INTERNATIONAL

NOTIZIARIO ALLERGOLOGICO

ISSN 2038-2553

2025 • Vol. 43 • n. 3

Intelligenza Artificiale: focus su allergia

Artificial Intelligence: focus on allergy

Inteligencia Artificial: enfoque en alergia

Allergia a farmaci: focus sui beta-lattamici

Drug allergy: focus on beta-lactams

Alergia a medicamentos: enfoque en beta-lactámicos

Le interazioni tra allergeni e ligandi

Interactions between allergens and ligands

Interacciones entre alérgenos y ligandos

NOTIZIARIO ALLERGOLOGICO

2025 • Vol. 43 • n. 3

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Registrazione Tribunale di Milano n. 306 dell' 1.8.1980
Pubblicazione quadrimestrale

Registration with the Court of Milan n. 306 of 1.8.1980
Four-montlhy publication

Registro en el Tribunal de Milán n. 306 de 1.8.1980
Publicación cuatrimestral

Il **Notiziario Allergologico** è on-line su
The Notiziario Allergologico is on-line at
El Notiziario Allergologico está en-línea en

www.lofarma.it

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SUMMARY

Notiziario Allergologico, 2025 Vol. 43, n. 3

Notiziario Allergologico

PDF VERSION

Notiziario Allergologico has been alive and well for over forty years. Today, it becomes international with a new layout that includes the translation of all content into three languages. The purpose remains unchanged if not implemented: to promote allergology culture by offering readers the possibility of an in-depth study and update on various allergology topics, also with a view to the future, thanks to the competence and authority of the authors of the articles published. The popular character of the articles contributes to making them accessible to a vast number of specialists, not only allergologists but also pulmonologists, paediatricians, dermatologists, etc.

ENGLISH

EDITORIAL

edited by **Gianni Mistrello**

Dear readers,
I hope you had a pleasant and rejuvenating holiday and that your return to work has been smooth. I invite you to read the contents of this new issue of the Newsletter, which I hope will meet your expectations.
Let's start with an article on an increasingly pervasive topic: Artificial Intelligence.
Although perhaps not everyone is aware of it, there are numerous examples (virtual assistants such as Alexa, automatic text translation systems, image analysis software, etc.) that demonstrate how artificial intelligence (AI) is already part of our daily lives. AI is a set of digital technologies based on the use of 'machines' capable of 'learning' and 'analysing' large amounts of data, far exceeding the capabilities of human beings, and then identifying patterns and relationships that might escape human observation. AI is evolving rapidly, with revolutionary potential in many sectors, including, of course, healthcare. In this issue of Notiziario Allergologico, we publish an article that specifically addresses the transformative potential of AI in the field of allergy. Asthma, rhinitis, food allergy and atopic dermatitis are the most common allergic diseases and can often co-exist in their development, manifesting complex associations that are not easily identifiable. Being able to identify these intricacies would improve our understanding of their pathogenesis and have a significant impact on their clinical management. The author of the article (Ing. Luca Filigheddu, Sellverge), after initially providing an overview of AI, illustrating its basic principles (machine learning, deep learning, etc.) in a simple and accessible way even for those unfamiliar with computer science, focuses his attention on the main clinical applications of AI already in use or in the experimental phase in the field of allergology. In particular, he highlights the innovative elements that can result in terms of supporting the medical profession in formulating more accurate diagnoses and defining personalised therapies, as well as becoming a key tool for optimising patient recruitment in clinical trials. The author concludes his contribution by emphasising that AI will not replace the clinical judgement of the allergist who, if able to exploit its potential, will find it a fundamental support in improving the quality of care for patients with allergic diseases.
This is followed by a contribution on drug allergies.
In general, drug allergy is defined as a disorder that develops, particularly in genetically predisposed individuals, following involvement of the immune system, which mistakenly recognises these substances as harmful and produces specific IgE antibodies against them. Allergy should be distinguished from the term adverse drug reaction, which refers to any undesirable effect (not immunologically mediated) caused by a drug at doses commonly used for diagnosis, prophylaxis or therapy. Potentially any drug can induce an allergic reaction, but antibiotics are among the most common. Drug allergy usually manifests itself with skin or systemic symptoms, including extremely severe ones such as anaphylaxis. In the extremely comprehensive article we are publishing, the authors (Prof. Enrico Heffler and Dr. Giovanni Paoletti, Humanitas University and IRCCS Humanitas Research Hospital) focus their attention on beta-lactams (which include penicillins, cephalosporins, carbapenems and monobactams), which are the most widely prescribed class of antibiotics in the world due to their therapeutic efficacy and safety profile. They emphasise the importance of

the diagnostic approach to beta-lactam allergy, which must be based on a thorough history of the circumstances of the reaction, symptoms, any confounding factors (concomitant viral infections) and, in particular, timing, in order to distinguish a genuine allergic reaction from pseudo-allergic reactions or intolerance. In addition, the diagnostic process may include the use of allergy tests (specific IgE dosage, skin tests and oral provocation tests) for confirmation. An important aspect that is widely discussed is the 'overdiagnosis' of beta-lactam allergy, with significant clinical, social and economic consequences, compounded by the risk of promoting the spread of resistant strains in the event of inappropriate use. Another aspect highlighted by the authors is the 'delabelling' of beta-lactam allergy, a process which, based on robust clinical evidence, should allow the removal of inappropriate allergy labels and possibly be implemented by careful risk assessment (which also takes into account the fact that IgE-mediated beta-lactam allergy may diminish over time).

In recent years, there has been a huge increase in the number of protein molecules (allergens) involved in allergic sensitisation phenomena. For many of these, thanks in part to the use of appropriate technologies to obtain them in purified form, our understanding of their biochemical characteristics, structure and IgE-binding determinants, as well as their interaction with the immune system, has improved.

However, it is still not entirely clear what mechanisms enable these molecules (which are harmless in themselves) to induce allergic sensitisation in genetically predisposed individuals. Many of the studies that have been carried out have focused on the structural properties of allergenic molecules as isolated entities, without considering that, when they come into contact with humans, they are contained in a complex mixture of components, including small ones (e.g., carbohydrates, lipids, etc.), such as those found in a pollen grain. More recently, researchers have focused their attention on studying whether some of these components (ligands) can interact directly with allergenic molecules and play an important role in inducing allergic sensitisation.

The author of the third article (Prof. Karin Hoffmann-Sommergruber, CePII Medical University of Vienna) focuses on current knowledge about the types of ligands that can interact with allergenic molecules and the impact that these derivatives can have on the immune system. In particular, consideration is given to certain families of allergens that are more characterised in terms of 'ligand-binding properties', such as lipocalins, non-specific lipid transfer proteins (whose most representative allergen is the major allergen in peaches, known as LTP), Niemann-Pick-type C2 proteins (whose best-known representatives are group 2 dust mite allergens), PR-10 proteins (the best-known representative of which is Bet v1, serum albumins). For each of these, the ligands (often consisting of lipids or their derivatives) with which they can interact are described and the possible effects of this interaction are discussed in terms of alterations in the physicochemical characteristics of the allergens involved, such as resistance to enzymatic degradation, i.e. changes in their conformation and stability, and their relevance to the immunological response, which can result in the activation of the Th2 response and thus favor the allergic response.

I wish everyone a good read.

Artificial intelligence in allergology: new perspectives for diagnosis, therapy and research

Ing. Luca Filigheddu
Founder of Sellverge

1. Introduction

In recent years, medicine has witnessed a radical transformation due to the introduction of advanced digital technologies. Among these, artificial intelligence (AI) has rapidly emerged as a tool with revolutionary potential, capable of supporting clinicians in analysing large volumes of data, formulating more precise diagnoses and defining personalised therapies. In allergology, a field that has always been characterised by a complex interplay between genetics, environment and immunology, AI offers new perspectives to better understand the mechanisms of allergic diseases, improve the diagnostic-therapeutic pathway and foster translational research.

The aim of this article is to introduce artificial intelligence in a simple and accessible way to allergologists unfamiliar with computer science, illustrating the main clinical and scientific applications already underway and offering insights for a conscious and profitable use of these emerging technologies.

2. What is artificial intelligence?

Artificial intelligence is a set of computer techniques that enables a machine to learn from experience, recognise patterns in data and perform human-like "intelligent" operations, such as recognising images, understanding natural language or predicting future events.

Underlying AI are mathematical algorithms that are "trained" on large amounts of data: for example, medical records, laboratory tests, diagnostic images or responses to treatments. Through this process, called machine learning (ML), the system is able to extract implicit rules and generalisations that often escape even the expert eye of the physician.

A subset of ML is deep learning (DL), which is based on artificial neural networks composed of numerous "layers". These layers process information in a hierarchical manner: the first recognise simple elements, subsequent layers build more complex relationships. This approach has been used, for example, to analyse skin images, respiratory auscultations or even patterns in allergy tests.

Another fundamental concept is that of supervised vs. unsupervised learning. In the first case, the data are provided already labelled (e.g. known diagnosis), and the algorithm learns to recognise them; in the second case, the system explores the data on its own and discovers hidden groupings (clusters), which is useful, for instance, to identify new clinical phenotypes. This has led, in allergology, to defining groups of patients with similar characteristics but not easily classifiable according to traditional approaches.

It is essential to emphasise a fundamental principle of AI: "garbage in, garbage out". If the input data are incomplete, inaccurate or unrepresentative, even the best model will generate inaccurate results. Artificial intelligence can be a real superpower in the hands of the physician, but only if it is fed with quality data and used with awareness; otherwise, it risks producing misleading or unhelpful suggestions.

3. Big Data in medicine and allergology

The concept of Big Data refers to extremely large, heterogeneous and con-

ABSTRACT

Keywords and related acronyms

• Artificial Intelligence (AI), Machine Learning (ML) • Deep Learning (DL) • Allergology • Assisted Diagnosis • Phenotyping • Immunotherapy • Personalised Medicine • Neural Networks • Allergen Immunotherapy (AIT) • Computer Aided Diagnosis (CAD) • Artificial Neural Networks (ANN)

Artificial Intelligence (AI) represents one of the most important technological innovations of recent years, with transformative potential also in the medical field. In allergology, AI offers tools to improve diagnosis (Computer-Aided Diagnosis, CAD), identify complex phenotypes by means of machine learning (ML) and unsupervised clustering, predict disease progression (e.g. asthma, allergic rhinitis, atopic dermatitis) and optimise treatments through personalised predictive models.

This article provides a popular overview of AI, explaining its basic principles (including deep learning, DL, and artificial neural networks, ANN) and illustrating the main applications already in place or in the experimental phase, including algorithms for skin image analysis, prediction of allergen-specific immunotherapy (AIT) response, and patient stratification in clinical registries.

Concrete examples are presented, supported by the international scientific literature, with a focus on opportunities for the allergist in daily clinical practice and translational research. The aim is to offer an initial orientation for understanding the potential of these digital tools, even in the absence of specific computer skills.

stantly updated collections of data. In modern medicine, these data come from a variety of sources: clinical reports, diagnostic examinations, laboratory results, epidemiological investigations, but also environmental and genetic data. In allergology, we think of the millions of data related to pollen, pollution, skin tests, specific IgE, food and respiratory profiles of patients, responses to drugs, and even interactions with climatic factors.

AI allows these data to be processed and integrated to build predictive models, identify subgroups of patients with common characteristics, or even suggest personalised treatment paths.

One of the main challenges of Big Data is the quality of the information: many sources are noisy, incomplete or collected with different standards. Therefore, a preliminary work of cleaning and harmonising the data is essential to allow the algorithms to work properly. A poorly maintained dataset can generate misleading results: this is where the concept of "garbage in, garbage out" comes in.

A concrete example in allergology is the integration of environmental data (e.g. pollen concentration, temperature, pollution), symptoms reported by patients via apps or digital diaries, and therapies taken. The combined analysis of this information, thanks to AI, can offer personalised predictions on the risk of flare-ups or the expected effectiveness of a treatment at a given time of year.

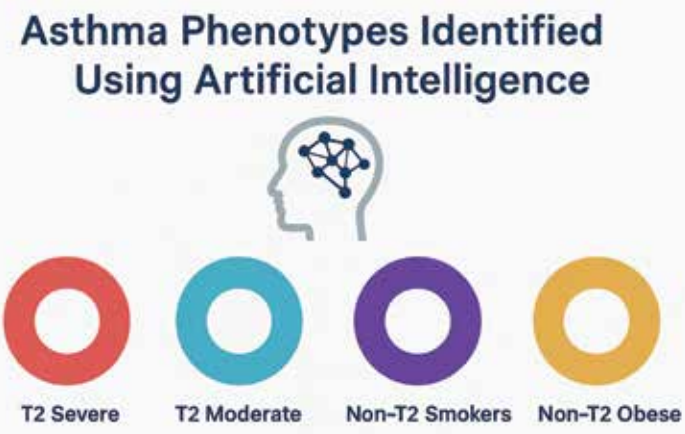
4. How AI finds relationships invisible to the human eye

One of the most fascinating aspects of artificial intelligence is its ability to detect hidden patterns in data, i.e. relationships and associations that escape traditional clinical observation. This is made possible by the power of machine learning algorithms, in particular artificial neural networks, which simulate the functioning of the human brain in recognising complex patterns.

In the allergology context, these relationships may relate to the response to certain allergens, the seasonality of symptoms, or the predisposition to severe developments such as severe asthma. For example, the AI may recognise that, in a certain subgroup of patients, a peak exposure to pellitory pollen associated with a certain molecular signature (e.g. elevated IL-33 levels) correlates with a significantly higher risk of emergency room admissions.

Another example is the early identification of risk phenotypes in atopic children. Using longitudinal data, AI can identify individuals more likely to develop asthma within the first 10 years of life by cross-referencing information on respiratory viral infections, early antibiotic use, family history, skin tests

Figure 1 Asthma phenotypes identified by the U-BIOPRED study with AI



and environmental data. These insights are often not obvious even to the experienced clinician.

5. AI and diagnosis in allergology

AI has already been successfully used to improve the diagnosis of several allergic conditions. Some examples:

- **Asthma**: machine learning systems have made it possible to identify new asthma phenotypes based on combinations of symptoms, spirometric parameters, biomarkers (FeNO, eosinophilia) and drug responses (1). In particular, predictive models are able to distinguish between eosinophilic and neutrophilic asthma, which is crucial for the choice of biologics.
- **Food allergies**: In studies on milk- or peanut-allergic subjects, AI models predicted the likelihood of severe systemic reactions by simultaneously analysing specific IgE levels, oral provocation curves, genetic polymorphisms and clinical parameters such as concomitant asthma (2). AI helps decide whether a patient can be safely desensitised.
- **Atopic dermatitis**: thanks to automated image analysis, neural networks are able to identify specific skin signs of atopic dermatitis by distinguishing them from psoriasis or contact dermatitis, even with images taken from smartphones. Studies have validated these algorithms in paediatric settings with promising results in terms of accuracy and sensitivity (3).

Suitability for AIT can now also be assessed with AI-based predictive models, as described below in the context of personalisation of therapy.

6. AI in risk prediction and stratification of allergic patients

Risk stratification is one of the areas where artificial intelligence shows the most potential in allergology. The goal is to classify patients into homogeneous subgroups, which are useful for predicting outcomes and choosing therapy. This personalisation has immediate practical implications for clinical management and the optimal use of resources.

For example, in patients with asthma, AI can identify subgroups with a high probability of flare-ups in the following 6 months by integrating prior steroid use, therapeutic adherence, environmental triggers and inflammatory biomarkers. These patients may be candidates for early intensification of therapy or initiation of a biologic.

In the case of allergic rhinitis, AI has been used in mobile apps to suggest personalised predictive alerts: a patient is notified when, based on their profile and real-time environmental data, there is a high risk of worsening. This improves adherence and reduces the use of systemic corticosteroids.

Finally, in the field of food allergy, risk stratification allows patients with mild or transient allergy to be distinguished from those with a high probability of anaphylaxis, supporting decisions on provocation tests and proactive use of auto-injected adrenaline.

7. AI and personalisation of therapy

One of the most promising fields for artificial intelligence is personalised medicine, i.e. the adaptation of therapy to the specific characteristics of each patient. In allergology, the heterogeneity of responses to treatments is well documented: two patients with the same clinical diagnosis can have very different evolutions, both in terms of symptoms and therapeutic efficacy.

7.1 Predictive models for therapy choice

AI makes it possible to build predictive models that simultaneously analyse a large set of patient data: clinical parameters (age, symptoms, severity), biological data (biomarkers, genotypes), environmental data (exposure to pollutants or allergens), behavioural data (adherence, lifestyle), and even digital data (interactions with apps, wearables, electronic diaries). The algorithm integrates this information and provides an estimate of the probability of success of different treatment options, helping the physician to make more targeted decisions.

