of asthma control level: very poor, poor, fair, good, and excellent, achieving an accuracy of 100% (Cohen's coefficient k = 1) [8]. These findings suggest that the combination of clinical features (i.e., spirometry) and the subjective information provided by asthmatic patients can improve the performance of classification of SC models and help physicians to manage their patients more effectively. Another study demonstrates how SC models, through a training and pattern-recognition approach, can solve classification problems even when the specific category is not well-defined. In this regard, in a pilot study Jaing et al. [21] proposed the use of ANN to identify children's behavior categories representing different approaches of asthma management. This review also revealed that SC models are potentially useful for estimating pharmacokinetics performances. In two clinical trials, De Matas et al. [22, 23] used the ANN to model in vitro and in vivo data to predict the clinical effects of dry powder inhaler formulations containing salbutamol sulfate in individual subjects. In the first study, on healthy subjects the trained ANN was able to predict 83% of cumulative urinary excretion of salbutamol and metabolite 0–24 h post-inhalation. The correct prediction of ANN for mild asthmatic patients was 84%. In the second study, the ANN model was used to forecast the bronchodilator response defined as ΔFEV1 (%) measured at both 10 (T10) and 20 (T20) minutes after receiving each of the four doses of salbutamol sulfate puffs for each of the three different particle sizes (1.5, 3 and 6 μm).The average error between predicted and observed ΔFEV1(%) for individual subjects was <4% across the cumulative dosing regimen. These findings provide further evidence that ANNs supply a suitable approach for modeling complex biological data sets and have the potential to generate predictable models that can provide reliable estimations of clinical response to inhaled drug products in humans. The last clinical study about SC models and asthma disease was proposed by Kharroubi et al. [24], who explored the use of a non-parametric Bayesian method to classify the health state of people with asthma. The work presents the results of the non-parametric

Table 3 Overview of clinical studies related to SC models and allergic diseases

Type of allergy	No. of studies	Overall accuracy (%)	Application	ANN	SVM	BN	FL
Asthma	18	82.44 ± 23.71	To classify exacerbations [10–14]	1	1	2	1
			To classify severity [6, 15, 16]	–	–	1	2
			To classify pathologic vs control [4, 17–19]	2	1	–	1
			To classify asthma control level [8, 20]	1	–	–	1
			To classify how manage their pathology [21]	1	–	–	–
			To predict the clinical effect of salbutamol [22, 23]	2	–	–	–
			To classify health state[24]	–	–	1	–
ADR	6	94.5 ± 2.12	To predict the posterior probability of a drug (BARDI tool) [28–30]	–	–	3	–
			To predict hypersensitivity reaction (AERS database) [25–27]	–	–	3	–
Allergic rhinitis	1	88.31	To classify pathologic vs control [31]	1	–	–	–
Allergic conjunctivitis	1	100	To classify pathologic vs control [32]	1	–	–	–
Atopic dermatitis	1	96.4	To classify pathologic vs control [33]	1	–	–	–

model compared to the original model estimated using a conventional parametric random-effects model. This work evidenced that the non-parametric Bayesian models are theoretically more appropriate than previously used parametric models and provide better estimates of asthma quality of life.

Studies on adverse drug reactions

This review examined results about the use of SC models and adverse drug reactions (ADRs). Three studies evaluated the ADRs from the public version of the food and drug administration (FDA) adverse event reporting system (AERS). A large dataset of 1,644,220 case reports from 2004 to 2009 was analyzed through the use of a BN. In particular, Gandhi et al. [25] identified 10 combination cases of thrombotic events associated with the use of one C1 esterase inhibitor product (Cinryze) in patients with hereditary angioedema. Kadoyama et al. [26] evaluated the susceptibility to hypersensitivity reactions to anticancer agents using parameters based on a Bayesian confidence propagation neural network, and the empirical Bayes geometric mean. These indexes of pharmacovigilance provided signals of mild, severe and lethal hypersensitivity reactions associated with paclitaxel and docetaxel agents. In another study [27], the same group demonstrated with the same approach that carboplatin and oxaliplatin caused mild, severe, and lethal hypersensitivity reactions, whereas cisplatin did not. The use of dexamethasone affected oxaliplatin-induced mild hypersensitivity reactions, but had lesser effects on severe and lethal reactions. These findings highlight the significant potential of SC models to analyze huge amounts of data and the ability to discover patterns deeply hidden within the data. Another three studies highlighted the importance of a diagnostic tool for assessment of adverse drug events (BARDI). This computer program is based on BN and is able to perform a differential diagnosis on cutaneous reactions suspected of being drug-induced [28–30].

