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La Citologia Nasale in Rinoallergologia
Nasal Cytology in Rhinoallergology
Citología nasal en Rinoalergología

Immunoterapia specifica e tolleranza: le ILC2 regolatorie
Specific immunotherapy and tolerance: regulatory ILC2s
Inmunoterapia específica y tolerancia: las ILC2 reguladoras

Anafilassi: 10 passi per riconoscere e gestire questa emergenza
Anaphylaxis: 10 steps to recognise and manage this emergency
Anafilaxia: 10 pasos para reconocer y manejar esta emergencia

VUOI VEDERE GLI ACARI DA VICINO?
WANT TO SEE MITES UP CLOSE?
¿QUIERES VER LOS ÁCAROS DE CERCA?

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COPERTINA • COVER • PORTADA

Gli acari sono artropodi della classe degli aracnidi a cui appartengono anche i ragni. Nel mondo si conoscono più di 60.000 specie acaridiche diverse; alcune di queste rivestono un'importanza particolare perché rappresentano una fonte di molecole (allergeni) che possono provocare nell'uomo reazioni allergiche specifiche. In base alla loro ubiquità e rilevanza allergologica gli acari possono essere divisi in due principali categorie: a) pyroglyphidae, a cui appartengono gli acari della polvere di casa (House Dust Mites, HDM), cioè i Dermatophagoides farinae e pteronyssinus; b) non-pyroglyphidae o acari delle derrate alimentari (storage mites, SM).
Quelli più facilmente riscontrabili nelle derrate alimentari stoccate nei magazzini e nei silos sono *T. putrescentiae*, *A. siro*, *L. destructor* e *G. domesticus*. Essi possono essere responsabili di allergie respiratorie occupazionali che colpiscono i lavoratori addetti alla produzione di prodotti alimentari o alla movimentazione dei cereali o farine.
In Italia, in provincia di Parma, si produce uno dei prosciutti più apprezzati nel mondo, cioè il prosciutto crudo. Ebbene, sulle pareti e sui pavimenti dei locali adibiti alla loro stagionatura si può notare, a causa delle particolari condizioni di umidità e temperatura, la presenza di una polverina biancastra formata da acari stessi e dalle muffe di cui si nutrono. Si dice che questa polverina conferirebbe al prodotto un tipico aroma. In questo caso l'acaro buongustaio è il *T. putrescentiae*, una delle specie più diffuse e cosmopolite di SM.
Nella cover è mostrato un dettaglio di un esemplare di *T. putrescentiae* come appare al microscopio elettronico. Scansionando poi il QR code è possibile visionare un video di esemplari di *T. putrescentiae* allevati nel nostro reparto di Acarologia.

Mites are arthropods of the arachnid class, to which spiders also belong. More than 60,000 different mite species are known worldwide; some of these are of particular importance because they are a source of molecules (allergens) that can cause specific allergic reactions in humans. On the basis of their ubiquitousness and allergological relevance, mites can be divided into two main categories: a) pyroglyphidae, to which House Dust Mites (HDM) belong, i.e., Dermatophagoides farinae and pteronyssinus; b) non-pyroglyphidae or storage mites (SM).
Those most easily found in foodstuffs stored in warehouses and silos are *T. putrescentiae*, *A. siro*, *L. destructor*, and *G. domesticus*. They may be responsible for occupational respiratory allergies affecting workers engaged in food production or handling grain or flour.
In Italy, in the province of Parma, one of the world's most prized hams, prosciutto crudo, is produced. Well, on the walls and floors of the rooms used for curing these hams, one can notice, due to the particular humidity and temperature conditions, the presence of a whitish powder formed by the mites themselves and the mold on which they feed. This powder is said to impart a typical aroma to the product. In this case, the gourmet mite is *T. putrescentiae*, one of the most widespread and cosmopolitan species of SM.
The cover shows a detail of a specimen of *T. putrescentiae* as it appears under an electron microscope. By then scanning the QR code it is possible to view a video of specimens of *T. putrescentiae* reared in our Acarology department.

Los ácaros son artrópodos de la clase de los arácnidos, a la que también pertenecen las arañas. Se conocen más de 60.000 especies diferentes de ácaros en todo el mundo; algunas de ellas son de especial importancia porque representan una fuente de moléculas (alérgenos) que pueden provocar reacciones alérgicas específicas en los seres humanos. En función de su ubicuidad y relevancia alergológica, los ácaros pueden dividirse en dos categorías principales: a) los pyroglyphidae, a los que pertenecen los ácaros del polvo doméstico (House Dust Mites, HDM), es decir, Dermatophagoides farinae y pteronyssinus; b) los no pyroglyphidae o ácaros de almacén (storage mites, SM).
Los que se encuentran con más facilidad en los alimentos almacenados en almacenes y silos son *T. putrescentiae*, *A. siro*, *L. destructor* y *G. domesticus*. Pueden ser responsables de alergias respiratorias ocupacionales que afectan a trabajadores dedicados a la producción de alimentos o a la manipulación de grano o harina.
En Italia, en la provincia de Parma, se produce uno de los jamones más apreciados del mundo, el prosciutto crudo. Pues bien, en las paredes y suelos de las salas utilizadas para la curación de estos jamones se puede observar, debido a las particulares condiciones de humedad y temperatura, la presencia de un polvo blanquecino formado por los propios ácaros y el moho del que se alimentan. Se dice que este polvo confiere al producto un aroma típico. En este caso, el ácaro gourmet es *T. putrescentiae*, una de las especies de EM más extendidas y cosmopolitas.
La portada muestra un detalle de un ejemplar de *T. putrescentiae* tal como aparece bajo un microscopio electrónico. Luego escaneando el código QR es posible visualizar un video de ejemplares de *T. putrescentiae* criados en nuestro departamento de Acarología.