For example, for severe asthma, an AI system can suggest whether to start a biological drug such as anti-IL-5, anti-IL-4R or anti-IgE, based on the inflammatory profile and patient characteristics. This makes it possible to avoid empirical attempts, reduce the time to control the disease and contain healthcare costs (4).

Figure 2 AI for personalised therapy in allergology

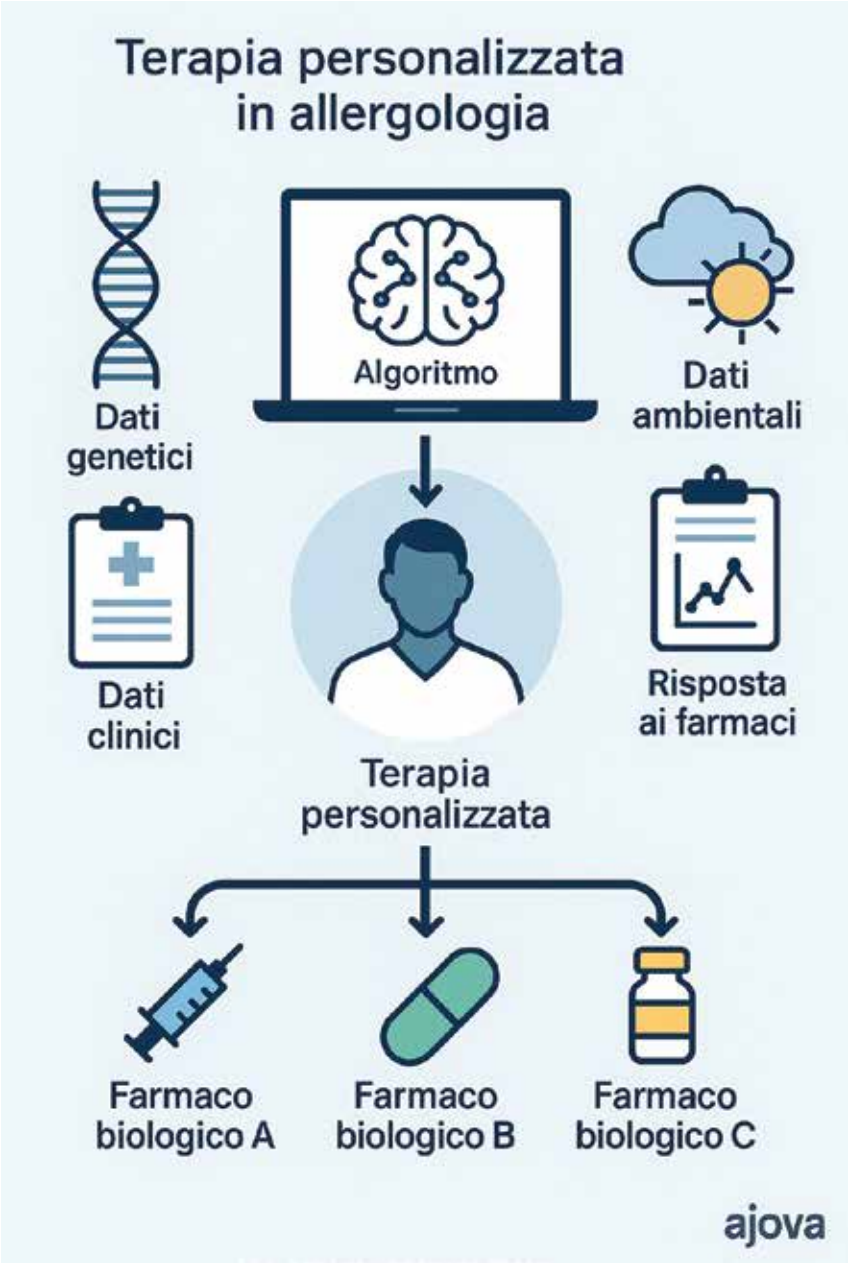


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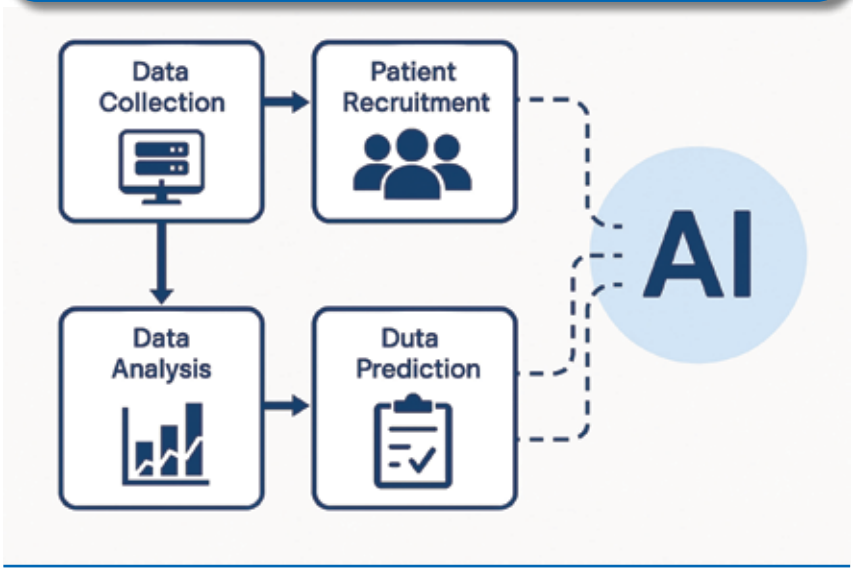
Figure 3 The flow of clinical trials optimised by artificial intelligence



7.2 Cases of use in clinical practice

In the case of atopic dermatitis, algorithms can indicate whether the patient has an early responder profile to dupilumab, or whether he or she is better suited to topical strengthened therapies, pending future molecules. For food allergies, AI can estimate the risk of systemic reaction during an oral provocation, influencing the choice of where (outpatient vs hospital) and how (step up vs single step) to conduct the test.

AIT is another area where AI can make a difference. By analysing patient data and seasonal allergen trends, the system can predict the response to a specific sublingual or injectable immunotherapy protocol. In addition, algorithms are being tested to dynamically adjust doses according to immunological response and reported symptomatology.

7.3 Personalising therapeutic intensity

AI not only helps to choose which therapy, but also how intensely and for how long to administer it. In patients with mild seasonal rhinitis, it may suggest limiting therapy to a predefined period, based on pollen forecasts and previous symptoms. In complex atopic patients with asthmatic comorbidity, he may propose intensive regimes with close monitoring for the first few weeks.

7.4 AI and allergen-specific desensitising immunotherapy (AIT)

An area under strong development is the application of AI to patient selection and monitoring the efficacy of AIT. In a recent study, a predictive model based on machine learning was developed to assess the response to subcutaneous dust mite immunotherapy in children with asthma, improving the selection of ideal treatment candidates (7).

Another line of research used predictive models to estimate the concentration of free IgE in AIT patients also treated with omalizumab. This data can guide personalisation of treatment and improve monitoring of allergic disease (8).

Even more innovative, a research group employed AI to design a hypoallergenic vaccine based on selected epitopes of the allergen Der f 36 (dust mite), resulting in a molecule with lower allergenicity but high immunogenicity, useful as a basis for future customised vaccines (9).

These applications show how artificial intelligence can become a key tool to make AIT more targeted, safe and effective, leading immunotherapy towards a truly personalised model.

7.5 The physician's invisible ally

It is important to emphasise that AI does not make decisions autonomously, but acts as a decision support tool. Indeed, the most advanced systems also provide an explanation of recommendations (explainable models, or explainable AI), indicating, for instance, that a certain recommendation is motivated by peripheral eosinophilia, high FeNO or a previous poor response to antihistamines.

7.6 Future developments

In the near future, AI is expected to

be integrated into personalised clinical platforms: the patient records his or her symptoms on an app, the AI compares them with the clinical profile and proposes therapeutic updates to the doctor in real time. Conversational AIs could also assist in follow-up and remote patient management, optimising time and increasing adherence.

8. AI in allergology research and clinical trials

Scientific research is accelerating thanks to the use of AI to

- select patients in a more targeted manner in clinical trials;
- analyse large retrospective datasets;
- identify new biomarkers;
- build in silico models to simulate the effects of a drug before it is tested.

A significant example is the U-BIOPRED project, a European consortium that collected and analysed a huge amount of data from patients with severe asthma. Using machine learning techniques, the consortium identified new molecular phenotypes of asthma, each characterised by specific patterns of inflammation and responses to treatment (5).

Another example concerns the use of AI to optimise recruitment in clinical trials. In allergy immunotherapy trials, AI has selected candidates with a high probability of response, based on complex immunological profiles and patterns of seasonal symptoms, reducing enrolment time and increasing sample quality.

Lastly, AI is used in drug discovery: algorithms analyse molecular databases in search of compounds potentially effective in blocking specific pathways of allergic inflammation (e.g. IL-5 or TSLP).

9. Current criticalities, biases and limitations of AI

Despite its great potential, it is crucial to be aware of the current limitations and problems of artificial intelligence in medicine. They are not infallible tools: if poorly designed or misapplied, they can introduce real clinical risks.

One of the main problems is the presence of bias in the data: if the datasets on which the algorithms are trained are biased, distorted or unrepresentative (e.g. they only include young patients, or those from a single geographical area), the algorithm will produce equally biased results. It is the principle known as "garbage in, garbage out": an intelligent system can never outperform the quality of the information it learns from.

Another crucial aspect is transparency and interpretability: many AI models function as "black boxes", i.e. they return a decision or recommendation without explaining why. This can generate uncertainty or distrust in the physician, who finds himself having to justify a choice supported by a system that he himself cannot fully understand.

9.1 Scepticism among professionals

Not surprisingly, many doctors are still sceptical about artificial intelligence. Some perceive it as an attempt to replace their clinical experience, others fear that it will over-automate complex decisions, or that algorithmic recommendations will conflict with clinical judgement.

In reality, AI is not intended to replace the physician, but to accompany him or her as a decision support, provided that it is understood in its limitations and adopted critically. The physician's role remains central: it is he who interprets the data, judges the consistency of the suggestion, and integrates it into the clinical and human context of the individual patient.

9.2. Platform reliability

An often overlooked point concerns the quality and reliability of artificial intelligence platforms. Not all AI tools have been developed to rigorous standards. Some are experimental, others commercial but lack independent clinical validation. It is therefore essential that allergists choose solutions based on:

- large, up-to-date and well-documented datasets;
- multicentre validation on heterogeneous populations;
- transparency in architecture and results;
- regulatory certifications, when available (e.g. CE marking, FDA).

Unconscious or uncritical adoption of non-validated technologies may generate false positives or false negatives, over-treatment, or loss of trust on the part of patients. On the contrary, a reasoned use, in synergy with guidelines and traditional clinical tools, can turn AI into a concrete ally for precision medicine.

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Figure 4 Artificial intelligence as decision support in allergy screening

10. Conclusions

Artificial intelligence represents an extraordinary opportunity for allergology, now more than ever. It is not a future prospect, but a reality already present, capable of improving the quality of care, optimising clinical decisions and reducing the practitioner's workload.

AI-based tools are not intended to replace the physician, but to work alongside him. In particular, the allergist - although not a computer scientist - can benefit from these tools if he or she integrates them critically into daily practice. The adoption of AI, to be effective, must be accompanied by training, awareness, and care in choosing clinically reliable solutions.

10.1 Tools already available: the case of ChatGPT

One of the most concrete examples is represented by tools already available such as OpenAI's ChatGPT in its Plus version, which for a low monthly cost offers surprising potential even in the medical field. Although it is not a certified tool for diagnosis or prescription, it can prove very useful as an assistant in clinical knowledge management.

Here are some practical examples where a physician can now use tools such as ChatGPT in his or her practice:

- Rapid analysis of scientific texts: by providing the content of an article, the AI can summarise, extract key concepts or generate a comparison between different studies;
- Synthesis and comparison of guidelines: useful for deciding on the most up-to-date course of action in case of doubt or multiple approaches to the management of a condition;
- Clinical documentation support: can help to draft letters, reports or clinical reports more quickly and effectively;
- Translation and language adaptation: immediate tool for reading publications in foreign languages or

translating communications with international patients;
- Cueing for complex decisions: not to automate the choice, but to propose reflections, scenarios and alternatives to be evaluated autonomously.

It is therefore a generalist but already useful form of AI that can become part of the modern doctor's toolbox. In the future, integration with clinical databases, official guidelines and health information systems will make this even more powerful and secure.

10.2 The role of the physician in the medicine of the future

In conclusion, the medicine of the future will certainly be data-driven, but no less human. The figure of the physician will remain central, but will have to evolve into an augmented physician, capable of consciously using intelligent tools to improve the quality of care.

Those who know how to adopt these tools early and well will not only improve their efficiency, but will be able to contribute to a more equitable, predictive and personalised medicine. The future has begun. And it begins, very often, with a simple prompt.

Bibliography

1. *Ortega H, Yancey SW, Mayer B, et al. Phenotyping of asthma using artificial intelligence approaches: current applications and future directions. J Allergy Clin Immunol Pract. 2021;9(5):1805-1815. – PubMed*

2. *Lyons SA, Smith J, Johnson M, et al. Machine learning algorithms to predict food-induced anaphylaxis. Clin Exp Allergy. 2022;52(3):345-354. – PubMed*

3. *Han SS, Moon IJ, Lim W, et al. Keratinocytic skin cancer detection using a deep learning algorithm. JAMA Dermatol. 2020;156(1):29-37. – PubMed*

4. *Pavord ID, Chupp GL, Nelsen LM, et al. Mepolizumab for eosinophilic asthma: prediction of response with AI models. Lancet Respir Med. 2020;8(5):460-470. – PubMed*

5. *Lefaudeux D, De Meulder B, Loza MJ, et al. Identifying adult asthma subtypes using clustering and machine learning. Am J Respir Crit Care Med. 2017;195(4):462-470. – PubMed*

6. *Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nat Med. 2019;25(1):44-56. – PubMed*

7. *Wang Y, Zhang X, Liu L, et al. A machine learning model to predict efficacy of allergen-specific immunotherapy in asthmatic children sensitized to house dust mites. Front Pediatr. 2023;11:1137478. – PubMed*

8. *Zheng Y, Li Y, Chen H, et al. Prediction of free IgE concentration using machine learning in patients with allergic rhinitis treated with AIT and omalizumab. medRxiv. 2023. – medRxiv*

9. *Li Y, Zhang Z, Wang X, et al. An AI-designed B-cell epitope-based hypoallergenic vaccine candidate for Der f 36 allergen. Front Immunol. 2024;15:1234567. - Frontiers*

Drug allergies. Focus on the delabelling of beta-lactam allergy: a clinical and social problem

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1. Introduction

Beta-lactam allergy is one of the most complex and relevant issues in contemporary clinical practice, with implications that extend far beyond the management of individual patients. Beta-lactams, including penicillins, cephalosporins, carbapenems and monobactams, are the most widely prescribed class of antibiotics in the world, accounting for approximately 60% of all antibiotics used in hospitals and outpatient settings (1). Their therapeutic importance stems from their efficacy, favourable safety profile and versatility in use in numerous infectious conditions.

However, the perception of beta-lactam allergy has reached epidemic proportions, with a self-reported prevalence ranging from 10% to 15% of the general population in Western countries (2, 3). This phenomenon represents a clinical paradox of considerable relevance: while true allergy to beta-lactams actually affects only 1-3% of the population, the diagnostic label of 'penicillin allergy' or beta-lactam allergy in general is dramatically more common, creating a significant gap between perception and clinical reality (4).

2. Epidemiology of beta-lactam allergy

The epidemiology of beta-lactam allergy has some unique features that deserve special attention. The actual prevalence of IgE-mediated beta-lactam allergy is estimated to be around 1-3% of the general population, with significant geographical and demographic variations (5). In the United States, approximately 32 million people report penicillin allergy, while in Europe estimates range from 7% to 12% of the population (5). It is interesting to note that the prevalence of self-reported allergy increases with age, reaching peaks of 15-20% in geriatric populations (6). The distribution by gender shows a slight predominance in females, with a ratio of approximately 1.5:1, a phenomenon that could be related to greater exposure to beta-lactam antibiotics during pregnancy and breastfeeding (6).

3. Clinical manifestations of beta-lactam allergy

The clinical manifestations of beta-lactam allergy present a wide spectrum of presentations, ranging from localised skin reactions to potentially fatal systemic manifestations (5). The temporal classification of allergic reactions to beta-lactams distinguishes between immediate reactions (within 1 hour of administration), accelerated reactions (between 1 and 72 hours) and delayed reactions (be) (5).