Studies on other allergic diseases

Finally, the review revealed that few studies about SC models and other allergic diseases are present in literature. In 2015 Christopher et al. [31]. presented a CDSS based on ANN to assist junior clinicians to diagnoses the presence or absence of allergic rhinitis analyzing reports of intradermal skin tests. The trained neural network achieved an accuracy of 88, 31%. In another work Goulart et al. [32] proposed the ANN to study an allergic conjunctivitis screening questionnaire. In this work the ANN predicted allergic diagnosis in 100% of cases using 7 of the 15 existent items. A study conducted in Japan by Takahashi et al. [33], proposed the use of ANN to predict the effects of atopic dermatitis in infancy from an epidemiological survey. A total of 4610 answered surveys were received: 2714 from mothers of infants (12 months old) and 1896 from mothers of children (2 years old). The sensitivity, specificity and predictive accuracy of the ANN model were respectively 88.6, 99.5 and 96.4%.

Discussion

Main findings

This systematic literature review explored the main SC methodologies to investigate allergic diseases. Results were obtained after an exhaustive literature research and examination of hundreds of papers focused on clinical trials. Several studies provided results about SC systems focused on early diagnosis of allergic diseases and the classification of illness categories (i.e., exacerbation, severity) obtaining a mean accuracy of 86.5%. More specifically, this review revealed that the SC approach could have an important impact on the analysis of an enormous amount of screening questionnaires and in the prediction of allergic diseases, discovering patterns deeply hidden within the still-unexplored data [32, 33]. This is possible because the SC models are flexible and able to generalize and predict on an individual basis the probability of diagnosis related to the specific disease of questionnaire respondents. In this specific case, the ANN and BN models are particularly suitable. Another important issue that the use of SC models can resolve concerns missing data in clinical trials. Frequently patients do not complete their follow-up according to a protocol for a variety of reasons, making data analysis more difficult. The BN is a good example of models naturally suitable for handling missing data, as suggested by Carpenter et al. [34, 35]. Another important result emerging from our review is that the SC models could have an important role in CDSS. Indeed, they provide an opportunity to assess the overall information of the main phenomena coming from patients (i.e., identifying information, family history, environmental exposure, perceived treatment efficacy, disease-related quality of life questionnaire) and clinicians (i.e., laboratory tests, values of spirometry), underlying critical features of the disease, treatment planning and the provision of warnings by adding new evidence through associative recall from historical data. In this regard, studies on clinical emergence coupling CDSS-human interaction with the clinician's knowledge and the suggestions of feedback signals are undertaken in order to generate patient-specific advice and to assist clinicians at the point of care [14, 31, 36]. SC models are also suitable for analyzing data when the likelihood is not defined and statistical tests are not appropriate. More specifically, the choice of an FL approach in substitution of other SC models can provide valuable support, since it starts from

the context of a lack of experience data and entangled cause-and-effect relationships, which make it difficult to assess or diagnosis allergic diseases at an early stage [8, 16]. FL is extremely flexible, allowing the decision maker to use a broad range of linguistic variables and modifiers for finer discrimination. It is also a useful system in the case of the presence of a series of sub-decisions where available data is based on vagueness, uncertainty and opinion, such as questionnaires.

Strengths and limitations

Strengths of this review are related to an exhaustive literature search in PubMed and ScienceDirect databases. The research was performed following PRISMA guidelines and using recommended search queries with consensus finding. Moreover the protocol of this review was registered within the PROSPERO database, with the code CRD42016038894. We found most of SC works deal with asthma, six studies about ADR, and few studies about other allergic diseases. In this regard, in some cases their accuracy although high was quantitatively synthesized on few works. This result reveals how the SC approach is widely used to diagnose asthma, but it is still largely unexplored for other hypersensitivity diseases. Moreover, there are also limitations with the studies themselves. To date, a comparative analysis of SC performances with classic statistical methods was not possible due to the lack of studies comparing these models against a benchmark. This is due to the fact that in most cases the use of these advanced techniques needs to overcome obstacles including the need to establish multidisciplinary teams [37], the resistance to change in working practice especially from older clinicians [38] and the lack of appropriate gold standard clinical assessment procedures [39].