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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**

We were all surprised during the pandemic period at the unprecedented speed with which the development of effective mRNA vaccines against Covid-19 was reached. All of this was achieved thanks to the fundamental contribution of two researchers, Katalin Karikò and Drew Weissman, for which they were awarded the 2023 Nobel Prize in Medicine (see photo). During their research, the awardees discovered that nucleosides, the building blocks of RNA, could undergo special chemical modifications and the derivative produced was able to interact with the immune system without activating innate immunity, that would otherwise degrade it. The two researchers also observed that, by introducing the same modification into synthetic mRNAs, they could introduce themselves into cells by promoting the synthesis of the desired protein; the latter, once the mRNA vaccine was administered into the body, would be recognized as foreign thus stimulating the immune system to produce specific antibodies toward it while activating memory cells. This scientific background acquired over the years by the two researchers thus laid the foundation for the use of mRNAs as therapeutic agents. Notably, in the case of Covid-19, several other factors helped "skip ahead" in the development of a specific mRNA vaccine. Crucial steps turned out to be the knowledge of the sequence of the virus genome and subsequently the sequence of the Spike protein (the target antigen), the identification of procedures that could significantly improve the stability of the vaccine by preventing it from being "scrapped" before it had reached its destination, and, not insignificantly, the huge amount of economic resources, both public and private, made available worldwide to achieve such a goal.

Clinical experience during the pandemic demonstrated the enormous therapeutic potential of this technology, providing a solid basis for future applications in other diseases.

Is it conceivable to extend this technology in the case of allergies as well? Indeed, allergen-encoding mRNA vaccines could be potential candidates even from the perspective of a preventive therapy, given the level of safety of these products. In this sense, some preliminary experiments carried out in animal models have proved promising (1,2). Of course, in this case it would be necessary to develop mRNAs coding for multiple allergens, given the complexity of the extracts and the clinical relevance of the different allergens in it, and from a technical point of view, within certain limits, it would be possible. The problems that currently seem to be an obstacle to the development of such a vaccine would be related to the large investments to be borne, which would also include a substantial change in the production structure of conventional allergy vaccine companies.

While waiting for such a perspective to open up in the allergology field in the future, we "come down to earth". In this issue of the Notiziario Allergologico we present a series of articles that, in some ways, constitute an update on topics already covered in previous years.

We begin with the very timely and exhaustive contribution of Dr. Imperatore (Head of U.O. Allergology - Nasal Cytology P.A.T. Loreto Crispi Asl Napoli) on nasal cytology, a test that is becoming increasingly popular as a very useful tool both in the early identification of any alterations that accompany rhinopathies and in the constant monitoring of their progress, so as to identify the most appropriate therapy to be adopted from time to time. Nasal cytology is a simple, noninvasive, painless method that requires little time to perform and can be executed even on children. As the author points out, the success of this diagnostic tool has gone hand in hand with the advancement over time of differential staining techniques that have enabled the recognition of the different cell types present in the sample taken from inside the patient's nostrils. In fact, on the basis of the type of cells present in the sample, a classification of the different rhinopathies can be made, which is a fundamental element in the setting of the most targeted therapy to be implemented. Particularly interesting are the author's observations on the use of nasal cytology as an integrating element to complete the diagnostic framing of patients with allergic rhinitis and candidates for allergen-specific immunotherapy (AIT). It is noteworthy how the existence of allergic rhinitis superimposed on nonallergic rhinitis

2023 Nobel Prize winners Katalin Karikò and Drew Weissman.
© Nobel Prize Disclosure. Photo: Clement Morin

could underlie the failure of AIT, as observed in some studies. The author thus concludes her article by pointing out that a more widespread use of a simple and objective method such as nasal cytology could have an enormous impact in real-life, allowing a better identification of the most suitable patients to undergo AIT and thus contributing to an improvement to its therapeutic success.

We continue with the article by Prof. Cosmi (Director SODc Immunoallergology AOU Careggi, Florence), which is full of very interesting insights on AIT and the immunological changes it induces. After recalling that AIT is the only therapeutic option capable of modifying the natural course of allergic diseases, the author pointed out how it has evolved over time thanks to improved standardization of allergenic extracts, the introduction of molecular allergy diagnostics that has allowed a better definition of patients who are candidates for AIT and to the advent of alternative administrations to parenteral, particularly the sublingual route, characterized by a high safety profile. All these elements have contributed to the increased frequency of AIT therapeutic success. The author then focuses his attention on the mechanisms by which AIT induces modulation of the allergen-specific response that can be associated with subsequent clinical benefit. Such modulation involves numerous components of innate and adaptive (or specific) immunity, and the author describes their specificities. Among these, the author focuses on a particular heterogeneous population of cells of innate immunity, the so-called Innate Lymphoid Cells (ILCs), which includes three different groups of cells, each with different roles in allergic diseases. Over the past few years, numerous evidences have demonstrated a possible pathogenetic role of the ILC2 subset in both the initiation and maintenance of allergic inflammation, probably associated with both their submucosal localization and their ability to be activated in response to particular exogenous stimuli, including several epithelial cytokines. It follows, as the author concludes, that these cells could represent a potential therapeutic target for allergic diseases.

This issue concludes with the contribution by Dr. González-de-Olano (Ramón y Cajal University Hospital, IRYCIS, Madrid, Spain) on anaphylaxis. As is well known, anaphylaxis is the most severe and life-threatening manifestation of an allergic reaction. The article is characterized by very useful practical implications for the recognition of this condition and how best to manage it. Anaphylaxis can be triggered by several triggers (allergens, hymenoptera venom, medications, etc.), and a number of co-factors can amplify its risk and degree of severity. Symptomatically, anaphylaxis is a rapid-onset reaction (due to the release of a series of mediators by mainly mast cells and basophils) and can affect countless organs. Because it is associated with the risk of a fatal development by cardiovascular collapse or asphyxia due to laryngeal edema, early recognition of the event is of paramount importance for the setting of an appropriate therapeutic intervention. In severe cases, the first-line treatment is the timely administration of intramuscular adrenaline, to be combined later with other second- and third-line interventions. The tryptase assay remains the only useful parameter for diagnostic purposes. The author concludes the article by emphasizing the importance, once the acute phase has resolved, of subjecting the patient to close allergologic follow-up to identify the cause of the episode and to set up an action plan that can prevent its recurrence.