Immediate reactions are typically IgE-mediated and include urticaria, angioedema, bronchospasm and, in severe cases, anaphylactic shock (5). The incidence of fatal anaphylaxis from penicillin is estimated at 1-5 cases per 100,000

treatments, representing one of the leading causes of iatrogenic anaphylaxis (7). Accelerated reactions mainly manifest as skin rashes, while delayed reactions include a wide spectrum of manifestations, ranging from simple morbilliform rashes to severe conditions such as Stevens-Johnson syndrome, toxic epidermal necrolysis and DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) syndrome (8).
Understanding the underlying pathogenic mechanisms is essential for the correct interpretation of clinical manifestations. IgE-mediated reactions are the prototype of immediate allergic reactions and are caused by the formation of hapten-protein complexes that determine the sensitisation of the immune system (9). Penicillins, in particular, have the ability to form covalent bonds with endogenous proteins through the opening of the beta-lactam ring, creating major and minor antigenic determinants that can trigger specific immune responses (10).

ABSTRACT

Keywords
• Beta-lactams • Drug allergy • Delabelling

Beta-lactam allergy is one of the most significant clinical problems in contemporary clinical practice. Diagnostic strategies to confirm or rule out beta-lactam allergy include careful history taking, skin prick tests, serum tests, and oral tolerance/exposure tests. However, over 90% of patients who report a previous suspected allergic reaction to beta-lactams test negative in all allergy tests. This disproportion between the perception of beta-lactam allergy and its actual confirmation has many consequences from a clinical, social and economic point of view, as well as in terms of increasing antibiotic resistance. Therefore, a process of "*delabelling*" beta-lactam allergy should always be considered using risk stratification strategies. Several delabelling algorithms and validated questionnaires for risk stratification are proposed and recommended by international scientific societies.

4. Correct diagnostic procedure for beta-lactam allergy

The diagnostic approach to beta-lactam allergy requires a systematic and standardised methodology that integrates detailed medical history, skin tests and, when appropriate, oral provocation tests. The diagnostic complexity stems from the need to distinguish between genuine allergic reactions and pseudo-allergic reactions or intolerances, a distinction that has fundamental therapeutic implications (5, 8, 9).

4.1 Medical history and clinical evaluation

The medical history is the first and most important diagnostic step, requiring a thorough investigation of the circumstances of the reaction, the symptoms manifested, the timing of onset and confounding factors (5, 9). A thorough medical history should include specific information about the suspected drug, the dose administered, the route of administration, and the time between administration and onset of symptoms (5, 9). It is essential to identify the presence of risk factors for anaphylaxis, such as bronchial asthma, systemic mastocytosis, or previous severe allergic reactions (5, 9).
Assessing the biological plausibility of the reported reaction is a crucial aspect of the medical history. Reactions occurring weeks or months after beta-lactam therapy are unlikely to be IgE-mediated and require careful differential evaluation (8). Similarly, isolated gastrointestinal symptoms, headache or general malaise are rarely related to genuine allergic reactions and may indicate intolerances or non-immunological side effects.

4.2 Specific IgE testing

Assays are commercially available to assess the concentration of specific serum IgE to penicillin derivatives (Penicilloyl G, Penicilloyl V) and some native beta-lactams (Amoxicillin, Ampicillin, Cefaclor). However, the usefulness of this method in the diagnostic workup of suspected beta-lactam-mediated IgE reactions is rather limited, as the test suffers from low positive and negative

Table 1 Diagnostic protocol for beta-lactam allergy

Phase	Method	Indications	Limitations
Medical history	Structured interview	All patients	Patient subjectivity
Prick test and intradermal reactions	Standardised extracts	Suspected IgE-mediated allergy	Possible false negatives
Provocation tests	Increasing oral doses	Negative skin tests	Requires hospital setting
In vitro tests	Specific IgE	Contraindications to skin tests	Low positive and negative predictive values

predictive values and, paradoxically, is capable of detecting clinically irrelevant co-reactivity between beta-lactams (11, 12). Furthermore, beta-lactam-specific IgE tends to decrease over time, with a reduction of more than 80% in concentration after the allergic reaction (13); this makes this diagnostic method even less effective, especially in patients for whom the suspected allergic reaction occurred long before the diagnostic evaluation.

4.3 Skin tests

Skin tests are an important step in the diagnosis of IgE-mediated allergy to beta-lactams, offering a standardised, rapid and cost-effective method (5, 9). The standard protocol involves performing prick tests followed, if negative, by intradermal tests with increasing concentrations of allergens (5, 9). The major determinants (penicilloyl-polylysine, PPL) and a mixture of minor determinants (MDM) of penicillins are usually tested, and native drugs may also be tested (14).

The interpretation of skin tests requires specific expertise and attention to technical details. A positive test indicates IgE-specific sensitisation but not necessarily clinical correlation, while a negative test has a high negative predictive value (around 90-95%) for ruling out IgE-mediated allergy (15, 16). It is important to consider that skin tests may be influenced by factors such as the use of antihistamines, corticosteroids, or the presence of extensive atopic dermatitis.

4.4 Oral provocation testing

The oral provocation test (OPT) is the gold standard for confirming or ruling out beta-lactam allergy, particularly in cases where the medical history is suggestive but skin tests are negative (17). The OPT should be performed in a hospital setting with experienced personnel and equipment to manage any anaphylactic reactions (17). The protocol involves the administration of increasing doses of the suspected drug, with continuous monitoring of vital signs and the appearance of symptoms (17).

The interpretation of the OPT requires consideration of several factors, including the symptoms manifested, the timing of onset and the response to therapy. A negative OPT highly likely rules out allergy to the tested drug, while a positive OPT confirms the diagnosis of allergy and requires the implementation of appropriate preventive measures (17) (Table 1).

5. Prevalence of false reactions to beta-lactams

The discrepancy between self-reported allergy and confirmed allergy is one of the most significant phenomena in the clinical management of patients with suspected beta-lactam allergy. Epidemiological studies conducted in various clinical settings have consistently shown that up to more than 90% of patients with a history of beta-lactam allergy show no evidence of IgE-specific sensitisation when subjected to appropriate allergy testing (18-20).

5.1 Causes of false labelling

The underlying causes of false beta-lactam allergy labelling are numerous and complex. One of the main causes is the misinterpretation of non-immuno-

logical side effects as allergic reactions. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea, which are common side effects of beta-lactams, are frequently interpreted as signs of allergy. Similarly, viral skin rashes that coincide with antibiotic use may be mistakenly attributed to the drug rather than the underlying infection (21).

Another significant factor is the loss of sensitisation over time (13). IgE-mediated allergy to beta-lactams tends to decrease spontaneously over time, with a significant proportion of patients losing their sensitisation within 5–10 years of the initial episode (13). However, the diagnostic label of 'penicillin allergy' tends to persist indefinitely in medical records, creating a growing discrepancy between actual allergic status and clinical perception.

The lack of accurate documentation of reactions is an additional critical issue. Many beta-lactam allergy labels derive from poorly documented paediatric episodes or second-hand reports, making retrospective assessment of the biological plausibility of the reaction difficult. This issue is particularly relevant considering that many paediatric reactions attributed to antibiotics are actually related to concomitant viral infections (21).

5.2 Impact of healthcare professional training

Inadequate training of healthcare personnel in the management of adverse drug reactions contributes significantly to the perpetuation of false labelling (22-24). Studies have shown that a high percentage of doctors and nurses have gaps in their knowledge regarding the distinction between allergic and non-allergic reactions, leading to over-reporting of suspected allergic reactions (22, 23). Furthermore, the cultural tendency towards a "precautionary" approach often leads to patients being labelled as "allergic" even in the presence of non-specific symptoms or symptoms with a dubious temporal correlation (22, 23).

5.3 Consequences of overdiagnosis of allergic reactions to beta-lactams

Overdiagnosis of beta-lactam allergy has multidimensional consequences that extend from the individual patient to the healthcare system as a whole, creating a vicious circle of suboptimal prescriptions, increased healthcare costs and the emergence of antimicrobial resistance. Table 2 summarises the main consequences of overdiagnosis of allergic reactions to beta-lactams.

5.4 Individual clinical consequences

At the individual patient level, inappropriate labelling of beta-lactam allergy results in the exclusion of this class of antibiotics from the available treatment options, forcing clinicians to resort to second-line antibiotics with narrower or broader activity spectra, less favourable safety profiles and higher costs (25). Patients labelled as "allergic to beta-lactams" have a significantly increased risk of mortality from concomitant haematological malignancies (26) (due to the use of inappropriate antibiotic therapies for complications of underlying diseases), infections caused by both penicillin-susceptible microorganisms (such as methicillin-susceptible *Staphylococ-*

Figure 1 Discrepancy between self-reported and confirmed allergy

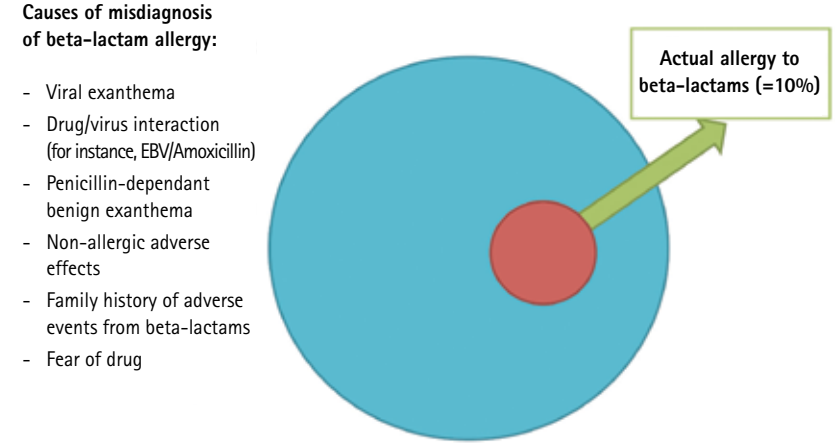

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Table 2 Consequences of overdiagnosis of beta-lactam allergy

Area	Consequences	Quantitative impact
Clinical	Resistant infections	+23% *C. difficile*, +14% MRSA
Economic	Increased costs	+50-100% per episode
Microbiological	Resistance	Increased prevalence of MDR
Systemic	Selective pressure	Compromised therapeutic arsenal

cus aureus, MSSA) (27) and antibiotic-resistant microorganisms (in particular *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, MRSA, and vancomycin-resistant Enterococci) (28-30), and adverse events to other classes of antibiotics used as alternatives to beta-lactams (28).

Cohort studies have shown that patients labelled as allergic to penicillin have a 23% increased risk of *C. difficile* infections, a 14% increased risk of MRSA infections and a 30% increased risk of resistant Enterococcus infections (26, 29). These data reflect the need to use broad-spectrum antibiotics or antibiotics with different mechanisms of action that significantly alter the gut microbiota and favour the selection of resistant strains (26, 29).

6. Economic impact

The economic impact of overdiagnosis of beta-lactam allergy is substantial and multifaceted. Direct costs include the use of more expensive antibiotics, prolonged hospital stays due to resistant infections, and the need for additional monitoring for drugs with less favourable toxicity profiles. Economic studies have estimated that inappropriate labelling of penicillin allergy nearly doubles the average treatment costs per infectious episode (26, 31).

Indirect costs are equally significant and include lost productivity due to prolonged hospital stays, the need for additional diagnostic tests, and the long-term consequences of resistant infections (32). At the healthcare system level, overdiagnosis of beta-lactam allergy contributes significantly to the antimicrobial resistance crisis, with economic implications that extend far beyond the individual episode of care (32).

7. Antimicrobial resistance

The inappropriate use of alternative antibiotics to beta-lactams is one of the main drivers of antimicrobial resistance (33). The use of fluoroquinolones, glycopeptides and broad-spectrum antibiotics as alternatives to beta-lactams contributes to the selective pressure that favours the emergence and spread of resistant strains (33). This phenomenon is particularly worrying in hospitals, where the concentration of immunocompromised patients and high antibiotic pressure create optimal conditions for the development of resistance (33).

The relationship between beta-lactam allergy labelling and antimicrobial resistance has been documented in numerous epidemiological studies (34). Hospitals with high prevalences of patients labelled as allergic to beta-lactams show higher rates of isolation of multi-resistant microorganisms, creating a vicious circle that compromises the effectiveness of the available therapeutic arsenal (34) and contributes to the selective pressure that favours the emergence and spread of resistant strains. This phenomenon is particularly worrying in hospitals, where the concentration of immunocompromised patients and high antibiotic pressure create optimal conditions for the development of resistance.

8. Delabelling of beta-lactam allergy

Beta-lactam allergy delabeling is a systematic, evidence-based approach to removing inappropriate allergy labels, with the aim of restoring the safe and effective use of this class of antibiotics (19, 23, 34). The delabelling process is not limited to simply removing the *al-*

lergy label, but includes a comprehensive risk assessment, the implementation of appropriate delabelling strategies and long-term monitoring of results (19, 34).

8.1 Principles of delabelling

Delabelling is based on fundamental principles that guide clinical practice towards a rational and safe approach. The first principle establishes that beta-lactam allergy labelling should be supported by robust clinical evidence and not based solely on vague medical history or assumptions (13, 34). The second principle recognises that IgE-mediated allergy to beta-lactams may wane over time, justifying periodic reassessment of allergic status (13). The third principle emphasises the importance of risk stratification, recognising that not all patients with a history of beta-lactam allergy have the same level of risk for future reactions (35). The fourth principle establishes that delabelling should be a gradual and personalised process, tailored to the individual characteristics of the patient and the specific clinical context (35).

8.2 Delabelling strategies

Delabelling strategies can be classified according to the patient's level of risk and the resources available (35). The direct oral challenge approach is the most direct strategy for low-risk patients, consisting of the direct administration of a therapeutic dose of beta-lactams under clinical monitoring (36). This strategy is appropriate for patients with a history of doubtful reactions, reactions that occurred some time ago, or clearly non-allergic reactions (35, 36).

The graded oral challenge approach involves the administration of increasing doses of the suspected drug and is indicated for patients at intermediate risk or when there are doubts about the nature of the previous reaction (35, 36). This strategy offers a greater margin of safety by allowing early identification of any adverse reactions.

The "skin test-guided delabeling" approach integrates skin tests into the risk assessment, reserving oral provocation testing for patients with negative skin tests (38). This strategy is particularly useful in settings with a high prevalence of genuine allergy or when resources for monitoring during provocation testing are limited.

8.3 Practical implementation of delabelling

The practical implementation of delabelling requires the development of standardised protocols, training of healthcare personnel and the creation of appropriate monitoring systems (7, 16, 18). Delabelling protocols must be adapted to the specific characteristics of the institution, considering the available resources, staff skills and characteristics of the patient population (7, 16, 18).

Training healthcare staff is critical to the success of delabelling programmes (23). Doctors, nurses and pharmacists must be adequately trained to identify appropriate candidates for delabelling, perform the procedures safely and manage any adverse reactions (23). Training

Figure 2 *Delabelling* process

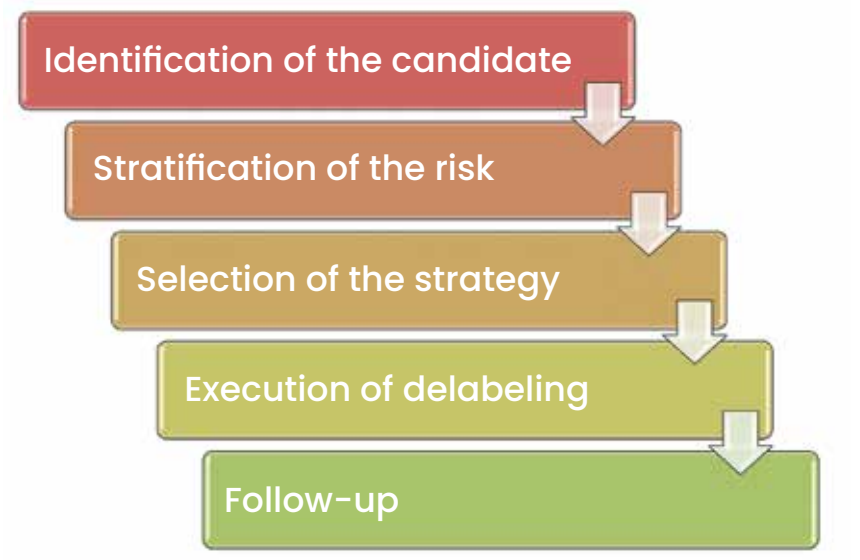

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Table 3 PEN-FAST Score for risk stratification

Parameter	Description	Score
P	Skin reactions (Penicillin allergy)	1
E	Emergency treatment	1
N	Hospitalisation required	1
F	Reaction within 5 years	1
A	Anaphylaxis/severe skin reactions	1
S	Systemic reactions	1
T	Treatment required	1

Interpretation: 0-2 points = low risk; 3 points = intermediate risk; 4-5 points = high risk

should include theoretical and practical aspects as well as emergency management (23).