Conclusions

This systematic review, analyzing clinical trials employing SC methodologies, shows as these methodologies have been used in allergy field for several purposes such as for detecting patients with asthma exacerbations, to prompt clinicians to identify guideline-eligible patients, to evaluate putative ADR, to discriminate drug from nondrug-induced reactions, to improve the diagnostic accuracy and to enhance the management of patients with hypersensitivity reactions. The review also identify promising trends, especially in the diagnosis, prognosis and treatment of some allergic diseases, but also the need for a more extensive application as occurs in genetic association studies [40–43].

Such methods enable a new concept for modeling allergic diseases that combines the collection and mining of multimodal clinical evidence, with dynamic modeling of causal factors. We believe that the introduction of SC models can ease the exploration of big clinical data sets to enable better understanding of allergic disease subgroups, their pathophysiology and optimization of existing treatments. Clinicians should improve evidence by undertaking more randomized controlled trials to prove the efficacy of SC methodologies. In this regard, they should be trained to be more confident with the new perspective provided by these advanced techniques going over their standard methods of choice in interpreting medical data. The review provides evidence that SC methodologies can play a key role in predicting the onset, diagnosing, evaluating pathogenesis and prognosis and managing most of allergic diseases. Moreover, these methods can discover new patterns and evidence about early recognition of inflammatory markers and their relation to allergic diseases [44]. Nowadays most studies deal with asthma, however it is to be hoped that in the near future SC methodologies could be used to investigate all allergic diseases, with a particular attention to those pathologies with a huge burden on health for their impact on quality of life and their severity, such as urticaria and anaphylaxis.

Oral food challenge outcomes in a pediatric tertiary care center

Abstract

Background: Oral food challenges are the clinical standard for diagnosis of food allergy. Little data exist on predictors of oral challenge failure and reaction severity.

Methods: A retrospective chart review was done on all pediatric patients who had oral food challenges in a tertiary care pediatric allergy clinic from 2008 to 2010.

Results: 313 oral challenges were performed, of which the majority were to peanut (105), egg (71), milk (41) and tree nuts (29). There were 104 (33%) oral challenge failures. Children were more likely to fail an oral challenge if they were older (P = .04), had asthma (P = .001) or had atopic dermatitis (P = .03). Risk of challenge failure was significantly different between food allergens, with more failures noted for peanut than for tree nuts, milk or egg (P = .001). Among challenge failures, 19% met criteria for anaphylaxis. Significantly more tree nut and peanut challenges met criteria for anaphylaxis than milk or egg (P < .001). Skin test size and specific IgE level were significantly higher in those who failed oral challenges (P < .001). The highest rate of challenge failure and severity of failure was to cashew, with 63% of cashew challenges reacting, of which 80% met clinical criteria for anaphylaxis.

Conclusion: The risk of challenge failure differed with type of food studied, with peanut and tree nut having a higher risk of challenge failure and anaphylaxis. Cashew in particular carried a high risk and caution must be exercised when performing these types of oral challenges in children.

Keywords: Food allergy, Oral food challenge, Anaphylaxis

Background

Food allergy affects 2–10% of the population, and is more common in children than adults [1]. The diagnosis of food allergy is often based on results of a careful history, skin prick testing (SPT) and serum food-specific IgE [2]. Oral food challenges (OFCs) assist in the diagnosis of food allergy, and are essential to determine whether an allergy has been outgrown [3]. However, OFCs do carry the risk of a systemic allergic reaction [3]. While the double blind placebo controlled food challenge is the most accurate and a true 'gold standard' for diagnosis of food allergy, it is time consuming and costly [3]. The open oral food challenge is often used instead, although it is subject to patient bias [3].

Previous studies have examined the feasibility and safety of oral food challenges, as well as diagnostic levels at which to consider food challenges based on results of serum food-specific IgE and/or epicutaneous testing [4–10]. However, there is a paucity of literature examining other predictors of food challenge outcomes.

We performed a retrospective chart review to examine whether oral food challenge outcomes varied by characteristics such as food being challenged, patient characteristics (age, atopy), and results of skin prick testing or serum food-specific IgE.