I wish everyone a good read.

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ENG

Nasal Cytology in Rhinoallergology

Dr Clara Imperatore
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National Adviser and Secretary of the Italian Academy of Nasal Cytology (A.I.C.NA.)

INTRODUCTION

The study of nasal cellularity was born in 1863 when the German pathologist Friederich Daniel von Recklinghausen, a professor at the University of Strasbourg who linked his name to the description of neurofibromatosis type 1 and generalised cystic fibrosis osteitis, both known as 'von Recklinghausen's disease', first described 'granular-looking cells'. His view is unfortunately 'black and white' because he lacked the tools to accurately discriminate the different 'granularities'.
We have to wait until the beginning of the 'colour period of science', as Prof. Matteo Gelardi calls it, for a more definite and precise description of these cells. The beginning of the chromatic period of science falls in 1878 when Paul Erlich graduated from the University of Leipzig, discussing and then publishing his dissertation 'The Methods of Colouring'. In his thesis, Erlich emphasised the extreme importance of the application of staining techniques in medicine. As a microbiologist, immunologist, chemist and future winner of the Nobel Prize in Medicine for discovering the use of Salvasartan in the treatment of syphilis, Erlich developed precise cell-staining techniques, which also formed the basis of the later Gram staining. Amongst other things, he described cells, which he called Mastzellen (well-nourished cell), with a cytoplasm rich in dense granules that made the nucleus underneath almost invisible. These cells stained violet with toluidine blue (metachromasia: the phenomenon whereby certain tissue and/or cellular elements take on a different colour from the dye with which they are treated). He also defined another cell type with cytoplasmic granules with a high affinity for

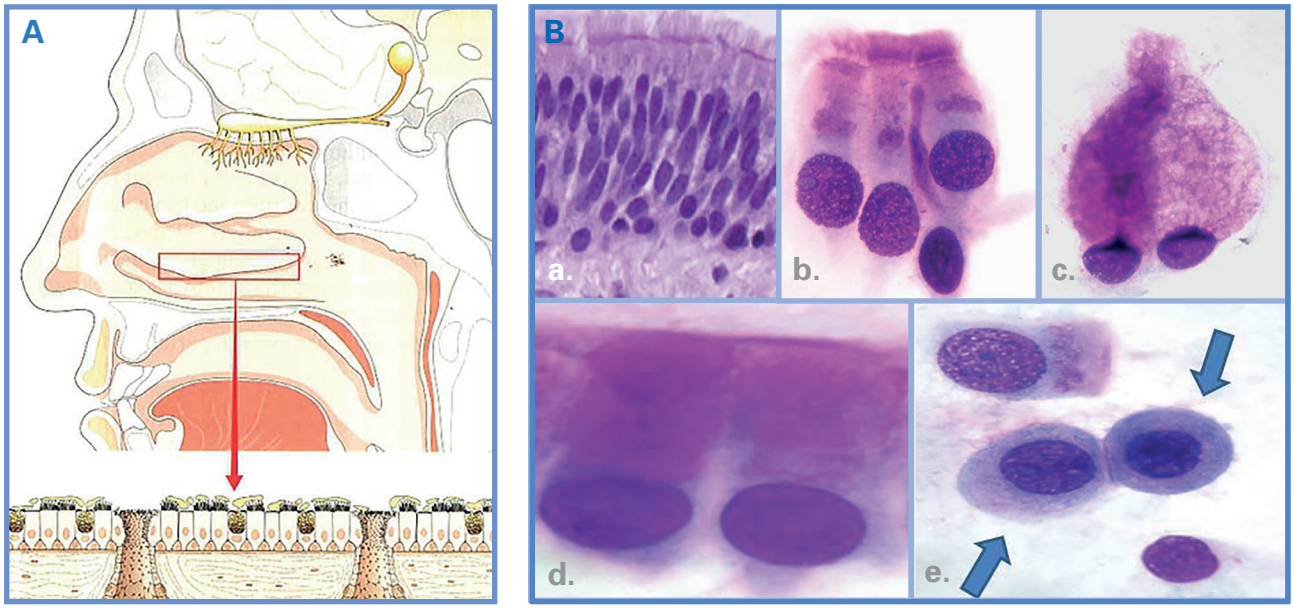
SUMMARY

Keywords
• **allergic rhinitis** • **cellular rhinitis** • **nasal cytology** • **polyposis** • **asthma** • **precision medicine**

Acronyms
• **CRS** chronic rhinosinusitis • **CRSwNP** chronic rhinosinusitis with nasal polyposis
• **CRSsNP** chronic rhinosinusitis without nasal polyposis
• **NARES** non-allergic eosinophilic rhinitis
• **NARESMA** non-allergic eosinophilic-mastocytic rhinitis
• **NARNE** non-allergic neutrophilic rhinitis • **NARMA** non-allergic mastocytic rhinitis
• **NAR** non-allergic rhinitis • **AR** allergic rhinitis • **MGG** May-Grunwald Giemsa

In the field of etiopathogenetic diagnostics of rhinopathies, the use of nasal cytology has become increasingly important.
Although the study of nasal cellularity has ancient roots, dating back to the late 1800s, it is only with discontinuity that we find evidence of it in later years.
Studies resumed systematically in the 1990s, with the use of nasal cytology as a diagnostic tool in rhinopathies demonstrating its usefulness both diagnostically/therapeutically and prognostically.
We will then discuss its use in applied rhinoallergology and in research.