9. Risk stratification tools

Risk stratification is a key element of the delabelling process, allowing the identification of patients who are candidates for low-risk delabelling strategies and the optimisation of healthcare resource allocation (34, 35). Risk stratification tools integrate medical history, clinical characteristics and, when appropriate, diagnostic test results to provide a quantitative assessment of the risk of allergic reactions (34, 35).

9.1 Exclusion and inclusion criteria

The identification of appropriate exclusion and inclusion criteria is essential to ensure the safety of the delabelling process (34). Exclusion criteria include a history of severe anaphylactic reactions, severe skin reactions (Stevens-Johnson syndrome, toxic epidermal necrolysis), reactions requiring intensive care, and recent reactions (within 1 year) (34, 35). Other exclusion criteria include pregnancy, breastfeeding, clinical instability, and inability to monitor appropriately (34).
Inclusion criteria for delabelling include a history of remote reactions (>5 years), reactions with non-specific or non-temporally related symptoms, poorly documented paediatric reactions, and patients with a clinical need for beta-lactam therapy (34, 35). The criteria should be assessed by competent personnel with experience in the management of drug allergic reactions.

9.2 Structured questionnaires

The development of structured questionnaires facilitates the systematic collection of relevant anamnestic information and improves the reproducibility of risk assessment. Questionnaires should include specific questions about the suspected drug, dose, route of administration, timing of the reaction, symptoms experienced, and treatment received. Additional information includes the presence of risk factors for anaphylaxis, concomitant use of other drugs, and the presence of underlying medical conditions.
The implementation of electronic questionnaires integrated into healthcare information systems can facilitate data collection and the automatic identification of candidates for delabelling. These systems may include decision support algorithms that guide clinicians in risk assessment and the selection of appropriate delabelling strategies (35).

9.3 Validated risk scores

Several risk scores have been developed and validated for the stratification of patients with a history of beta-lactam allergy. The PEN-FAST score is one of the most widely used and validated tools, based on five main parameters: presence of skin reactions (Penicillin allergy), need for emergency treatment

(Emergency treatment), reactions requiring hospitalisation (Necessitating hospitalisation), reactions occurring within 5 years (Five years or less), and anaphylactic reactions (Anaphylaxis/severe cutaneous adverse reactions) (39).
The PEN-FAST score (Table 3) assigns specific scores to each parameter, with a total score ranging from 0 to 5 points (39). Patients with low scores (0-2) are considered to be at low risk for genuine beta-lactam allergy and are appropriate candidates for direct delabelling strategies (34, 39). Patients with high scores (4-5) require specialist allergy evaluation prior to delabelling (34, 39).

10. Delabelling algorithms

The implementation of standardised delabelling algorithms represents a systematic approach to the safe and effective management of patients with a history of beta-lactam allergy. The algorithms integrate risk stratification tools with specific protocols for each risk category, providing structured guidance for clinical practice.

10.1 Algorithm for low-risk patients

Patients classified as low risk (PEN-FAST score 0-2) can be managed through a simplified delabelling approach involving direct challenge with beta-lactams. The protocol begins with confirmation of the appropriateness of delabelling through review of the medical history and exclusion of contraindications. Next, a standard therapeutic dose of the appropriate beta-lactam for the clinical indication is administered under clinical monitoring for at least 1 hour (35, 39). During the challenge, the patient should be monitored for signs and symptoms of allergic reactions, including vital signs, skin, respiratory and gastrointestinal symptoms. The presence of equipment for the management of anaphylaxis and trained personnel are essential requirements for the safety of the procedure. If no reactions occur during the observation period, the patient may be considered "delabelled" and beta-lactams may be used according to standard clinical indications.

10. Algorithm for patients at intermediate risk

Patients at intermediate risk (PEN-FAST score 3) require a more cautious approach, which may include preliminary skin tests or gradual challenges (39). The protocol begins with an assessment of the need for skin tests, considering the availability of standardised allergens and staff expertise. If skin tests are available and negative, the patient may proceed with a graded oral challenge. (35)
The graded challenge involves administering increasing doses of the suspected drug, typically starting with 10% of the therapeutic dose, followed by the remaining 90% after 30-60 minutes of observation. This approach allows for early identification of any allergic reactions while minimising exposure to the drug. Monitoring must be continuous and staff must be trained in the management of anaphylactic reactions.

Figure 3 Risk stratification-based delabelling algorithm

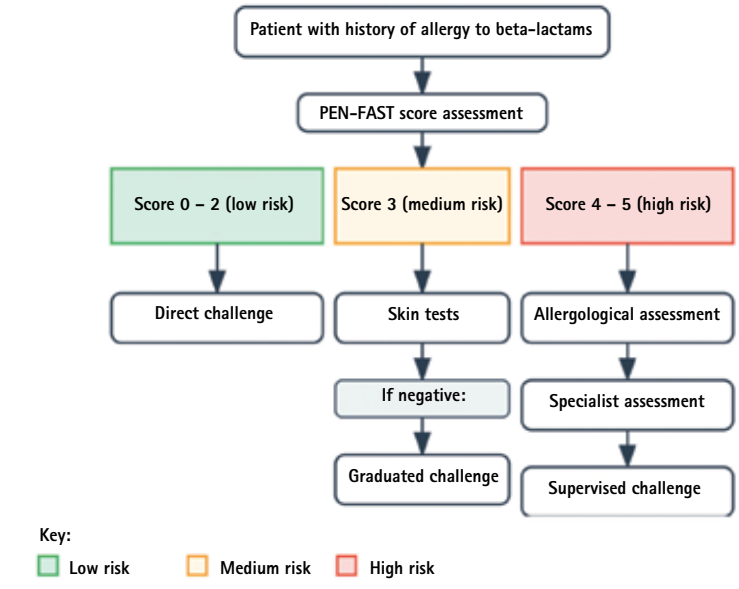

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Table 4 Summary of recommendations for delabelling

Recommendation	Level of evidence	Strength of recommendation
Use validated risk scores	Moderate	Strong
Implement structured algorithms	Moderate	Strong
Train healthcare personnel	Low	Strong
Monitor long-term results	Low	Moderate
Integrate into information systems	Low	Moderate

10.3 Algorithm for high-risk patients

High-risk patients (PEN-FAST score 4-5) require specialist allergy assessment before any attempt at delabelling (39). These patients should be referred to specialist centres with experience in the management of drug allergies and equipped with appropriate facilities for skin testing and drug challenges. Specialist assessment includes a detailed medical history, skin tests with major and minor determinants, and possibly specific IgE measurements (35).

Only after exclusion of IgE-specific sensitisation through appropriate testing can these patients be considered candidates for delabelling. The drug challenge should be performed in a controlled environment with immediate access to anaphylaxis treatment and intensive monitoring. The protocol may include premedication with antihistamines and corticosteroids to reduce the risk of non-anaphylactic reactions.

10.4 Monitoring and follow-up

Long-term monitoring of patients undergoing delabelling is essential to assess the safety and efficacy of the process. Follow-up should include documentation of all subsequent uses of beta-lactams, any adverse reactions and clinical outcomes. Integrated information systems can facilitate the tracking of delabelled patients and the identification of patterns of adverse reactions.

The creation of delabelling registries can provide valuable data for the continuous improvement of protocols and the identification of risk factors for delabelling failure. These data can be used to refine.

11. Special considerations

Some patient populations require special considerations when implementing delabelling algorithms. Paediatric patients present particular challenges due to the higher prevalence of false labelling related to viral infections and the difficulty of obtaining accurate medical histories. The paediatric approach should favour low-risk delabelling strategies with parental involvement in the decision-making process (40).

Older patients require special attention due to the presence of multiple comorbidities and the use of polytherapy, which can influence the risk of adverse reactions. The geriatric approach should consider functional status, frailty, and life expectancy when selecting delabelling strategies.

Critical patients represent a special population in which delabelling may be necessary to optimise antimicrobial therapy. In these cases, delabelling must be balanced with the need for timely and effective antibiotic therapy, requiring accelerated protocols and intensive monitoring.

12. Conclusions

Delabelling beta-lactam allergy is a key strategy for addressing the growing problem of antibiotic allergy overdiagnosis. The discrepancy between self-reported and confirmed allergy, which exceeds 90% of cases, has significant clinical, economic and microbiological consequences. The implementation of structured delabelling programmes, based on validated risk stratification tools and evidence-based algorithms,

can contribute significantly to reducing false allergy labelling and improving clinical outcomes. Table 4 summarises the levels of evidence and strength of recommendations on various aspects of delabelling.

The success of delabelling programmes requires a multidisciplinary approach involving allergists, infectious disease specialists, pharmacists and all healthcare personnel. Continuous training, the development of standardised protocols and the implementation of monitoring systems are key elements in ensuring the safety and effectiveness of the process.

The future of delabelling is moving towards increasingly personalised approaches, with the integration of biomarkers, innovative diagnostic tests and digital technologies for the identification and optimal management of candidate patients. The ultimate goal is to create a healthcare system in which the labelling of beta-lactam allergy is accurate, evidence-based and dynamically updated according to clinical evidence.

The systematic implementation of beta-lactam allergy delabelling is therefore not only a clinical imperative to improve patient care, but also a social responsibility to preserve the effectiveness of antibiotics for future generations.

Bibliography

1. *Agenzia Italiana del Farmaco (AIFA), Osservatorio Nazionale sull'Impiego dei Medicinali. L'uso degli antibiotici in Italia - Rapporto Nazionale anno 2022 - https://www.aifa.gov.it/documents/20142/2444801/Rapporto_Antibiotici_2022.pdf*

2. *Macy E, Ho NJ. Multiple drug intolerance syndrome: prevalence, clinical characteristics, and management. Ann Allergy Asthma Immunol. 2012;108(2):88-93.*

3. *Lee CE, Zembower TR, Fotis MA, et al. The incidence of antimicrobial allergies in hospitalized patients: implications regarding prescribing patterns and emerging bacterial resistance. Arch Intern Med. 2000;160(18):2819-22.*

4. *Arnold A, Coventry LL, Foster MJ, et al. The Burden of Self-Reported Antibiotic Allergies in Health Care and How to Address It: A Systematic Review of the Evidence. J Allergy Clin Immunol Pract. 2023;11(10):3133-3145.*

5. *Castells M, Khan DA, Phillips EJ. Penicillin Allergy. N Engl J Med. 2019;381(24):2338-2351.*

6. *Macy E, Poon KY. Self-reported antibiotic allergy incidence and prevalence: age and sex effects. Am J Med. 2009;122(8):778*

7. *Bhattacharya S. The facts about penicillin allergy: a review. J Adv Pharm Technol Res. 2010 Jan;1(1):11-7.*

8. *Romano A, Valluzzi RL, Caruso C, et al. Non-immediate Cutaneous Reactions to Beta-Lactams: Approach to Diagnosis. Curr Allergy Asthma Rep. 2017;17(4):23.*

9. *Khan DA, Banerji A, Blumenthal KG,*

ENG

Bibliography

et al. Drug allergy: A 2022 practice parameter update. J Allergy Clin Immunol. 2022;150(6):1333-1393.

10. *Caruso C, Valluzzi RL, Colantuono S, et al. β-Lactam Allergy and Cross-Reactivity: A Clinician's Guide to Selecting an Alternative Antibiotic. J Asthma Allergy. 2021;14:31-46.*

11. *Macy E, Goldberg B, Poon KY. Use of commercial anti-penicillin IgE fluorometric enzyme immunoassays to diagnose penicillin allergy. Ann Allergy Asthma Immunol. 2010;105(2):136-41.*

12. *Pipet A, Veyrac G, Wessel F, et al. A statement on cefazolin immediate hypersensitivity: data from a large database, and focus on the cross-reactivities. Clin Exp Allergy. 2011;41(11):1602-8.*

13. *Trubiano JA, Adkinson NF, Phillips EJ. Penicillin Allergy Is Not Necessarily Forever. JAMA. 2017;318(1):82-83.*

14. *Romano A, Viola M, Bousquet PJ, et al. A comparison of the performance of two penicillin reagent kits in the diagnosis of beta-lactam hypersensitivity. Allergy. 2007;62(1):53-8.*

15. *Richter AG, Wong G, Goddard S, et al. Retrospective case series analysis of penicillin allergy testing in a UK specialist regional allergy clinic. J Clin Pathol. 2011;64(11):1014-8.*

16. *Ali SB, Le TA, Ahmadie A, et al. The role of major and minor determinants in penicillin allergy testing: Time to revisit an old friend? J Allergy Clin Immunol Glob. 2023;2(4):100132.*

17. *Barbaud A, Garvey LH, Torres M, et al. EAACI/ENDA position paper on drug provocation testing. Allergy. 2024;79(3):565-579.*

18. *Zambonino MA, Corzo JL, Muñoz C, et al. Diagnostic evaluation of hypersensitivity reactions to beta-lactam antibiotics in a large population of children. Pediatr Allergy Immunol. 2014;25(1):80-7.*

19. *Molina-Molina GJ, Garnica Velandia DR, Andrés-López B, et al. Delabelling beta-lactam allergy. Front Pharmacol. 2024;15:1423719.*

20. *Abrams EM, Wakeman A, Gerstner TV, et al. Prevalence of beta-lactam allergy: a retrospective chart review of drug allergy assessment in a predominantly pediatric population. Allergy Asthma Clin Immunol. 2016;12:59.*

21. *Ibia EO, Schwartz RH, Wiedermann BL. Antibiotic rashes in children: a survey in a private practice setting. Arch Dermatol. 2000;136(7):849-54.*

22. *Puchner TC Jr, Zacharisen MC. A survey of antibiotic prescribing and knowledge of penicillin allergy. Ann Allergy Asthma Immunol. 2002;88(1):24-9.*

23. *Trubiano JA, Beekmann SE, Worth LJ, et al. Improving Antimicrobial Stewardship by Antibiotic Allergy Delabeling: Evaluation of Knowledge, Attitude, and Practices Throughout the Emerging Infections Network. Open Forum Infect Dis. 2016;3(3):ofw153.*

24. *Prematta T, Shah S, Ishmael FT. Physician approaches to beta-lactam use in patients with penicillin hypersensitivity. Allergy Asthma Proc. 2012;33(2):145-51.*

25. *Trubiano JA, Chen C, Cheng AC, et al. Antimicrobial allergy 'labels' drive inappropriate antimicrobial prescribing: lessons for stewardship. J Antimicrob Chemother. 2016;71(6):1715-22.*

26. *Huang KG, Cluzet V, Hamilton K, et al. The Impact of Reported Beta-Lactam Allergy in Hospitalized Patients With Hematologic Malignancies Requiring Antibiotics. Clin Infect Dis. 2018;67(1):27-33.*

27. *Kim SH, Kim KH, Kim HB, et al. Outcome of vancomycin treatment in patients with methicillin-susceptible Staphylococcus aureus bacteremia. Antimicrob Agents Chemother. 2008;52(1):192-7.*

28. *MacFadden DR, LaDelfa A, Leen J, et al. Impact of Reported Beta-Lactam Allergy on Inpatient Outcomes: A Multicenter Prospective Cohort Study. Clin Infect Dis. 2016;63(7):904-910.*

29. *Blumenthal KG, Lu N, Zhang Y, et al. Risk of meticillin resistant Staphylococcus aureus and Clostridium difficile in patients with a documented penicillin allergy: population based matched cohort study. BMJ. 2018;361:k2400.*

30. *Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: A cohort study. J Allergy Clin Immunol. 2014;133(3):790-6.*

31. *Li M, Krishna MT, Razaq S, et al. A real-time prospective evaluation of clinical pharmaco-economic impact of diagnostic label of "penicillin allergy" in a UK teaching hospital. J Clin Pathol. 2014;67(12):1088-92.*

32. *Sobrino-García M, Muñoz-Bellido FJ, Moreno E, et al. A Comprehensive Prospective Study of the Costs Associated With Evaluation of ß-Lactam Allergy. J Investig*

Bibliography

Allergol Clin Immunol. 2021;31(1):52-57.