Methods

A retrospective chart review was performed on all open oral food challenges between January 1, 2008 and December 31, 2010 at the University of Manitoba pediatric allergy clinic. Oral food challenges were performed based on the clinical decision of the attending physician,

with consideration of clinical history, results of epicutaneous testing, and/or results of serum food-specific IgE. Challenges were performed to confirm food allergy, or when there was a suspicion of oral tolerance after a period of avoidance in a food allergic child.

This study met the criteria for a waiver of informed consent by the research ethics board at the University of Manitoba as it was an internal quality improvement study.

The oral challenge was administered as half-log (base 10) incremental doses (starting at .1 mg for solids and .1 mL for liquids) every 15–20 min until a final dose of 10 g (30–100 mL for cows' milk) was tolerated. In children with asthma, oral challenges were only performed if asthma was well controlled. Challenges were terminated and considered positive if there were objective symptoms noted by the attending physician, or, on occasion, when only worrisome subjective symptoms (subjective oropharyngeal symptoms) were reported by the patient. Patients were observed for an hour after the final dose. If there was a reaction, patients were observed for a minimum of an hour, until objective signs of the reaction had resolved. Patients were asked to notify the attending physician should there be any delayed reaction after discharge.

Treatment of challenge failures was at the discretion of the attending physician, and based on reaction severity. If the patient met the criteria for anaphylaxis, .01 mg/kg of intramuscular epinephrine (1:1000) was administered. A repeat dose was given in 10–15 min if there was no symptom resolution. Other treatment of positive challenges was at the discretion of the attending physician and included an age-appropriate dose of antihistamine for cutaneous symptoms, 2.5–5 mg inhaled albuterol for respiratory symptoms refractory to epinephrine, and an age appropriate dose of prednisone (.1 mg/kg).

Statistical methods

Statistical analyses were performed by using SAS version 9.3 (SAS Institute, Cary NC). Pearson's Chi square test was used for categorical variables, Kruskal–Wallis test was used for comparing continuous distributions between groups, and relative risk was used as a measure of association. $P < .05$ was considered to be statistically significant.

Results

There were 313 oral food challenges performed between January 1, 2008 and December 31, 2010 at the University of Manitoba Pediatric Allergy Clinic. There were 105 peanut, 71 egg, 41 milk, 29 tree nut (6 almond, 1 brazil nut, 8 cashew, 6 hazelnut, 1 macadamia nut, 2 pecan, 5 walnut), 10 finned fish, 14 shellfish, 9 soy, and 34 other challenges performed. Seventeen patients underwent oral challenges to more than one food during this time (although never more than one food each day), and eleven patients had more than one oral challenge to the same food. Some peanut and tree nut challenges were masked (often in pudding).

Table 1 shows the characteristics of the study population. There were 104 oral food challenge failures (33% of food challenges), of which 82 were objective and 22 were subjective failures (predominantly subjective oropharyngeal symptoms).

Median patient age was 5.5 years (range 8 months–18 years). Older children were significantly more likely to fail an oral challenge than younger children (median age 73 months vs 58 months; $P = .04$). There was no difference in overall rate of atopy (defined as atopic dermatitis, other food allergy, asthma, or aeroallergen sensitization) between those who failed and those who passed oral challenges. Overall rate of other atopic disease was high at 74%. Rate of physician diagnosed atopic dermatitis was significantly higher among those who failed oral challenges (60% vs 74%; $P = .03$). Rate of asthma was also significantly higher among those who failed oral challenges (47% vs 72%; $P = .001$). Rate of multiple food allergy and aeroallergen sensitization were not significantly different among those who failed oral challenges.

Clinical characteristics of challenge failures are noted in Table 2. Risk of challenge failure was significantly different between food allergens ($P = .001$), with more failures noted for peanut than for tree nut, milk or egg ($P = .001$). Among challenge failures, 20/104 (19%) met the criteria for anaphylaxis (epinephrine administration or multi-organ involvement). Significantly more tree nut and peanut challenges met the criteria for anaphylaxis than milk or egg ($P < .001$). There were no documented incidences of biphasic reactions and no reactions that included hypotension or required hospital admission.