Figure 1 Nasal cytology: easy to perform, non-invasive, quick to learn, useful for differentiating various cell prevalence levels, useful in follow-up, important for research purposes



A. *Anatomical site for nasal scraping: mucosal surface of the medial side of the inferior turbinate.*
B. *Panel a, normal nasal mucosa (stained with MGG; 400×).*
Panel b, ciliated cells (stained with MGG; 1000× with camera magnification factor 2×).
Panel c, muciparous goblet cells (stained with MGG; 1000× with camera magnification factor 2×).
Panel d, striated cells (stained with MGG; 1000× at 2× magnification factor of the camera).
Panel e, basal epithelial cells (stained with MGG; 1000× camera magnification factor 2×).
Images and description taken from article (3) and source (4).

eosin that he called Eosinophil.
Much later, in 1889, with the finding by H. Gollash of the presence of numerous eosinophils in the nasal secretion of a patient suffering from bronchial asthma, there will be the first clue supporting the role of these cells in this disease. When Charles H. Eyermann demonstrated the presence of eosinophils in the nasal secretion of patients with allergic rhinitis in 1927, the first real connection between allergic rhinopathy and nasal cellularity was made (1).
However, we had to wait until the 1990s, which marked the beginning of modern nasal cytology, when Prof. M. Gelardi's research focused on the systematic study of the use of this method in clinical practice as well.
The fact that research programmes involving nasal cytology are very often interdisciplinary, involving allergologists, otorhinolaryngologists, immunologists, rheumatologists, anatomopathologists, ophthalmologists, paediatricians, microbiologists and virologists, testifies to the extreme transversality of this method, which participates in the global care of the patient.
Nasal cytology is an objective, simple,

ENG

Table 1 General classification of rhinopathies according to Gelardi M. Taken from source (6)

Infectious	• Acute • Chronic	• Viral • Bacterial • Mycotic	
Inflammatory	• From physico-chemical-atmospheric agents		
Vasomotor	• Allergic	• Seasonal • Perennial	Replaced by ARIA classification (intermittent/persistent)
	• Non-allergic ("cellular")	• Neutrophilic (NARNE) • Eosinophilic (NARES) • Mastocytic (NARMA) • Eosinophilic-mastocytic (NARESMA)	
Hyperplastic/ Granulomatous	• Nasal polyposis, Anthro-coanal polyp, Wegener's granules, Sarcoidosis. Churg-Strauss		
Tumors	• Papilloma Inverted-Fibroma-Chondroma-Angioma-Carcinoma-Sarcoma etc.		
Atrophic	• Senile or from chronicity of particular rhinitic processes		
Iatrogenic	• From abuse of α-adrenergics, α-blockers, cocaine, clonidine, ACE inhibitors, Oral contraceptives, Antiepileptics, Neuroleptics, Aspirin and other NSAIDs, calcium channel blockers		
Hormonal	• Hypothyroidism • Pregnancy • Pre-menstrual		
Other	• Adrenergic or angiospastic - Cholinergic • Gustatory (oro-nasal syndrome) • Mechanical (Septal dev., foreign bodies, choanal atresia, adenoidism, etc.) • Decubitus - from exercise • Occupational (allergic and nonallergic) • Psychotic - Emotional - Sexual arousal • Ciliary dyskinesia - Cystic fibrosis - Meningoencephalocele		

non-invasive and inexpensive diagnostic method for identifying and phenotyping vasomotor rhinitis in allergic rhinitis, non-allergic cellular rhinitis and infectious rhinitis (bacterial, sporigenic, viral) (2) (Figure 1).

As part of the diagnostic tools for respiratory pathologies, together with the SPT (Skin Prick Test) and, where indicated and necessary, the search for specific IgE against the suspected allergen (RAST) and the search for molecular specific IgE, nasal cytology has proved indispensable for an early and precise diagnosis that generally results in a better prognosis.

Indeed, even in the latest Italian update of the Lybra 2023 Project, nasal cytology appears to be a pivot point in the diagnostic algorithm of inflammatory rhinopathies.

It is recommended for patients suffering from rhinitis, conjunctivitis, asthma, nasal polyposis and also for younger patients suffering from recurrent respiratory infections.

A pharmacological wash-out (antihistamines and corticosteroids) must be carried out prior to nasal cytology to exclude the possibility that the therapy performed may interfere with the expression of immunophlogosis. Only isotonic or hypertonic nasal sprays will be permitted during this period.

The cytological technique involves several steps: collection, processing (which includes fixation and staining) and microscopic observation. Cytological sampling consists of collecting superficial cells of the nasal mucosa using a small disposable plastic curette (nasal scraping). The collection should be performed at the middle portion of the inferior turbinate under anterior rhinoscopy and with good illumination. The cytological specimen must be laid out on the coverslip, fixed by air-drying and then stained, generally, according to the May-Grunwald Giemsa (MGG) method, which is able to stain all cellular components of the nasal mucosa and all cells of the immunophlogosis (eosinophils, mast cells, neutrophils, lymphocytes) bacteria, fungal spores and fungal hyphae. With the coverslip attached, observation is carried out using an optical microscope equipped with 1000x immersion optics. At least 50 fields of the slide are read, and the percentage of cellular elements found is calculated. Nasal cytology allows us to make a differential diagnosis in the field of vasomotor rhinopathies (Table 1), to monitor the course of the pathology, to assess the effectiveness of drug therapy, to select candidate patients for allergen immunotherapy (AIT) and to evaluate its effectiveness (5).

1. Differential diagnosis

Through the use of nasal cytology, it was possible to phenotype all those non-allergic rhinitis previously defined non-specifically as 'vasomotor' and classify them into eosinophilic, mastocytic, eosinophilic-mastocytic and neutrophilic (Table 1) (6).

In patients with allergic rhinitis with overlapping NAR, clinical pictures of Severe Chronic Upper Airway Disease (SCUAD) often occur, in which the symptoms of rhinitis or rhinitis remain uncontrolled despite maximum drug therapy according to guidelines (Figure 2) (7-8).

The timing of the sampling remains of crucial importance for the study of nasal cytology.