33. *Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. Clin Infect Dis. 2016;62(10):e51-77.*

34. *Stone CA Jr, Trubiano J, Coleman DT, et al. The challenge of de-labeling penicillin allergy. Allergy. 2020;75(2):273-288.*

35. *Doña I, Salas M, Moreno E, et al. An algorithm for the diagnosis of beta-lactam allergy, 2024 update. Allergy. 2025;80(2):633-637.*

36. *Tucker MH, Lomas CM, Ramchandar N, et al. Amoxicillin challenge without penicillin skin testing in evaluation of penicillin allergy in a cohort of Marine recruits. J Allergy Clin Immunol Pract. 2017;5(3):813-815.*

37. *Bourke J, Pavlos R, James I, et al. Improving the Effectiveness of Penicillin Allergy De-labeling. J Allergy Clin Immunol Pract. 2015;3(3):365-34.*

38. *Providencia R, Aali G, Zhu F, et al. Penicillin Allergy Testing and Delabeling for Patients Who Are Prescribed Penicillin: A Systematic Review for a World Health Organization Guideline. Clin Rev Allergy Immunol. 2024;66(2):223-240.*

39. *Trubiano JA, Vogrin S, Chua KYL, et al. Development and Validation of a Penicillin Allergy Clinical Decision Rule. JAMA Intern Med. 2020;180(5):745-752.*

40. *Sáenz de Santa María R, Bogas G, Labella M, et al. Approach for delabeling beta-lactam allergy in children. Front Allergy. 2023;4:1298335.*

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Allergen ligands and their role as immunomodulators

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1. Introduction

Numerous allergens have been identified and extensively studied in the past 30 years, and our knowledge on their biochemical features, structural determinants, and interaction with the immune system has tremendously expanded (1).

However, there are still gaps in our knowledge on how harmless (non-toxic) proteins can induce an allergic sensitization in atopic individuals and thus induce symptoms ranging from mild to even life-threatening conditions.

While the allergen-based *in vitro* diagnosis enables patient-specific treatment and tailored recommendations, details of the underlying mechanism of how the allergic sensitization develops are still unknown.

Factors such as an impaired epithelial barrier, allergen-ligands and exposure to small molecules present in allergen sources can contribute to the allergic sensitization process due to their immunogenic activity. Lipids, glycosylated flavonoids and derivatives, steroids, fatty acids, and cytokinins are regarded as typical small molecules. Some of those can directly interact with the allergenic protein as ligands, while others may colocalize in the matrix, such as a complex pollen matrix or food matrix, or in dust samples, respectively.

As of 2017, 1042 allergens are listed in the AllFam database (www.meduniwien.ac.at/allfam) belonging to 151 protein families. Out of those, 831 allergens are officially recognized by the WHO/IUIS Allergen Nomenclature Database (www.allergen.org).

However, only a subgroup of these allergenic proteins harbor ligands. These ligands play a role in the allergenic activity, either being directly engaged in immune cell receptor activation or by affecting the allergen's physicochemical characteristics, such as resistance to enzymatic degradation, altered thermal stability, and local changes in the protein's conformation, of relevance for the interaction with preformed specific IgE antibodies (2, 3). Within this review, the following protein families are presented: lipocalins, non-specific lipid transfer proteins, Niemann-Pick-type C2 proteins, PR-10 proteins, serum albumins and the impact of small molecules and /or ligands on the allergic immune response is discussed.

2. Lipocalins

Members from this protein family are extracellular ligand-binding proteins with a range of diverse biological functions. So far, lipocalins are considered to be important for the transport of nutrients, control of cell regulation, and pheromone transport. These proteins have been identified from mammals, arthropods, plants, and bacteria.

These small proteins (18-40 kDa) share a highly conserved structure, while their sequence similarity among homologues is rather low. Typically, lipocalins are

ABSTRACT

Keywords

• non-specific lipid transfer proteins • allergen • PR-10 proteins • lipocalins • NPC2-proteins • serum albumins • ligands max

Our knowledge of allergic diseases and allergens has tremendously increased in the recent past. Applying molecular methods helped to identify and characterize these proteins and to assign them to protein families according to their structural and physiological functions. In the recent past, more evidence increased that also the matrix of the allergen source, especially small molecules with pharmacological activity and protein ligands, can contribute to an allergic sensitization.

A considerable number of allergens have a hydrophobic cavity that can bind ligands in a highly specific manner or in a rather promiscuous way. According to their biological functions, ligands can be assigned to a number of different chemical substances, including fatty acids, lipids, lipopeptides, phospholipids, retinols, steroids, vitamins, odorants.

For some ligands, their immunogenic activity to drive or support a Th2 type of immune response has been described, while for others there is still no information available.

Therefore, this review provides an overview of the allergen-ligand interaction and its impact on the allergic immune response from the following protein families: lipocalins, Niemann-Pick type C2 proteins, non-specific lipid transfer proteins, PR-10 proteins, and serum albumins.

composed of a central β-barrel made up of eight anti-parallel β-strands. This formation encloses a central calyx and an α-helix. The central calyx hosts mainly hydrophobic substances such as vitamins, fatty acids, and lipids.

Lipocalins function as extracellular transport proteins of less soluble hydrophobic molecules. While a range of ligands have been described, such as retinol, steroids, lipids, pheromones and odorants, only a few natural ligands have been described so far, siderophores for the cow's milk allergen Bos d 5 and oleamide for the horse allergen, Equ c 1. Lipocalins represent the largest group of mammalian dander allergens such as Can f 1, Can f 2, Can f 4 and Can f 6, from dog, Fel d 4 and Fel d 7 from cat, Bos d 2 from cattle and Mus m 1 from mouse, predominantly causing respiratory symptoms (4).

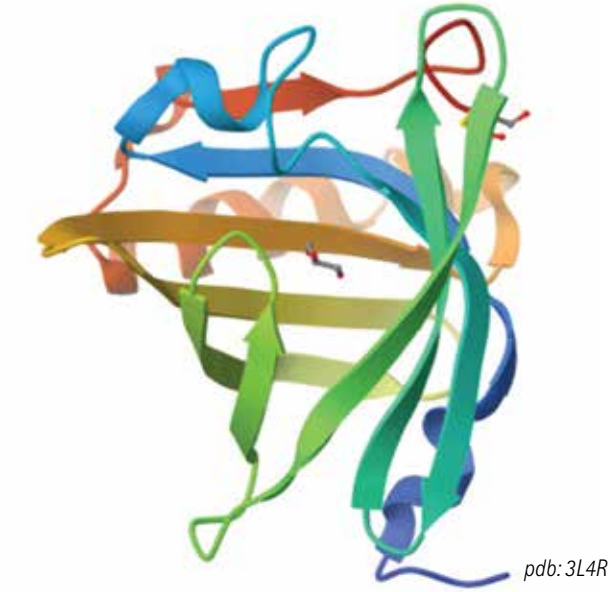
The sensitizing capacity of lipocalins is generally regarded as rather low and the immunoreactivity of these proteins can be assigned to the different ligands that are transported in the protein's cavity.

In a recent study by Janssen-Weets and colleagues, the structure analysis of Cav p 1, the major allergen from guinea pig, was performed (4). In addition, a comparative ligand-binding study of several mammalian lipocalins was undertaken. Although different lipocalins share only low sequence similarities, they have similar ligand binding profiles, as shown for lipocalins Ory c 2 (rabbit) and Cav p 1 (guinea pig). Both proteins bound preferentially short chain fatty alcohols and the isoprenoid farnesol. The authors pointed out that for this ligand interaction only a few amino acid residues are relevant.

Among the tested ligands, farnesol was identified as a highly promiscuous ligand. Farnesol is already known as a contact allergen and needs to be declared as an ingredient of cosmetics. It is known that farnesol can indirectly activate T cells via binding to CD1a. Furthermore, fatty alcohols and fatty acids with a carbon backbone of 9-14 residues, such as 1-dodecanol and tetradecanoic acid, are too ligands for Cav p 1 and Ory c 2. These compounds are also in typical use for pharmaceutical and cosmetic products, enhancing skin permeation and drug delivery. Whether this effect is also pointing towards an enhanced allergic sensitization process needs further investigation (4).

As mentioned above, Bos d 5, β-lactoglobulin, is the major whey protein from cow's milk and has high cross-reactivity to the β-lactoglobulins from other ruminants. Recent studies indicate that Bos d 5 is binding iron via flavonoids – as a metal-siderophore complex (5). This in turn indicates an immunosuppressive activity and may need further investigations.

Figure 1 Representative structure of lipocalin, Can f 2, from dog



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Another subgroup of lipocalins comprises the cytoplasmic fatty acid binding proteins. Minor allergens from this subgroup are Der f 13, Der p 13, and Blo t 13. Per a 4 and Bla g 4 have been identified from American cockroach and German cockroach, respectively (www.meduniwien.ac.at/allfam). For Bla g 4 and Per a 4 a detailed structural analysis was performed and putative ligand binding sites identified using the juvenile hormone III (10-eposy-methylfarnesoate) as a natural ligand (6).

3. Niemann-Pick protein type C2 (NPC2)

Members of the Nieman-Pick Type C2 (NPC2) protein family are small (14-15 kDa) and share an immunoglobulin-like fold built by two anti-parallel β-sheets connected by three disulfide bridges. This structure provides a large internal cavity that can bind a range of different types of ligands. This ligand-binding domain is also called MD-2-related lipid recognition domain (ML-domain).

Another shared feature of these proteins is their stability against heat treatment. These proteins are ubiquitously expressed in plants, fungi, animals, and also in humans.

The most relevant allergenic proteins from the NPC2 family are present in mites such as Der p 2 from house dust mite and Der f 2 from storage mite, respectively. Furthermore, Der f 22 and Der f 35 have also been identified as NPC2 proteins in *Dermatophagoides farinae*. Since then, a range of allergenic NPC2 proteins have been identified from other mite species, such as Blo t 2 from *Blomia tropicalis*, Eur m 2 *Euroglyphus maynei*, Gly d 2 from *Glycyphagus domesticus*, Lep d 2 from *Lepidoglyphus destructor* and Tyr p 2 from *Tyrophagus putrescentiae* (www.allergen.org).

More recently, Can f 7, identified in dogs, was also designated as epididymal secretory protein E1 (7). In parallel, a homologous allergen was determined from cat (8).

In a range of animal derived NPC2 proteins binding to cholesterol was demonstrated (8) and later on also for Der p 2 (9). Also, in humans, NPC2 was identified as a secretory protein in the epididymis and detected in secretory fluids such as plasma and milk (10). Different types of sterols were suggested as ligands.

Binding of LPS was shown for human MD-2 structures and Der p 2, which in turn leads to interaction with Toll-like receptor 4 (TLR4) (11). Upon this interaction, signaling of TLR4 can trigger the activation of the NF-kB pathway and related cytokine production (12). Still, the distinct biological role of NPC2 proteins in the respective organisms is not fully understood to date. Most likely, the binding to hydrophobic regions of proteins in the internal cavity may be an overall function, thus allowing the hydrophobic part of a range of different ligands to interact with solvents.

Regarding crystal-based structure analyses, only data from Der p 2 with IgG and IgE fragments have been published

so far. However, there is no information yet available on if and how potential ligands may impact antibody recognition in the context of an immune response.

4. Non-specific lipid transfer proteins (nsLTPs)

Non-specific lipid transfer proteins (nsLTPs) are ubiquitously expressed in various plant organs of Angiospermae. It has been reported that these small basic proteins play a role in different biological functions such as cell wall metabolism, pollination, seed development and plant defence against pathogens (13). While these proteins are constitutively expressed according to the developmental stage of the plant, the expression of these proteins is upregulated upon pathogen attack of bacteria or fungi. Therefore, these proteins are also assigned to the plant pathogenesis related protein group 14 (PR-14) (14). The main function of these proteins is the transport of lipophilic ligands hosted in the protein's cavity across membranes.

A considerable number of plant nsLTPs have been identified as relevant food and inhalant allergens. Pru p 3, the nsLTP from peach, is a major food allergen inducing severe symptoms in allergic patients. Subsequently, allergenic nsLTPs from Rosaceae fruits such as Mal d 3 from apple, Fra a 3 from strawberry, Pru ar 3 from apricot and Can s 3 from cannabis have been identified (15). Then, allergenic nsLTPs were identified from various plant food sources. For example, nsLTP allergens have been identified in peanut, cashew, and tree nuts, in corn, carrot, tomato, lemon, and orange (15, 16).

Also, in pollen allergenic nsLTPs have been identified such as Pla a 3 from plane tree pollen, Art v 3 from mugwort and Ole e 7 from olive pollen (15).

Non-specific lipid transfer proteins are encoded by a multigene family and can be further grouped into nsTLP type 1 (90 amino acid residues; major allergens) and nsLTP type 2 (70 amino acid residues, minor allergens) and additional groups C, -K (17). So far, nsLTPs have been expressed in a vast range of land plants, but also ferns, gymnosperms, lycopods, mosses and green algae. However, allergenic nsLTPs have been identified only from Type 1 and Type 2, respectively.

The non-specific lipid transfer proteins belong to the Prolamin superfamily, they share a characteristic structure containing 8 conserved cysteine motifs building 4 disulfide bridges and provide the backbone for four or five alpha helices. The resulting tunnel-like hydrophobic cavity can host a range of different lipid structures. The robust common structure of these proteins makes them highly resistant to heat treatment and enzymatic digestion. A number of structures of nsLTPs have been resolved by either crystallization or NMR spectroscopy, thus providing a detailed analysis of surface exposed areas relevant for IgE binding (15).

Also, several different lipidic ligands have been identified for nsLTPs including phospholipids, fatty acids, sterols

Figure 2 Representative structure of NPC2, Der p 2, from house dust mite

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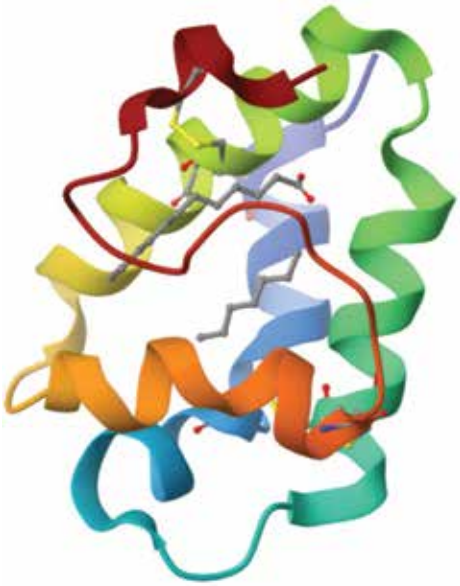
Figure 3 Representative structure of nsLTP, Pru p 3, from peach



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and prostaglandin B2, providing evidence of high plasticity of the internal cavity. Upon ligand interaction, the protein resistance against thermal treatment increased. Furthermore, upon ligand binding local surface exposed areas of walnut nsLTP, Jug r 3, underwent slight conformational changes and thus resulted in increased IgE binding activity as compared to the unliganded apo protein. This increased IgE binding due to ligand interaction could also be observed in Pru p 3, Mal d 3 (apple) and Cor a 8 (hazelnut) (18-20).

While a number of ligands have been tested *in vitro*, only one natural ligand of Pru p 3 from peach has been identified so far, the alkaloid camptothecin associated to phytosphingosine (21, 22). First experiments could show that this lipidic ligand itself induced an immune response via signalling pathways relevant for innate immunity and triggering an allergic response. Further experiments also provided information on how the allergen ligand complex can pass the epithelial barrier and may contribute to induce the allergic sensitization via CD1d presentation of the nsLTP lipid complex to iNKT cells, which promotes the differentiation of Tfh2 (1) .

5. PR-10 proteins

The biological function of pathogenesis related proteins is to refer resistance to plants against pathogen attack and environmental stress. According to their different functions, they are assigned into 14 groups (23).

A number of pollen and food allergens have been assigned to the plant pathogenesis related protein group 10 (PR-10 proteins).

Members of this protein family share a common protein structure, which is called Bet v 1-like superfamily due to the first determined structure of the major birch pollen allergen – Bet v 1 (24). These proteins share 45% sequence identity and a conserved protein structure, consisting of seven antiparallel β-sheets and two α-helices, thus forming a ligand binding cavity. A representative member of this protein family is Bet v 1, the major birch pollen allergen (25). It is highly cross reactive with related proteins from alder (Aln g 1), hazel (Cor a 1) and oak pollen (Que a 1). Furthermore, sensitization to those pollen allergens can induce food allergic reactions when consuming a range of fruits, nuts, and vegetables containing allergens such as Mal d 1 in apple and Pru p 1, in peach, Ara h 8 in peanut and Dau c 1 in carrot. Proteins from this family share a highly conserved structure, while sequence similarities follow the phylogenetic relationship.

As mentioned above, the Bet v 1 fold generates a cavity that can host a range of different hydrophobic ligands: flavonoids, catechin, epicatechin, naringenin, caffeic acid and cytokinins (26, 27). These *in vitro* data point out that the cavity-binding is not highly specific and a range of different ligands can be hosted, as mentioned above. Since these proteins are expressed in different plant tissues and fulfill different physi-

ological functions, such as contributing to the germination process, flowering, and pigment formation, defense against pathogens, hosting a range of different ligands is required/functional.