The characteristics of the type of reaction varied by food. Respiratory symptoms were present in 40% of those who failed tree nut challenges (all of whom received

Table 1 Patient demographics of failed versus passed oral challenges

	Passed	Failed	Total	P value
Median age (months)	58	73	66	.04
Female (%)	43	36	40	–
Overall atopy (%)	72	79	74	.16
Atopic dermatitis (%)	60	74	65	.03
Asthma (%)	47	72	55	.001
Multiple food allergy (%)	49	46	48	.62
Aeroallergen sensitization (%)	81	81	81	.96

Table 2 Clinical characteristics of challenge failures

	Milk	Egg	Peanut	Tree nut	Total	P value
Challenge failures (% per food)	34	28	47	34	33	.001
Anaphylaxis (%)	7	5	20	70	19	<.001
Urticaria (%)	29	55	55	70	52	.31
Angioedema (%)	0	0	12	30	11	.06
Gastrointestinal symptoms (%)	29	5	14	30	14	.11
Respiratory symptoms (%)	0	0	0	40	4	<.001
Subjective symptoms (%)	36	40	8	0	21	.001

epinephrine), but no patients who failed peanut, milk or egg challenges (P < .001). Subjective reactions (oropharyngeal or behavioural symptoms during ingestion period) were more common in egg and milk challenges than peanut or tree nut challenges (P = .001).

Skin prick testing was positive at initial or subsequent evaluations in 181 patients, and negative in 97 patients overall (SPT not done in 35 patients, who were followed by serial food serum-specific IgEs). Median skin test size was 3.8 mm for egg (range 0–17.5 mm), 5.8 mm for cows' milk (range 0–12.5 mm), 5.8 mm for peanut (range 0–17.5 mm), and 6.6 mm for cashew (range 3.5–10 mm). Serum food-specific IgEs were performed in 297 patients, and were positive at initial or subsequent evaluations in 147 patients.

Table 3 describes the SPT and specific IgE results of failed versus passed oral challenges. Skin test size was significantly higher in those who failed oral challenges overall (median wheal diameter 6.5 mm vs. 2.0 mm; P < .001). Skin test size was not significantly correlated with challenge failure rate for egg, milk, or tree nut but was significantly correlated for peanut (median wheal diameter 7.5 mm vs. 3.25 mm; P < .001).

Food specific IgE was significantly higher overall in those who failed oral challenges (median .7 kU/L vs < .35 kU/L; P < .001). Food specific IgE level was not significantly correlated with challenge failure for egg, cow's milk or tree nut, although there was a significant difference for peanut (median .78 kU/L vs < .35 kU/L; P < .0001).

Food dose eliciting a reaction in challenge failures was significantly different (P = .01) between milk, egg, peanut and tree nut, with many peanut and tree nut challenges reacting at low doses, and egg and milk challenges reacting at higher doses. Median final dose ingested prior to an allergic reaction for egg was 2.0 mg, for milk was 3.0 mL, for peanut was .30 mg and for tree nuts was .75 mg. There was no significant correlation between initial reaction characteristics (organ involvement) and reaction characteristics at oral challenge.

There were 5/8 (63%) failed cashew challenges. Cashew was significantly more likely to cause a reaction at oral challenge than the other tree nuts (63% versus 24%; P = .05). Cashew oral challenges were significantly more likely to cause anaphylaxis (P < .001) with a rate of 80% for cashew, compared with 17% overall. Of the cashew challenge failures, 3/5 (60%) had no prior known exposure to cashew, and were avoiding it due to peanut or other tree nut allergy.

Table 3 Median skin test and specific IgE results in failed versus passed oral challenges

	Passed	Failed	P value
Skin test size overall (mm)	2.0	6.5	<.001
Skin test size to peanut (mm)	3.25	7.5	<.001
Specific IgE overall (kU/L)	<.35	.70	<.001
Peanut specific IgE (kU/L)	<.35	.78	<.001

Discussion

Our study shares some findings that are similar to previous studies. Oral challenge failure rate of 33% is in keeping with other studies that have reported challenge failure rates varying from 18.8 to 43% [4–10]. Similar to other studies, we found increased risk of challenge failure in children with asthma and eczema. Perry et al's retrospective review of 604 oral challenges also noted increased risk in children with eczema or asthma, but not other atopic disease outcomes [9]. Our population, similar to Perry et al's study, is that of a tertiary care facility which may lead to higher atopic rates than seen in other primary or secondary care settings. Finally, similar to previous studies, we found that skin test sizes and serum food-specific IgE levels were significantly higher for failed than passed oral challenges [6, 9, 11].