The month of November is generally chosen to intercept NAR, during which the aeroallergic insult is generally limited. In November, the A.I.C.NA. (Italian Academy of Nasal Cytology) promotes the National Day of the Rebellious Nose dedicated precisely to the screening of non-allergic inflammatory rhinitis.

In patients with allergic rhinitis due to dust mite sensitisation with a history suggestive of superimposed NAR, it can also be useful to perform cytological sampling in the summer months, in which the finding of immunophlogosis may lead us to a diagnosis of superimposed non-allergic cellular rhinitis.

NARNEs constitute a diverse and constantly increasing group of NARs in which the dense neutrophilic infiltrate is not accompanied by the presence of bacteria and/or fungal spores. It is often found in individuals subjected to physical-chemical aggression: tobacco smokers, industrial workers, inhabitants of heavily industrialised regions as well as patients suffering from gastro-oesophageal reflux disease (GERD).

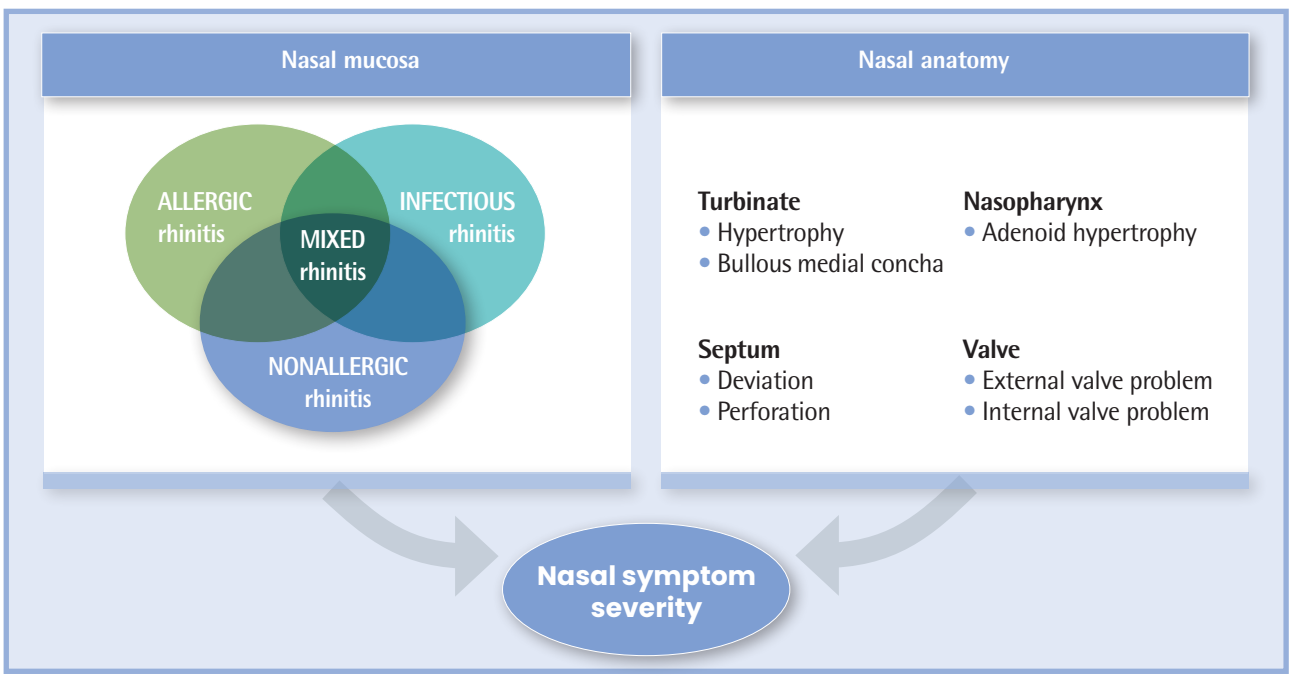
It is also possible to document in both AR and NAR the presence of bacterial and/or fungal overinfection, which often complicates and confuses the symptomatological picture. The occurrence of biofilms is also documented when these pathogenic microorganisms evolve

from the planktonic form (Figure 3).
The biofilm consists of 15% bacterial, fungal or mixed colonies and 85% of an organic matrix produced by them whose skeleton consists of exopolisaccharides (EPSs). Typical is the cyan colouring, in all its shades, which can be highlighted with MGG staining.
Viral infectious rhinitis can also be documented, characterised by a rich infiltrate of lymphocytes with a normal morphological appearance of both the nucleus and cytoplasm, by the detection of cells with multinucleation phenomena (ground glass image) and by the typical phenomenon called ciliocytophthora in the ciliated cells, an indirect sign of viral infection.
Ciliocytophthora includes a thickening of the nuclear chromatin, positioning at the margins of the nucleus, the appearance of inclusion material, the formation of a halo around the nucleus, the appearance of cytoplasmic granules and the narrowing of the cell body with consequent separation of the basal portion from the ciliated apical portion.

2. Monitoring the course of the disease

Nasal cytology is also an agile and effective tool in monitoring the course of the disease. Indeed, in patients with allergic rhinitis, the extent of clinical symptoms correlates with the degree of inflammation found in the nasal epithelium (10). The documented inflammation at the level of the nasal epithelium in patients suffering from allergic rhinitis caused by pollinosis is characterised by a rich eosinophilic infiltrate with impairment of the ciliated cells, which appear impoverished, and often present themselves as HSS negative, i.e. lacking the Hyperchromatic Supranuclear Stria that characterises them and which testifies to their wellbeing and functionality associated with an increase in muciparous cells. All of the above results in an alteration of the physiological 5/1 ciliate/muciparous cell ratio with a consequent