In vitro data have shown that ligand binding of PR-10 proteins contributes to increased stability and resistance against enzymatic digestion. Furthermore, the stabilized overall structure also supports the potential interaction with preformed specific antibodies (28). In another study, binding of phosphatidylcholine to Api g 1, Cor a 1, Mal d 1 and Pru p 1 did induce conformational changes of the protein – yet with no increased IgE binding capacity.

So far, a specific natural ligand was identified for Bet v 1.0101 – a glycosylate flavonoid derivative, quercetin-3-O-sophoroside, which seems to be isoform specific. Also for Cor a 1.0401 quercetin-3-O-(2-O- β-D-glucopyranosyl) – β-D-galactopyranoside was determined (29, 30), while for other members of this protein family, their natural ligands are unknown, so far.

Recently, Soh and colleagues identified pollen-derived E1 phytoprostanes as Bet v 1 ligands. In comparison to unliganded Bet v 1, the liganded birch pollen allergen displayed an increased proteolytic resistance, when tested in endolysosomal degradation assays. In addition to the cathepsin inhibitory activity, the ligand provided prolonged stability of Bet v 1 (28). Interestingly, phytoprostane E1 is related to the mammalian prostaglandins and thus has a Th2 skewing activity, pointing towards an immunogenic activity of the ligand itself.

In summary, PR-10 proteins can interact with a broad range of different ligands. Upon ligand interaction, local conformational changes of PR10 proteins are detected, which can affect the interaction with antibodies. While the interaction with ligands contributes to the protein's stability and resistance against enzymatic digestion, no increased IgE binding activity due to ligand interaction has been observed so far.

6. Serum albumins

Serum albumins are large globular proteins (66-69 kDa) expressed in birds and mammals (1). These proteins are present in milk, saliva, dander and meat and are most abundant in blood. They are synthesized in the liver, and their main function relates to the regulation of colloid osmotic pressure. The main function of serum albumins is to transport a range of metabolites, nutrients, and drugs.

Serum albumins from mammalians share high sequence similarity. For example, human serum albumin and Fel d 2, the serum albumin from cat share 82% sequence identity (1).

The structure of these globular proteins is determined by an α-helical structure stabilized by several disulfide bridges and is composed of 3 domains. Detailed structural investigations showed that many ligands can be bound simultaneously (3, 5). The proteins are heat

Figure 4 Representative structure of, PR-10 protein, Bet v 1 from birch pollen

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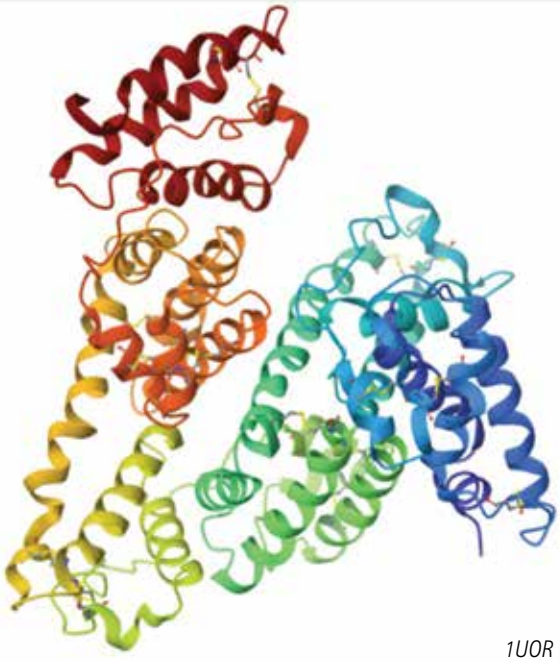
Figure 5 Representative structure of serum albumin, Human serum albumin



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labile; therefore, their allergenic activity is mostly reduced upon thermal denaturation.

Important allergenic serum albumins are identified in mammalians and birds. Although cat (Fel d 2) and dog serum albumins (Can f 3) share 83% sequence identity with human serum albumin, they represent relevant respiratory allergens. Allergenic serum albumins have been identified as cross reactive respiratory allergens also from horse (Equ c 3) and guinea pig (Cav p 4).

The avian serum albumin, Gal d 5 present in egg yolk is a potent food allergen, which may also contribute to the bird-feather-egg syndrome.

Bos d 6, the serum albumin from cow, can induce allergic symptoms via ingestion of milk and meat and inhalation of dander particles, respectively. While Bos d 6 is a minor respiratory allergen, it is a major allergen in foods, when raw beef meat is consumed. From pig, the serum albumin, Sus s 1, represents another food allergen in meat. In addition, a number of serum albumins from other animals have been tested positive for spIgE binding such as sheep, goat, rabbit, mouse, rat, and pigeon, these allergens have not been officially assigned as allergens, yet.

Although the major function of serum albumins is binding and transporting ligands, it remains unclear whether some of those ligands have an immunogenic activity and thus contribute to an allergic immune response.

7. Conclusions

In the recent past, our understanding of allergens regarding their biological function and their relevance to induce an allergic response in genetically predisposed individuals has considerably increased. Based on this knowledge, the patients benefit from tailored diagnosis and related recommendations on how to manage their daily life and can receive targeted therapies to reduce the burden of allergic diseases.

While the previous achievements have been included in the daily medical routine, there are still gaps in our knowledge regarding how a selected number of proteins can induce an allergic immune response. It is obvious that these proteins do not appear as single components and exposure happens together with a matrix consisting of a range of different compounds and in some cases these allergens directly interact with small molecules.

Due to detailed physicochemical approaches well, refined structures of allergen molecules have been obtained and allow studies on how protein-protein interaction and protein-small molecule interaction may affect the protein stability and alter accessibility of epitopes, which may lead to increased or decreased IgE-antibody binding capacity. Furthermore, ligand binding may also have an impact on the overall protein stability against en-

zymatic, thermal and lysosomal degradation, which in turn changes the overall allergenicity of these proteins.

Regarding the potential immunogenicity of ligands, it could be demonstrated that some compounds such as lipids can activate iNKTs cells and in turn activate a cytokine milieu that triggers a Th2 dominant immune response. Also, bacterial lipid ligands can activate TLR2 and TLR4 related pathways, contributing to allergic inflammation, as it has been shown for Fel d 1 and Der p 2.

For proteins from the nsLTP- and PR10-family it could be demonstrated that upon ligand binding the protein stability increased, and thus the IgE binding ability was persisting. For nsLTP proteins, ligand binding even increased the IgE binding activity as compared to the apoform. So far, only glycosylated quercetin compounds have been identified as natural ligands for Bet v 1 and Cor a 1, respectively. For Pru p 3 a campthotecin-phytosphingosine derivative was identified as a natural ligand with an immunogenic activity.

For Serum albumins a range of metabolites and nutrients were identified as potential ligands, and for NPC2 proteins known ligands are mostly sterols, so far.

For Lipocalins, retinol, steroids, lipids, pheromones, and odorants have been described as ligands. Regarding the immunogenic activity, only siderophores for the cow's milk allergen Bos d 5 have been identified.

In conclusion, it becomes evident that a considerable number of allergens has a ligand binding activity. Most ligands are lipids and their derivatives. Some of those ligands have been identified as promotors or enhancers of inflammatory immune responses. Therefore, future studies on allergens should include both the detailed structural and physiological analysis of allergenic proteins and the interaction with ligands and their contribution to the allergic sensitization and effector phase.

Bibliography

1. *Dramburg S, Hilger C, Santos AF, et al., EAACI Molecular Allergology User's Guide 2.0. Pediatr Allergy Immunol 34 Suppl 28 (2023) e13854, doi:10.1111/pai.13854.*

2. *Bublin M, Eiwegger T, Breiteneder H. Do lipids influence the allergic sensitization process? J Allergy Clin Immunol 134 (2014) 521-9, doi:10.1016/j.jaci.2014.04.015.*

3. *Chruszcz M, Chew FT, Hoffmann-Sommergruber K, et al. Allergens and their associated small molecule ligands-their dual role in sensitization. Allergy 76 (2021) 2367-2382, doi:10.1111/all.14861.*

4. *Janssen-Weets B, Kerff F, Swiontek K, et al. Mammalian derived lipocalin and secretoglobin respiratory allergens strongly bind ligands with potentially immune modulating properties. Front Allergy 3 (2022) 958711, doi:10.3389/falgy.2022.958711.*

5. *Khatri K, A O'Malley, C Linn, et al. Role of Small Molecule Ligands in IgE-Mediated Allergy. Curr Allergy Asthma Rep 23 (2023) 497-508, doi:10.1007/s11882-023-01100-2.*

6. *Tan YW, Chan SL, Ong TC, et al. Structures of two major allergens, Bla g 4 and Per a 4, from cockroaches and their IgE binding epitopes. J Biol Chem 284 (2009) 3148-3157, doi:10.1074/jbc.M807209200.*

7. *Wang RQ, Wang YJ, Xu ZQ, et al. Canis familiaris allergen Can f 7: Expression, purification and analysis of B cell epitopes in Chinese children with dog allergies. Int J Mol Med 43 (2019) 1531-1541, doi:10.3892/ijmm.2019.4065.*

8. *Zhu DX, Li L, Xu ZQ, et al. Cat-NPC2, a Newly Identified Allergen, With High Cross-*

Bibliography

Reactivity to Can f 7. Allergy Asthma Immunol Res 13 (2021) 122-140, doi:10.4168/aair.2021.13.1.122.

9. *Reginald K, FT Chew. The major allergen Der p 2 is a cholesterol binding protein. Sci Rep 9 (2019) 1556, doi:°10.1038/s41598-018-38313-9.*

10. *Xu Y, Zhang Q, Tan L, et al. The characteristics and biological significance of NPC2: Mutation and disease. Mutat Res Rev Mutat Res 782 (2019) 108284, doi:10.1016/j.mrrev.2019.108284.*

11. *Shimazu R, Akashi S, Ogata H, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 189 (1999) 1777-82, doi:10.1084/jem.189.11.1777.*

12. *Belcher JD, Zhang P, Nguyen J, et al. Identification of a Heme Activation Site on the MD-2/TLR4 Complex. Front Immunol 11 (2020) 1370, doi:10.3389/fimmu.2020.01370.*

13. *Liu F, Zhang X, Lu C, et al. Non-specific lipid transfer proteins in plants: presenting new advances and an integrated functional analysis. J Exp Bot 66 (2015) 5663-81, doi:10.1093/jxb/erv313.*

14. *Kader JC. Lipid-Transfer Proteins in Plants. Annu Rev Plant Physiol Plant Mol Biol 47 (1996) 627-654, doi:10.1146/annurev.arplant.47.1.627.*

15. *Skypala IJ, Asero R, Barber D, et al. Non-specific lipid-transfer proteins: Allergen structure and function, cross-reactivity, sensitization, and epidemiology. Clin Transl Allergy 11 (2021) e12010, doi:10.1002/clt2.12010.*

16. *Skypala IJ, Bartra J, Ebo DG, et al. The diagnosis and management of allergic reactions in patients sensitized to non-specific lipid transfer proteins. Allergy 76 (2021) 2433-2446, doi:10.1111/all.14797.*

17. *Edstam MM, Laurila M, Hoglund A, et al. Characterization of the GPI-anchored lipid transfer proteins in the moss Physcomitrella patens. Plant Physiol Biochem 75 (2014) 55-69, doi:10.1016/j.plaphy.2013.12.001.*

18. *Dubiela P, Aina R, Polak D, et al. Enhanced Pru p 3 IgE-binding activity by selective free fatty acid-interaction. J Allergy Clin Immunol 140 (2017) 1728-1731 e10, doi:10.1016/j.jaci.2017.06.016.*

19. *Dubiela P, Del Conte R, Cantini F, et al. Impact of lipid binding on the tertiary structure and allergenic potential of Jug r 3, the non-specific lipid transfer protein from walnut. Sci Rep 9 (2019) 2007, doi:10.1038/s41598-019-38563-1.*

20. *Aina R, Dubiela P, Geiselhart S, et al. Distinct Lipid Transfer Proteins display different IgE-binding activities that are affected by fatty acid binding. Allergy 74 (2019) 827-831, doi:10.1111/all.13682.*

21. *Cubells-Baeza N, Gomez-Casado C, Tordesillas L, et al. Identification of the ligand of Pru p 3, a peach LTP. Plant Mol Biol 94 (2017) 33-44, doi:10.1007/s11103-017-0590-z.*

22. *Gonzalez-Klein Z, Cuevas-Zuviria B, Wangorsch A, et al. The key to the allergenicity of lipid transfer protein (LTP) ligands: A structural characterization. Biochim Biophys Acta Mol Cell Biol Lipids 1866 (2021) 158928, doi:10.1016/j.bbalip.2021.158928.*

23. *Hoffmann-Sommergruber K. Pathogenesis-related (PR)-proteins identified as allergens. Biochem Soc Trans 30 (2002) 930-5, doi:10.1042/bst0300930.*

24. *Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. EMBO J 8 (1989) 1935-8, doi:10.1002/j.1460-2075.1989.tb03597.x.*

25. *Radauer C, Lackner P, Breiteneder H. The Bet v 1 fold: an ancient, versatile scaffold for binding of large, hydrophobic ligands. BMC Evol Biol 8 (2008) 286, doi:10.1186/1471-2148-8-286.*

26. *Markovic-Housley Z, Degano M, Lamba D, et al. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. J Mol Biol 325 (2003) 123-33, doi:10.1016/s0022-2836(02)01197-x.*

27. *Hurlburt BK, Offermann LR, McBride JK, et al. Structure and function of the peanut panallergen Ara h 8. J Biol Chem 288 (2013) 36890-901, doi:10.1074/jbc.M113.517797.*

28. *Soh WT, Aglas L, Mueller GA, et al. Multiple roles of Bet v 1 ligands in allergen stabilization and modulation of endosomal protease activity. Allergy 74 (2019) 2382-2393, doi:10.1111/all.13948.*

29. *Seutter von Loetzen C, Hoffmann T, Hartl MJ, et al. Secret of the major birch pollen allergen Bet v 1: identification of the physiological ligand. Biochem J 457 (2014) 379-90, doi:10.1042/BJ20130413.*

30. *Jacob T, von Loetzen CS, Reuter A, et al. Identification of a natural ligand of the hazel allergen Cor a 1. Sci Rep 9 (2019) 8714, doi:10.1038/s41598-019-44999-2.*

Amaretti: delicious, but... watch out for armelline!

Armelline Almond Allergy: The First Reported Case

Alessi L et al. Investig Allergol Clin Immunol. 2025; 35(1): 73-75 doi: 10.18176/jiaci.1033

This paper describes the case of a 7-year-old boy who developed severe anaphylaxis immediately after eating a dessert made with hazelnuts, cocoa, milk, eggs and biscuits (amaretti). The symptoms resolved after administration of adrenaline, B2 agonist aerosol, oral corticosteroids and antihistamines. Considering that the patient regularly consumed foods containing milk, eggs and cocoa without any problems, the authors focused their attention on almonds, hazelnuts and the main components of amaretti biscuits, namely apricot armelline (the small oval-shaped kernels found inside apricot seeds, which resemble almonds in shape and colour) (Figure 1). The patient was then subjected to a series of allergy tests, including SPT and *prick by prick* tests for hazelnuts, almonds and amaretti biscuits and for the inner and outer parts of apricot kernels, as well as serological analysis to detect specific IgE antibodies to extracts and individual molecules of various tree nuts (pistachios, pine nuts, almonds, walnuts, peanuts and hazelnuts). All the results of these analyses were negative, including those for respiratory allergens.

No reaction was observed following the reintroduction of various tree nuts, including walnuts and peanuts, into the patient's diet, which were introduced for the first time and tolerated by the patient. However, the patient was advised to avoid foods containing apricot kernels. In an attempt to identify the potential allergens responsible for the allergic reaction, the authors performed a series of other experiments based on electrophoretic analysis and immunoblotting (with the patient's serum) of samples of water- and oil-soluble extracts of hazelnut, almond, walnut, peanut and bitter almonds. The IgE-reactive bands (one in the case of water-soluble apricot kernel extract and several others in the case of oil-soluble extracts) were then removed from the gel and subjected to further analytical techniques (HPLC combined with mass spectrometry). Using protein databases, it was then possible to demonstrate that the band observed in the water-soluble extract of bitter almonds was that of a protein known as cupin type-1 domain-containing protein, belonging to the cupin group (11S seed storage proteins), already known as potential allergens. Considering that these components show more than 90% homology with the almond allergen Pru du 6 (a food that was tolerated by the patient), the authors hypothesised that the aforementioned component (cupin) was not involved in triggering the allergic reaction. Other IgE-reactive components, in particular two low molecular weight oleosins, were detected in the oil-soluble extract of various tree nut extracts (most likely the result of cross-reactivity), but these were also not considered relevant by the authors, who instead decided to investigate the high molecular weight, highly IgE-reactive area observable in the immunoblotting profile.