Our study had some findings that were discrepant from previous studies on oral challenge outcomes. While the age gap was not wide, older age was a significant risk factor for challenge failure in our population, which is discrepant from Lieberman et al's findings of no age difference between the group that passed OFCs and the group that failed in their retrospective review [6].

We also found a strong difference in rate of oral challenge failure and severity of reaction based on food allergen. Oral food challenge failures were significantly more common for peanut than they were for milk, egg, or tree nuts (P = .001). To our knowledge, this has not been reported in previous studies. In contrast, Spergel et al's retrospective review noted milk, egg and peanut to be the most common causes of positive oral challenges, and also the most common cause of multi-organ involvement [10].

There was an overall anaphylaxis rate of 19%, which is higher than some other studies on oral food challenge outcomes [5, 6]. There were no biphasic reactions and no hospital admissions in our study, which has been echoed by other retrospective reviews as well [8]. As with Jarvinen et al's analysis, our study reveals that anaphylactic reactions were most common for peanut and tree nuts, suggesting that more caution is warranted in performing these challenges [5]. In contrast, Perry et al's retrospective review found no difference in reaction severity based on which food was challenged [8]. We did not find a correlation between reaction type at presentation and at oral challenge. Some studies have also found no correlation between reaction types [12] although Spergel et al's did [10].

Our study is unique in its inclusion of tree nuts—many previous retrospective reviews of oral challenges have focused on milk, egg, and peanut [4, 7, 11]. To our surprise, reactions to cashew were both common and severe. It is striking that, of the cashew challenge failures, 60% had no prior known exposure to cashew and were avoiding it due to peanut or other tree nut allergy. The literature on severity of cashew allergy is sparse although a recent systematic review on cashew allergy did note that anaphylactic reactions appear to be very frequent with cashew, and may be more frequent and/or more severe than peanut reactions [13]. To our knowledge this is the first study reporting oral food challenge outcomes on cashew and our results suggest a need for caution when performing an oral challenge to cashew.

There are some findings from our study that, to our knowledge, have not been reported in prior studies on oral challenge outcomes. For example, we report that subjective food challenge failures were high for milk and egg, but not for peanut or tree nuts. The reason for this is unclear but may be partially related to tolerance of the food in question as peanut and tree nut challenges were intermittently masked at the discretion of the attending allergist, often with pudding, while cow's milk and egg challenges traditionally were not. To our knowledge, this is the first study to stratify based on subjective or objective challenge failures, and the first to report that rate of subjective challenge failures differed by food type. We also found that eliciting dose varied by type of food. Children reacted at low doses to peanut and tree nut (median final dose .30 and .75 mg respectively) while they reacted at higher doses for egg and milk (median final dose 2.0 mg and 3.0 mL respectively). In our study population, children who did not react to the first few doses of peanut or tree nuts tended not to react, while they tended to react later in the protocol for milk and egg. To our knowledge this has not been reported in other studies to date.

There are several limitations to our study. It is retrospective in nature, although most studies on oral challenge outcomes share a similar study design. The challenges were open challenges, instead of double blind placebo controlled challenges, which would be the 'gold standard' although are typically not a practical approach. As our center is a tertiary care center, there is a high prevalence of other atopic disease which might make these patients higher risk. As the study was exclusively pediatric, results can only be applied to the pediatric population. While subjective symptoms were included, it is possible these symptoms could be due to anxiety as opposed to clinical reactivity. Some oral challenges to cow's milk were considered complete at a dose of 30 mL of cow's milk (approximately 1 g of milk protein) while typically protocols recommend a standard portion of cow's milk or 10 g solid cow's milk protein. Some oral challenges were done in children with negative skin prick testing, or had never eaten the food, and it is possible these children were not allergic at baseline, skewing results.

In conclusion, oral challenge failures occurred 33% of the time, and were more severe to peanut and tree nuts than to egg or milk. Children who reacted were older, had higher rates of eczema and asthma, and higher skin test sizes and/or serum specific-IgE levels to the food in question. Eliciting dose varied by food, with children reacting to lower doses of peanut and tree nuts than milk or egg. There was also a high subjective challenge failure rate to egg and milk. Finally, cashew challenges carried a high risk of severe reactivity, even in children with no prior history of cashew ingestion.

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