Figure 2 Phenotypes of chronic rhinitis. Image taken from article (9)



loss of the nose's main means of defense, the ciliary movement (11-12).
In a multi-center study, eosinophilic nasal inflammation was assessed by nasal cytology in patients allergic to *Parietaria* pollen. This work showed that the worsening of nasal inflammation is significantly associated with *Parietaria* flowering peaks, confirming the use of this method as a valid tool for monitoring nasal allergic inflammation (13).
In patients with allergic rhinitis caused by sensitisation to larger dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae) a persistent minimal inflammation is characteristic. This includes a rich neutrophilic infiltrate with rare eosinophils that may also occur in degranulation in the acute phases.
In NARES the eosinophilic infiltrate is pathognomonic, as is the mast cell infiltrate in NARMA. In NARESMA there is the most severe inflammation with a related obstructive symptomatology that is often particularly harsh. The detection of Charcot-Leiden crystals consisting of a protein, galectin-10, correlates with the severity of the eosinophilic infiltrate and symptomatology and, in rhinosinusitis with nasal polyposis, has been shown to be a reliable predictor of relapse (14).
Cellular rhinitis accounts for at least 25 per cent of all rhinitis patients and may coexist with allergic forms in at least 60 per cent of cases. They appear to be more common in women than in men and their clinical severity is related to nasal respiratory obstruction. Possible complications include CRSwNP or CRSsNP. As comorbidities we find bronchial asthma, aspirin intolerance and allergy. Patients with CRSwNP must also be evaluated with nasal cytology and by Clinical-Cytological Grading (CCG), which allows us to define the prognostic index of relapse. Appropriately, a strong correlation between CCG and galectin-10 expression, mainly colocalised with infiltrating eosinophils and mast cells, has also been demonstrated in patients with CRSwNP.
Patients with seasonal allergic rhinitis with a major component of moderate to severe nasal obstruction were assessed during the pollen season by symptom scoring, rhinomanometry and nasal cytology. IL-4, IL-5, IL-8 and IFN-γ were also assayed by immunoassay on fluids recovered from nasal lavage. A clear relationship emerged between allergic inflammation and nasal airflow. Furthermore, nasal allergic inflammation affiliated with allergic rhinitis was also associated with decreased lung function, in terms of bronchial symptoms, forced expiratory volume in 1s (FEV1) and methacholine provocative dose, and nasal cytology was also found to be a good predictor of early bronchial damage (15).
An elegant study on the role of nasal eosinophilia, assessed using the technique of nasal cytology as an indicator of eosinophilic inflammation in asthma, demonstrated that nasal eosinophilia can be a good predictor of eosinophilia in the sputum of subjects with asthma, promising a greatly simplified monitoring of inflammation in all airways with remarkable accuracy (16).

Figure 3 Bacterial biofilm. Original image

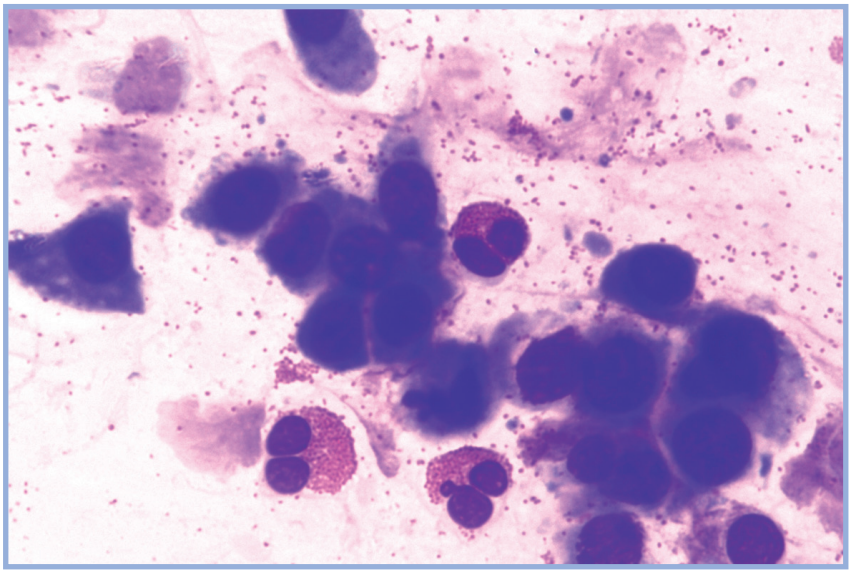


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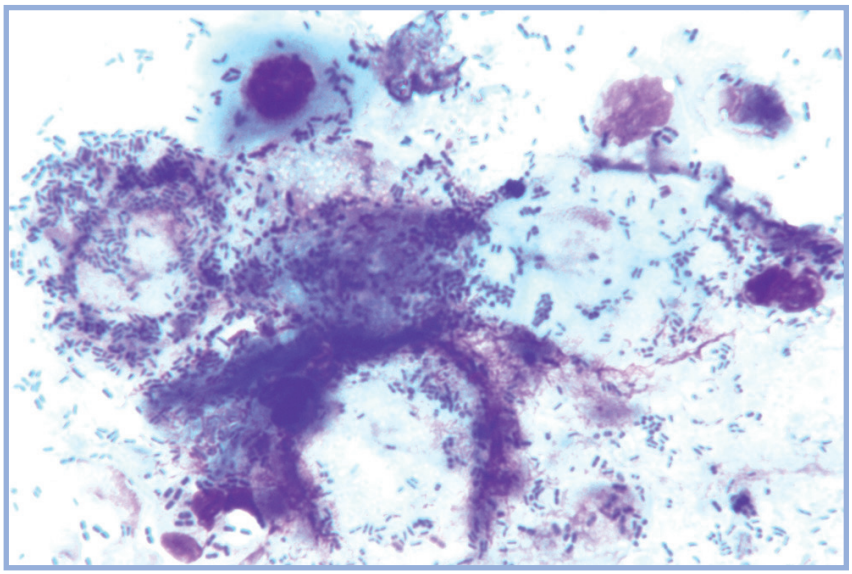
Figure 4 NARES. Original image



3. Evaluation of the effectiveness of drug therapy

A multicentre prospective observational study evaluated the use of Mepolizumab, an anti-IL-5 monoclonal antibody, in patients with severe asthma with CRSwNP and its impact on sino-nasal symptoms, polyp growth and asthma control in real life.