Using the analytical techniques mentioned above and with the help of specific databases, it was possible to identify the presence of a protein known as

Figure 1. Armelline (Apricot kernels).

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11-β-hydroxysteroid dehydrogenase (11β-OHSD2, belonging to the stereoleosin family) in the oil-soluble extract sample of apricot kernels, which the authors considered to be the most immunoreactive component observed in the immunoblotting profile.
Further investigations using a complex analysis based on the comparison of the amino acid sequence of 11β-OHSD2 with other proteins have shown that it has a certain homology (27.5% sequence identity, 63.8% sequence similarity) with a "seed maturation-like protein" already reported as a minor allergen present in an extract of *Sesamum indicum*. More recently, 11β-OHSD2 has been reported as a "novel wheat allergen" in patients with baker's respiratory allergy. The authors conclude the article by emphasising that this is the first case of primary allergic sensitisation to apricot kernels in a patient with no other allergies, given the patient's tolerance to multiple tree nuts, and that 11β-OHSD2, which is not detectable in other tree nuts, appears to be the allergen responsible for the allergic reaction observed in the case under study.

Immunotherapy against environmental fungi causing respiratory allergies

Immunotherapy against environmental fungi causing respiratory allergy

Gazi U, Bahceciler NN. J. of Medical Mycology. 2024;34,101517

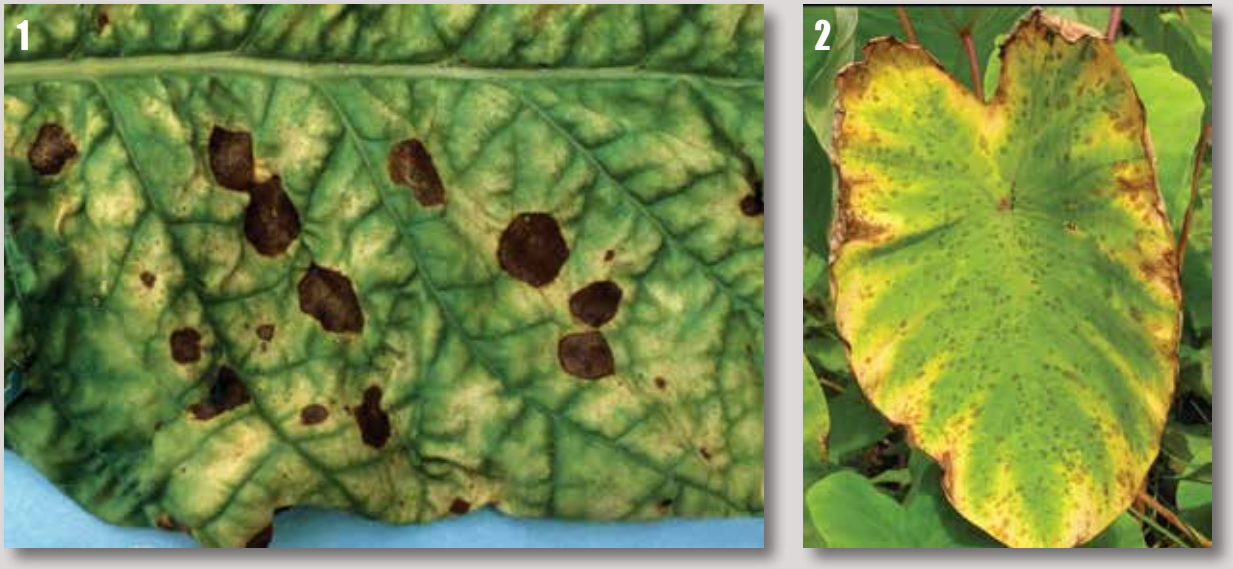
Among allergic diseases, those affecting the respiratory system (rhinitis and asthma, ARDs) represent one of the most serious public health problems worldwide. It should be noted that allergic rhinitis is a risk factor for the development of allergic asthma, a disease that negatively impacts the quality of life of those affected, representing the second leading cause of death.
The authors of this interesting review have focused their attention on one of the possible sources of allergens, namely fungal spores, which for some time have not been given sufficient consideration as a potential cause of allergy. Although their importance is now recognised, the prevalence of this disease in the general population, and in the atopic population in particular, is still uncertain. There are numerous known fungal species, but only a small percentage are associated with the risk of inducing allergic fungal airway diseases (AFADs), and among these, *Alternaria, Cladosporium, Penicillium* and *Aspergillus* are the four species most associated with this disease. Among the various therapeutic options, allergen-specific immunotherapy (AIT) is now generally recognised as the only treatment capable of interrupting the allergic march and inducing specific tolerance in patients. In the case of AIT with fungal extracts, clinical studies supporting their efficacy against AFADs are rather fragmented and have been conducted with a very small number of patients. The authors' effort to support the validity of this therapeutic option was to review the literature, selecting from 262 articles on various studies based on the use of fungal extracts, identified through a search of the PubMed database, only the most significant ones (32 articles) as they were conducted with standardised extracts. However, this approach limited the analysis to articles on *Alternaria* (in particular the *alternata* genus, which is the most representative of the species) and *Cladosporium* (*herbarum genus*), the two species of greatest environmental and clinical relevance for which, in cases selected by careful diagnosis, AIT is justified.
In the case of *Alternaria alternata*, it is very commendable that the various immunological parameters associated or not associated with the efficacy of AIT were also taken into consideration, even when these refer to various studies conducted with an extract consisting only of the allergen Alt a 1, by far the most clinically relevant allergen, given that sensitivity to it is greater than 80% in patients allergic to this fungal species. Contradicto-

ry results have been obtained on the role of IgG4 antibodies induced by AIT with Alt a 1. In the case of *Cladosporium*, there is clinical evidence supporting the therapeutic benefit (in terms of symptom reduction and drug consumption) of AIT with a *Cladosporium* extract observed in patients with asthma. Again, the increase in IgG4 antibodies observed does not appear to correlate with the therapeutic effect.

The authors conclude the article by expressing their hope that the clinical efficacy of AIT with fungal extracts (preferably of high quality, without excluding the use of chemically modified extracts, i.e. in allergen-free form) will be confirmed by double-blind placebo-controlled trials based on standardised protocols that include an appropriate number of patients and long-term follow-up.

Furthermore, the authors hope that controlled clinical studies will also be extended to other fungal species (*Penicillium* and *Aspergillus*, considered an important source of indoor allergens), including approaches to improve the indoor air quality to which patients are exposed.

Figure 1 Examples of *Alternaria* infection on a tobacco leaf (1) and *Cladosporium* infection on a taro leaf (2)



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2. Scot Nelson from USA - Taro: Cladosporium leaf spot, CC0, https://commons.wikimedia.org/w/index.php?curid=83967533

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Wheat allergy: importance of omega-5 gliadin and cofactors

Omega-5-gliadin-specific immunoglobulin E-positive, but wheat-specific immunoglobulin E-negative *wheat allergy dependent on augmentation fac*tors a frequent presentation

Faihs V et al. Explor Asthma Allergy. 2023;1:230–8. https://doi.org/10.37349/eaa.2023.00023

This study deals with a particular type of wheat food allergy (Figure 1) in which affected patients show IgE co-sensitisation to omega-5 (ω5) gliadin with allergic reactions associated with the presence of cofactors (known as augmentation factors or WALDA, wheat allergy dependent on augmentation factors). Among these, physical exercise is the most frequent, but alcohol consumption, non-steroidal anti-inflammatory drugs (NSAIDs), stress, infections and high temperatures can also play a role. Symptoms can range from mild or moderate (urticaria, angioedema) to severe, such as potentially life-threatening anaphylaxis. The diagnosis is based on medical history, IgE sensitisation profile, oral provocation test (OPT) with gluten and in the presence of augmentation factors. Often, the diagnosis of WALDA is recognised late due to an incomplete medical history or non-specific serological tests. Thanks to molecular diagnostics, it is now possible to identify cases of WALDA more accurately; in particular, the measurement of sIgE to ω5 is an effective screening tool. However, in some WALDA patients with positive IgE for ω5, the sIgE assay for total wheat extract may be negative. Faihs and colleagues therefore wanted to assess whether WALDA-positive patients (confirmed by positive sIgE for ω5 and OPT) showed clinical or timing differences in diagnosis based on sensitisation to total wheat extract.

The study was conducted on 36 patients aged between 23 and 81 years, with a history of allergic reactions to wheat in the presence of potentiating factors, sIgE positivity for ω5 and OPT positivity for wheat gluten with or without potentiating factors. The authors conducted a detailed clinical evaluation and measured total IgE, sIgE for wheat, gluten, ω5 and grass pollen, as well as baseline blood tryptase levels. In addition, prick-to-prick skin tests with wheat flour and gluten and OPT tests were also performed according to a protocol optimised by the group (1) that included different reaction thresholds, from 1 to 10, as outlined in Table 1.

The investigations revealed sIgE values for ω5 between 0.5 and 34.6 kUA/L (median 6.5 kUA/L) and significant

Table 1 Outline of the OPT protocol used in the study

Day	Factor	Exposure	Reaction threshold
1	Wheat gluten	Progressive oral administration of gluten: 8 g > 16 g > 32 g	 1, 2, 3
2	Gluten + ASA	1000 mg ASA + gluten 8 g > 16 g > 32 g	4, 5, 6
3	Gluten + ASA + alcohol + physical exercise	1000 mg ASA + 20 ml 95% ethanol in fruit infusion + gluten 32 g + 20 min anaerobic exercise on treadmill	 7 8
	Repetition with gluten + ASA + alcohol + physical exercise	Administration of 64 g gluten + ASA + alcohol + exercise	9 10

Figure 1. **Wheat ears** Credits to David von Diemar, Unsplash

reactions to OPT. Seventy-five per cent of patients had experienced episodes of systemic anaphylaxis, 25% had experienced urticaria and/or angioedema after consuming wheat with potentiating factors, and 13 patients had atopic comorbidities. On average, the time between the first allergic reaction and diagnosis was 5 years, but in 10 cases it exceeded 10 years, reaching up to 24 years. In the OPT test, 33% of patients reacted to gluten administration alone (including 2 patients with a reaction threshold of 1). Acetylsalicylic acid (ASA) as a cofactor induced reactions in 50% of cases (reaction thresholds 4 to 6). One patient developed urticaria after ASA, alcohol and gluten (threshold 7), while in 4 patients it was necessary to combine physical exercise to induce symptoms (threshold 8). No patient reached the highest thresholds (9 and 10). sIgE values for total wheat extract ranged from <0.1 to 4.65 kUA/L (median 0.54 kUA/L). A significant result of the study was that as many as 39% of patients (n = 14) were negative for IgE to wheat extract (<0.35 kUA/L) and, in half of these patients, the values were even below the detection limit (0.1 kUA/L). The prick-to-prick test with wheat flour gave positive results in 80% of cases (n = 29; mean wheal size = 5.4 mm), while the test with wheat gluten gave positive results in 86% of patients (n = 31, mean wheal size = 6.1 mm).

A correlation was found between sIgE levels to wheat extract and those to grass pollen, gluten, ω5, and also with the results of the prick-to-prick test with gluten. In particular, patients with negative tests for sIgE to wheat extract showed significantly lower values of total IgE and sIgE to ω5, gliadins, and wheat gluten (all $P < 0.001$), and lower wheal diameters in the prick-to-prick test with gluten ($P = 0.006$). No correlation was found with clinical characteristics, delays in diagnosis, presence of an atopic condition, severity of reactions, and OPT threshold. sIgE levels to grass pollen were correlated with the presence of atopic comorbidities and total IgE and sIgE to wheat extract, but not to gluten-related allergens.

In conclusion, this study highlights the problem of delayed diagnosis for patients with WANDA and, above all, emphasises the importance of using sIgE for ω5 as a screening parameter and the limited diagnostic value of sIgE to total wheat extract. In fact, almost 40% of patients with sIgE to ω5 were negative for sIgE to total wheat extract (perhaps because the allergens that trigger WALDA may be underrepresented).

Among the limitations of the study, the authors highlight the small number of patients and therefore call for further studies to confirm these data. They conclude their article by emphasising the importance of informing patients about triggering allergens and potentiating factors in order to prevent the onset of severe reactions.

Bibliography

1. *Faihs V, Kugler C, Schmalhofer V, et al. Wheat-dependent exercise-induced anaphylaxis: subtypes, diagnosis, and management. JDDG: Journal der Deutschen Dermatologischen Gesellschaft. 2023; 21: 1131–1135. https://doi.org/10.1111/ddg.15162*

ENG

Autism, allergies and eating disorders: a clinical case study

The Intersection of Autism Spectrum Disorder, Food Allergy, and Avoidant/Restrictive Food Intake Disorder: A Clinical Case Study

Proctor KB et al. J Pediatr. 2024; 269:113965. doi: 10.1016/j.jpeds.2024.113965

Autism spectrum disorder (ASD) negatively affects an individual's neurological development, leading to impairments in communication and social interaction, associated with restricted and repetitive behaviours. Children affected by ASD are also five times more likely to develop eating disorders than their healthy peers. In particular, almost all of them show strong food selectivity, often with a preference for starchy foods and snacks and a refusal to eat fruit and vegetables. In the most severe cases, ARFID (Avoidant/Restrictive Food Intake Disorder) may be diagnosed, a disorder that involves restricted food intake leading to significant weight loss and/or stunted growth, nutritional deficiencies, dependence on supplements/nutritional formulas, and impaired psychosocial relationships. ARFID is believed to arise in response to and be maintained by several factors such as sensory sensitivity, highly selective food preferences, lack of interest in food, or even fear of the negative consequences of eating (e.g., vomiting). ARFID can occur in conjunction with other medical conditions and disorders (e.g., gastrointestinal diseases, food allergies, ASD).

Children with ASD may show an aversion to food when they also suffer from food allergies. However, this population has been little studied despite the risk of serious health and psychosocial problems associated with the coexistence of ASD, food allergies, and ARFID. In this paper, a multidisciplinary team of specialists in paediatric nutrition, clinical psychology and allergology from Emory University in Atlanta (Georgia, USA) presents a representative clinical case: a 10-year-old boy (affected by three interrelated conditions, ASD, food allergies and ARFID) who was dependent on a hypoallergenic nutritional formula from which he received 100% of his nutritional needs. He accepted some solid foods, such as dried meat and chicken wings, which he chewed but spat out before swallowing. The authors described the medical history in detail, and the key information is summarised in

Table 1 Clinical and dietary history of the patient

Age/Period	Event/Diagnosis	Notes
5 months	Onset of eating disorders	Refusal to eat, crying,
12 months	Early intervention	Delayed development, aversion to food
13 months	Start of food therapy	Continued over the years
2–3 years	Food acceptance/rejection	Post-ingestion vomiting (e.g. yoghurt)
3 years	Diagnosis of ASD + milk allergy	Positive prick test, no follow-up
3 years	Gastrointestinal endoscopy	Negative
7–9 years	Allergy tests	Allergies to milk, eggs, nuts
School age	Dependence on nutritional formula	Only from specific cup (colour/brand)
2021–2022	Reduced and then ceased availability of formula	Severe malnutrition, hospitalisation
Hospitalisation 2022	Intravenous feeding	Then return to formula feeding
Post-hospitalisation	Formula replenishment through online fundraising, but limited supplies	No insurance coverage (since feeding was non-enteral)

Table 1. The subject's eating problems began at 5 months of age, and at 3 years of age he was diagnosed with an IgE-mediated allergy to milk. Further allergy tests were carried out later at the ages of 7 and 9, revealing allergic sensitisation to eggs and nuts as well.

In 2022, the child's persistent refusal to follow an acceptable nutritional formula led to severe malnutrition and subsequent hospitalisation. Subsequently, the family managed to find a hypoallergenic formula on the market that was acceptable to the child. However, the withdrawal of the formula from the market prompted the family to consult the specialists who authored the study. The child was urgently admitted to their intensive 8-week multidisciplinary programme (for the treatment of ARFID and other paediatric eating disorders). This included a nutritionally complete therapeutic menu that was safe for his allergic condition. At the time of assessment, the subject weighed 40.1 kg, was 143 cm tall and had a BMI in the 87th percentile.