Primary efficacy endpoints included the mean change in the Sino Nasal Outcomes Test (SNOT-22) and the Total Endoscopic Nasal Polyp Score (TENPS) from baseline, at 6 and 12 months of therapy. Secondary efficacy endpoints included change in blood eosinophil count, Prednisone intake, Asthma Control Test (ACT) score, Tiffeneau index (FEV1%), exhaled nitric oxide (FENO) at T0, T6, T12. In a subgroup of patients, nasal cytology was also performed by nasal scraping before (T0), after 6 months (T6) and after 12 months (T12) of Mepolizumab treatment. The results of nasal cytology suggested a significant reduction in the percentage of eosinophils after therapy and demonstrated a significant correlation with the primary and secondary endpoints, confirming the possible use of this method as a tool for monitoring the efficacy of drug therapy for Th2 inflammation of the upper and lower airways. No statistically significant downward trend was measured for FENO (17).

4. AIT Management

Introduced into clinical practice a century ago, AIT currently finds its application in both paediatric and adult populations in patients suffering from allergic rhinitis and rhinoconjunctivitis with or without allergic bronchial asthma. Moreover, numerous studies have shown that early use of AIT can contribute to the prevention of asthmatic pathology and even further sensitisation. Effectively selecting candidate patients for AIT is the first essential step towards therapeutic success.

Although Th2 inflammation and eosinophilic cellularity is common to allergic rhinitis (AR) and NARES, it is clear that the efficacy of AIT is specific and exclusive to allergic pathology.

For these reasons it becomes of paramount importance to complete the diagnostic framing of patients with allergic rhinitis who are candidates for allergen-specific desensitising immunotherapy with a nasal cytological examination in order to exclude and/or document the coexistence of a NAR. A recent study showed that 15% of patients with AR enrolled did not respond to AIT. This result appears to be consistent with previous studies that reported a response rate to AIT between 80 and 85% (18-19). It is therefore proposed to use nasal cytology, a simple, reliable and objective method, as a prognostic index to identify responders to AIT. Although AIT failure may depend on several factors such as poor adherence to treatment, early discontinuation, low allergen dosage or low quality of the allergen extract, the data identifying patients with allergic rhinitis with overlapping NARES as those in whom treatment failure has occurred, dictate early phenotyping into AR, NARES and mixed rhinitis.

5. Conclusions

In conclusion, this study highlights how the reason for the failure of AIT could depend on a defined phenotype, such as mixed rhinitis: AR associated with NARES (Figure 4). Nasal cytology could, therefore, also be a useful tool in the management of AIT (5-20).

Currently, no definitive biomarkers for the efficacy of AIT are available. Certainly, objective mechanisms of efficacy include, for example, an immune deviation towards the Th1-secreting IFN-γ response (20), but among others, nasal cytology could have a huge impact in real life, allowing the selection of the most suitable patients for this therapeutic option, which still remains the only therapeutic treatment capable of modifying the natural history of allergic respiratory diseases.

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ENG

Specific immunotherapy and tolerance: regulatory ILC2s

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1. The Immune System

The immune system consists of a collection of specialised cells and molecules, developed during evolution, whose physiological function is to defend the organism against infectious agents; however, foreign substances of a non-infectious nature (e.g. allergens, haptens) can also induce an immune response. The immune system's protective mechanisms can, in some cases, be themselves the cause of tissue damage and disease. The protective function of the immune system involves two closely cooperating components: innate (or natural/native) immunity and adaptive (or acquired/specific) immunity. Innate immunity is also possessed by plants and insects, as well as mammals (e.g. defensins, peptides toxic to bacteria and fungi common to plants and mammals, or Toll-like receptors, found in all forms of life, from insects to mammals), while adaptive immunity is specific to vertebrates.

Innate immunity forms the first line of defence against pathogens, recognises the body's self-cells when they are damaged or dead, eliminates them and initiates the process of tissue repair, and finally stimulates and makes the adaptive re-

SUMMARY

Keywords
• **bronchial asthma** • **cytokines** • **ILC** • **Th** • **AIT**

Acronyms
• **ILC** innate lymphoid cell • **ILC2** innate lymphoyd cell type 2
• **Th** T helper • **AIT** allergen-specific immunotherapy
• **SLIT** sublingual immunotherapy
• **SCIT** subcutaneous immunotherapy
• **TCR** T cell receptor • **APC** antigen presenting cell
• **CRSwNP** chronic rhinosinusitis with nasal polyps
• **mAbs** monoclonal antibodies

To date, allergen-specific immunotherapy (AIT) is the only treatment that can interfere with the natural history of respiratory allergies such as rhinitis and mild-to-moderate asthma, and a critically important aid in the treatment of patients with hymenoptera venom allergy. Over the last few years, AIT has made important progress, with the advent of sublingual immunotherapy (SLIT) in addition to subcutaneous immunotherapy (SCIT) in the treatment of respiratory diseases, which has made it possible to achieve a high safety profile, and consequently the possibility of treating patients with mild-to-moderate asthma. Moreover, the recent introduction of molecular allergy diagnostics has allowed a better definition of patients who are candidates for this type of treatment, increasing the frequency of therapeutic success. Finally, the increasing knowledge of the biological mechanisms by which AIT induces a modulation of the allergen-specific response has revealed how this treatment impacts the immune response in a comprehensive manner. In fact, the biological changes associated with the clinical benefits of AIT involve numerous components of specific and innate immunity, and are not always the same in every patient, demonstrating that there may be ▶

SUMMARY

differences in the mechanisms of action of the different preparations used. Recent findings unequivocally confirm how AIT is a precision medicine and personalised medicine approach indispensable for a modern and correct management of the allergic patient and for delivering in the hands of the immunoallergist an effective tool to modify the natural history of allergic diseases.