At the same time, the group's psychologist developed a behavioural analysis and intervention plan to promote oral food intake and encourage dietary diversity. The first goal of treatment was to achieve acceptance of a new hypoallergenic formula through gradual exposure. At first, the child showed strong resistance to accepting this treatment, leaving the room, crying and displaying aggressive behaviour. To help him overcome his anxiety and food refusal, an approach was adopted in which the child did not feed himself. Initially, he was given an empty spoon and clear instructions on how to behave, in the presence of a second therapist to ensure protection during the sessions. Gradually, other foods were introduced, increasing the requirements (e.g., formula/food volume) only when the subject showed that he was comfortable with the current nutritional plan. By the end of the second week of treatment, the child was drinking independently from a cup; in the third week, the medical team began to introduce various blended or naturally soft foods, and subsequently to introduce foods of greater consistency. At the end of the treatment, the subject seemed to enjoy his meals and participated in their preparation, consuming 54% of his nutritional requirements through blended foods that he consumed independently from a bowl with a spoon, while the remaining requirements were covered by formula, drunk from a cup. He no longer exhibited problematic behaviour during meals. His family (his mother had been instructed on the approach to follow and was able to implement it successfully in the clinic) was provided with a plan to gradually reduce the use of the nutritional formula.

The case described is representative of a potential population of vulnerable children, currently understudied, whose dietary restrictions are compounded by those necessary from a medical point of view (e.g. for food allergies) or avoidance behaviours associated with ASD in ARFID. In this case, the subject's delayed language skills and reduced cognitive abilities represented a significant complication in effective communication regarding the subject's food allergies.

The study shows how some of the distinctive features of ASD can complicate differential diagnosis and need to be assessed in children with clinical situations similar to the one described. To this end, the authors have included a table (on page 5 of the original paper) with examples of questions useful for recognising food avoidance beyond what would be expected as part of ASD and food allergy. The table can be useful in the early identification of ARFID cases and in facilitating referral to specialists in nutrition and/or paediatric psychology.

Finally, the authors conclude by emphasising how crucial a multidisciplinary approach is to successfully manage eating disorders in individuals with a condition similar to the one described in this case.

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Provide information, create a profession

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FeNO measurement beyond asthma

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FeNO (fractional exhaled nitric oxide) measurement is a test that determines the levels of exhaled NO. NO is a gas produced by nitric oxide synthase (NOS), of which there are three isoforms, two of which are constitutive: nNOS, found in neural tissue, and eNOS, expressed by the endothelium, epithelia, and striated muscle. Of particular relevance is the inducible isoform (iNOS), characterised by basal expression in the bronchial epithelium, pathologically increased in the chronic T2 inflammatory milieu, with the contribution of the endothelium, fibroblasts, and smooth muscle. It is now known that, precisely because of the overexpression of iNOS, the respiratory epithelium of atopic subjects is characterised by higher NO levels than others. This condition, sustained by interleukins (IL) 4 and 13, fuels the inflammatory picture by stimulating the recruitment of cellular mediators, such as eosinophils, mast cells, basophils and lymphocytes, but also through increased release of free radicals, greater bronchial hyperreactivity, reduced mucociliary clearance and increased vascular permeability. All these elements contribute to the T2 asthma scenario, in which eosinophils play a key role (1). However, while the role of FeNO as a surrogate marker of eosinophilic inflammation, response to therapy and risk of exacerbations is now universally recognised in asthma (1), its possible role in other T2-mediated conditions is somewhat more controversial.

In the case of allergic rhinitis (AR), some studies have reported higher FeNO levels in patients with nasal obstruction prevailing over rhinorrhoea (2). A correlation has also been found between high FeNO values in non-asthmatic AR patients and the pollen season (3). However, it should be noted that, given the anatomical continuity of the upper and lower airways, high FeNO levels could also be an expression of subclinical bronchial inflammation (1). What the literature seems to agree on, however, is the possible role of FeNO in assessing the response to H1-antihistamines, inverse agonists of iNOS activity, or to the combination of H1-antihistamines with a nasal corticosteroid (3). Still in the upper airways, higher FeNO values characterise non-asthmatic patients with chronic rhinosinusitis with nasal polyposis (CRSwNP) compared to subjects with CRS but without polyps (4). With regard to asthma-CRSwNP comorbidity, FeNO testing can be a valuable tool in selecting patients with T2 inflammation who are candidates for biological therapy (1, 5).

With regard to T2 diseases involving the gastrointestinal tract, the literature appears even less conclusive. In particular, in the case of eosinophilic oesophagitis (EoE), FeNO could re-

flect pathognomonic eosinophilic oesophageal inflammation; however, at present, there does not yet appear to be a clear response (1), although several studies have reported significantly higher FeNO values for patients with active EoE (6). For food allergies, it is very interesting to note the role that FeNO measurement could play in screening patients who are more susceptible to anaphylaxis during oral peanut tolerance testing, who are characterised by a decrease in FeNO during the test, compared to tolerant patients or those with minor allergic reactions. This finding could have a pathophysiological basis in airway oedema prior to the anaphylactic reaction, which would lead to a reduction in NO diffusion capacity (7).
With regard to patients with atopic dermatitis (AD), higher FeNO levels were found compared to healthy controls, but this could simply reflect a combination of increased allergic diathesis and exposure to allergens, as demonstrated by the direct proportionality between FeNO levels and the number of positive prick tests for aeroallergens (8).
In conclusion, beyond the still inconclusive data, the speed, safety and ease of performing FeNO testing certainly make this test worthy of further studies aimed at clarifying its various unresolved aspects and possible future applications.

Bibliography

1. *Maniscalco M et al. Exhaled Nitric Oxide as Biomarker of Type 2 Diseases. Cells. 2023;12(21):2518. Published 2023 Oct 25. doi:10.3390/cells12212518*

2. *Chen IC et al. Nasal Airflow Measured by Rhinomanometry Correlates with FeNO in Children with Asthma. PLoS One. 2016;11(10):e0165440. Published 2016 Oct 28. doi:10.1371/journal.pone.0165440*

3. *Antosova M et al. Exhaled and Nasal Nitric Oxide - Impact for Allergic Rhinitis. Physiol Res. 2020;69(Suppl 1):S123-S130. doi:10.33549/physiolres.934393*

4. *Kambara R et al. Lower Airway Inflammation in Eosinophilic Chronic Rhinosinusitis as Determined by Exhaled Nitric Oxide. Int Arch Allergy Immunol. 2017;173(4):225-232. doi:10.1159/000479387*

5. *Paoletti G et al. Very Rapid Improvement in Extended Nitric Oxide Parameters Is Associated With Clinical and Functional Improvement in Patients With Chronic Rhinosinusitis With Nasal Polyps Treated With Dupilumab. J Investig Allergol Clin Immunol. 2023;33(6):457-463. doi:10.18176/jiaci.0851*

6. *Josyabhatla R et al. Plasma Biomarkers and Fractional Exhaled Nitric Oxide in the Diagnosis of Eosinophilic Esophagitis. J Pediatr Gastroenterol Nutr. 2023;76(1):59-65. doi:10.1097/MPG.0000000000003634*

7. *Percival E et al. Change in exhaled nitric oxide during peanut challenge is related to severity of reaction. Allergy Asthma Clin Immunol. 2020;16:64. Published 2020 Jul 21. doi:10.1186/s13223-020-00464-8*

8. *Galiniak S et al. Fractional Exhaled Nitric Oxide in Teenagers and Adults with Atopic Dermatitis. Adv Respir Med. 2022;90(4):237-245. Published 2022 Jul 20. doi:10.3390/arm90040033*

Allergic phenotype in chronic rhinosinusitis with nasal polyposis (CRSwNP)

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Chronic rhinosinusitis with nasal polyposis (CRSwNP), one of the two main phenotypes into which chronic rhinosinusitis (CRS) is traditionally divided, is a heterogeneous condition whose symptoms have a significant impact on quality of life (1).
The association between CRS and conditions such as asthma and allergic rhinitis has long been known: approximately two-thirds of patients with CRS have atopy. In atopic individuals, the condition tends to present with more severe clinical symptoms due to greater tissue eosinophilic infiltration (2). However, there are CRSwNP phenotypes/endotypes in which the role of allergies is much more evident. Allergic Fungus RhinoSinusitis (AFRS) is a distinct endotype of CRSwNP whose

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pathogenesis is based on a localised allergic reaction to fungi colonising the nasal mucosa. Patients with AFRS are immunocompetent and have allergic sensitisation to one or more fungi. The clinical presentation is similar to that of classic CRSwNP, but more frequently it can lead to complications due to erosion of bone structures extending to areas outside the paranasal sinuses (3). Another subtype of CRSwNP is Central Compartment Atopic Disease (CCAD), characterised by the presence of polyps exclusively in the central compartment of the nasal cavity (middle turbinate, upper turbinate, nasal septum). CCAD is strongly associated (95% of cases) with sensitisation to inhaled allergens, especially weed pollen such as pellitory and ragweed and dust mites (4). It is thought that the deposition of allergens in these structures, which are more exposed to the external environment, may trigger the inflammatory cascade responsible for the development of CRS (5). From a therapeutic point of view, both CCAD and AFRS benefit from standard CRSwNP therapy: endoscopic surgery and local corticosteroids (2). Post-surgical recurrence is rare in patients with CCAD. Patients with resistant AFRS, on the other hand, may benefit from therapy with omalizumab, mepolizumab or dupilumab, depending on the case (2, 6). In all cases of CCAD and AFRS, it is essential to consider allergen-specific immunotherapy as a therapeutic approach, as it is the only true disease-modifying therapy available to us (2).

For these reasons, in the clinical practice of our PRGM-C Allergology and Clinical Immunology Centre at the "D. Casula" University Hospital in Monserrato (CA), directed by Professor Stefano Del Giacco, it is standard practice to perform skin prick tests on every patient with CRSwNP, followed by an evaluation of specific IgE levels in anticipation of a possible allergen-specific immunotherapy programme.

Bibliography

1. *Stevens WW, Schleimer RP, Kern RC. Chronic Rhinosinusitis with Nasal Polyps. J Allergy Clin Immunol Pract. 2016 Jul-Aug;4(4):565-72. doi: 10.1016/j.jaip.2016.04.012. PMID: 27393770; PMCID: PMC4939220.*

2. *Helman SN, Barrow E, Edwards T, DelGaudio JM, Levy JM, Wise SK. The Role of Allergic Rhinitis in Chronic Rhinosinusitis. Immunol Allergy Clin North Am. 2020 May;40(2):201-214. doi: 10.1016/j.iac.2019.12.010. Epub 2020 Jan 14. PMID: 32278445; PMCID: PMC7472891.*

3. *Dykewicz MS, Rodrigues JM, Slavin RG. Allergic fungal rhinosinusitis. J Allergy Clin Immunol. 2018 Aug;142(2):341-351. doi: 10.1016/j.jaci.2018.06.023. PMID: 30080526.*

4. *Davies, C.; Wu, F.; Huang, E.Y.; Takashima, M.; Rowan, N.R.; Ahmed, O.G. Central Compartment Atopic Disease as a Pathophysiologically Distinct Subtype of Chronic Rhinosinusitis: A Scoping Review. Sinusitis 2023, 7, 12-26. https://doi.org/10.3390/sinusitis7020003*

5. *Rubel KE, Lubner RJ, Lopez AA, Li P, Huang LC, Sheng Q, Wu J, Wise SK, DelGaudio JM, Chandra RK, Chowdhury N, Turner JH. Inflammatory characteristics of central compartment atopic disease. Int Forum Allergy Rhinol. 2023 Dec;13(12):2133-2143. doi: 10.1002/alr.23207. Epub 2023 Jul 1. PMID: 37302116; PMCID: PMC10711148.*

6. *Im YH, Stybayeva G, Hwang SH. Short-Term Efficacy of Biologics in Recalcitrant Allergic Fungal Rhinosinusitis: A Systematic Review and Meta-analysis. Otolaryngol Head Neck Surg. 2025 Jun 24. doi: 10.1002/ohn.1339. Epub ahead of print. PMID: 40552669.*

Anche se forse non tutti sono consapevoli, esistono numerosi esempi che dimostrano chiaramente come l'intelligenza artificiale (IA) faccia già parte della nostra quotidianità e lo sarà sempre di più in futuro. L'IA è un mondo in rapida evoluzione che sta rivoluzionando molteplici settori, incluso ovviamente quello sanitario. In questo numero del Notiziario Allergologico pubblichiamo un articolo che tratterà specificatamente dell'utilità della IA in campo allergologico, e su questo tema rimandiamo quindi il lettore alla lettura dello stesso.

Tra i vari campi di applicazione dell'IA (ovviamente di gran lunga meno importante di quello sanitario) ricordo quello per il quale sarebbe possibile, grazie all'IA, essere in grado di interpretare in futuro il linguaggio degli animali e capire mediante quali modalità gli animali comunicano tra loro. Come proprietario di un cane (Frodo, un trovatello simil pointer molto affettuoso che vorrebbe abbracciare chiunque incroci il suo sguardo) mi è capitato spesso di osservare che gli manchi solo la parola (immagino che molti proprietari di cani condividano con me questa sensazione). In effetti non è così; ai cani e in genere agli animali non manca la parola, ma semplicemente usano un linguaggio diverso da quello umano. A chi però non piacerebbe riuscire a decodificare il loro linguaggio e raggiungere così una maggiore profondità di rapporto con il proprio amico a 4 zampe comprendendo più a fondo il significato dei loro vocalizzi, di certi sguardi o della postura che tendono ad assumere, ovvero le emozioni o le situazioni di stress che provano e il loro stato di salute?

Per esempio, che cosa penserà Frodo quando seduto "a mo' di statua di porcellana" rivolge lo sguardo all'infinito? Starà meditando sul senso della vita? Ovviamente, non è realistico aspettarsi di saperlo anche in futuro.

Although perhaps not everyone is aware of it, there are numerous examples that clearly demonstrate how artificial intelligence (AI) is already part of our daily lives and will become increasingly so in the future. AI is a rapidly evolving field that is revolutionising many sectors, including, of course, healthcare. In this issue of Notiziario Allergologico, we are publishing an article that will specifically address the usefulness of

AI in the field of allergology, and we therefore refer readers to this article for more information on this topic.
Among the various fields of application of AI (obviously far less important than healthcare), I recall one in which, thanks to AI, it may be possible in the future to interpret the language of animals and understand how they communicate with each other.
As a dog owner (Frodo, a very affectionate pointer-like stray who would like to hug anyone who catches his eye), I have often observed that the only thing he lacks is speech (I imagine that many dog owners share this feeling with me). In fact, this is not the case; dogs and animals in general do not lack speech, but simply use a language different from that of humans. But who wouldn't like to be able to decode their language and thus achieve a deeper relationship with their four-legged friend by understanding more fully the meaning of their vocalisations, certain looks or the posture they tend to assume, i.e. the emotions or stressful situations they experience and their state of health?
For example, what is Frodo thinking when he sits 'like a porcelain statue' and stares into the distance? Is he meditating on the meaning of life? Obviously, it is not realistic to expect to know this in the future either.

Aunque quizá no todo el mundo lo sepa, hay numerosos ejemplos que demuestran claramente cómo la inteligencia artificial (IA) ya forma parte de nuestra vida cotidiana y lo hará cada vez más en el futuro. La IA es un mundo que evoluciona rápidamente y que está revolucionando muchos sectores, incluido, por supuesto, el ámbito sanitario. En este número del Notiziario Allergologico publicamos un artículo que trata específicamente de la utilidad de la IA en el campo de la alergología, por lo que remitimos al lector a dicho artículo.
Entre los diversos campos de aplicación de la IA (naturalmente mucho menos importantes que el de la salud), me gustaría mencionar aquél en el que sería posible, gracias a la IA, interpretar en un futuro el lenguaje de los animales y comprender las formas en que éstos se comunican entre ellos.
Como dueño de un perro (Frodo, un mestizo muy cariñoso parecido a un pointer al que le gustaría abrazar a cualquiera que se cruce en su camino), a menudo he sentido que sólo le falta hablar (me imagino que muchos dueños de perros comprenderán este sentimiento). De hecho, no es así; los perros y los animales en general no carecen de habla, simplemente utilizan un lenguaje distinto al de los humanos. Sin embargo, ¿a quién no le gustaría poder descifrar su lenguaje y lograr así una relación más profunda con su amigo de cuatro patas, comprendiendo más a fondo el significado de sus vocalizaciones, ciertas miradas o la postura que suele adoptar, o las emociones o situaciones de estrés que experimenta y su estado de salud?
Por ejemplo, ¿en qué pensará Frodo cuando se sienta "como una estatua de porcelana" y dirige su mirada al infinito? ¿Está meditando sobre el sentido de la vida? Por supuesto, no es realista esperar saberlo tampoco en el futuro.

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