sponse more effective. The components of innate immunity are: physical and chemical barriers such as lining epithelia, serum proteins such as complement and other mediators of inflammation, and a heterogeneous group of cells including phagocytes (e.g. neutrophil granulocytes and macrophages), mast cells, basophilic and eosinophilic granulocytes, dendritic cells, and ILCs. Specific immunity is present in all vertebrates and reaches its maximum development in mammals. It is defined as "adaptive" because it develops and adapts following infection, "specific" because it can distinguish closely related antigens, and "acquired" because it is able to respond more effectively to a second encounter with the same pathogen. The main characteristics of adaptive immunity are: specificity, i.e. the ability to generate different responses towards different epitopes; diversification, i.e. the ability to recognise an enormous number of antigens; tolerance towards the self, which consists in the non-response to the individual's own antigens; specialisation, which consists in the generation of optimal responses to counteract different pathogens; self-limitation, which translates into the attenuation of the response over time to avoid damage to the organism; and memory, i.e. the ability to increase the effectiveness of the response to subsequent encounters with the same antigen. Adaptive immune responses are essentially of two types: humoral immunity mediated by B lymphocytes and the molecules secreted by them, i.e. antibodies (Ab), and cellular immunity, mediated by T lymphocytes, which recognise the antigen via the TCR, after processing of the antigenic peptide by specialised cells called APCs. Mature T lymphocytes, on the basis of specific markers, are distinguishable into two subpopulations: CD4+ T lymphocytes, also called "helpers" due to their ability to induce B lymphocytes to produce antibodies, and CD8+ T lymphocytes, also called cytotoxic due to their cytolytic potential.

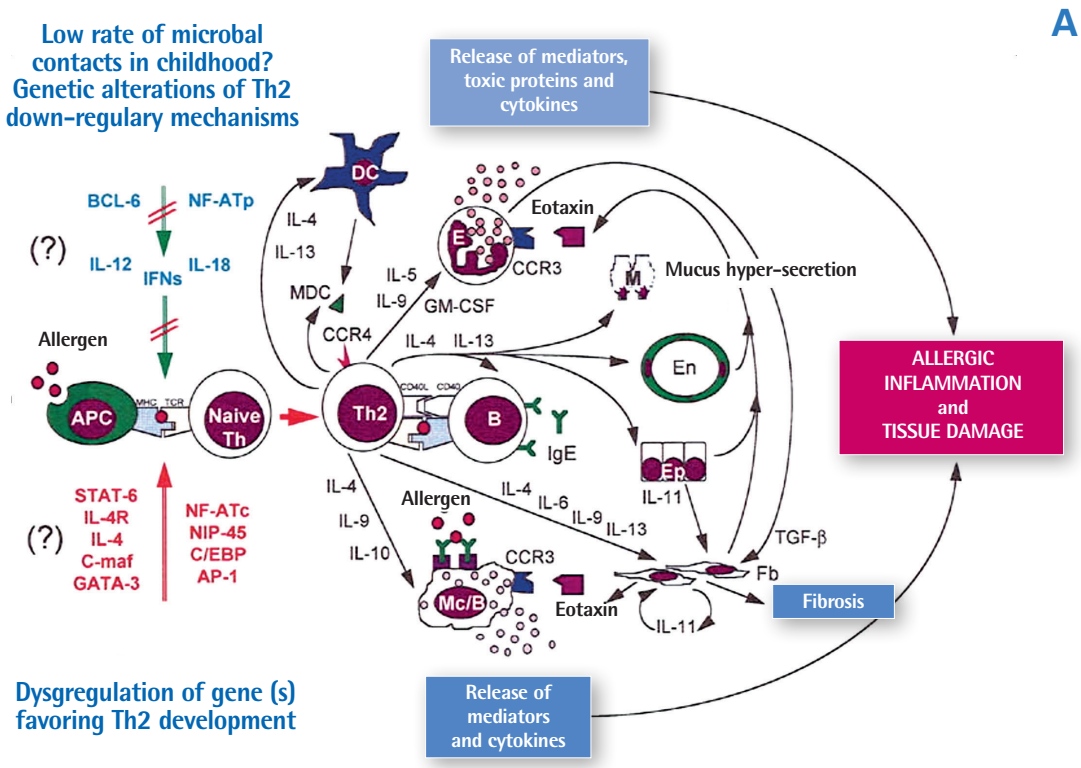
1.1 The three types of effector immunity

Based on the type of pathogen against which our immune system responds, one can basically distinguish three types of effector immunity: type 1 immunity, involved in responses against intracellular pathogens; type 2 immunity, involved in responses against helminths and venoms; and type 3 immunity, capable of responding to extracellular pathogens and fungi. CD4+ T helper lymphocytes are distinguished into different functional subpopulations, not only on the basis of their cytokine production profile, but also and above all for being the main players in the three aforementioned types of effector immunity. Th1 lymphocytes, involved in defence against intracellular viruses and bacteria, produce the cytokine IFN-γ, which activates macrophage functions and promotes the phagocyte-dependent response. Th2 lymphocytes, on the other hand, are characterised by the production of cytokines (IL-4, IL-5, IL-9, IL-13) that act on B lymphocytes, eosinophilic granulocytes and mast cells in the response against parasites. Finally, Th17 lymphocytes are implicated in the response towards mycophytes and extracellular bacteria, thanks to the production of the pro-inflammatory cytokine IL-17, which promotes the activation of neutrophil granulocytes. Each of these lymphocyte subpopulations may also be involved in the pathogenesis of various diseases. This occurs when the regulatory mechanisms of the immune response fail, or when the immune response is directed towards antigens that do not deserve it. Th2 responses to allergens constitute the biological primum movens of allergic diseases (1-3).

1.2 ILC2: from protective function to role in allergic diseases

ILCs are a heterogeneous population of cells of innate immunity that cooperate with their counterparts of specific im-

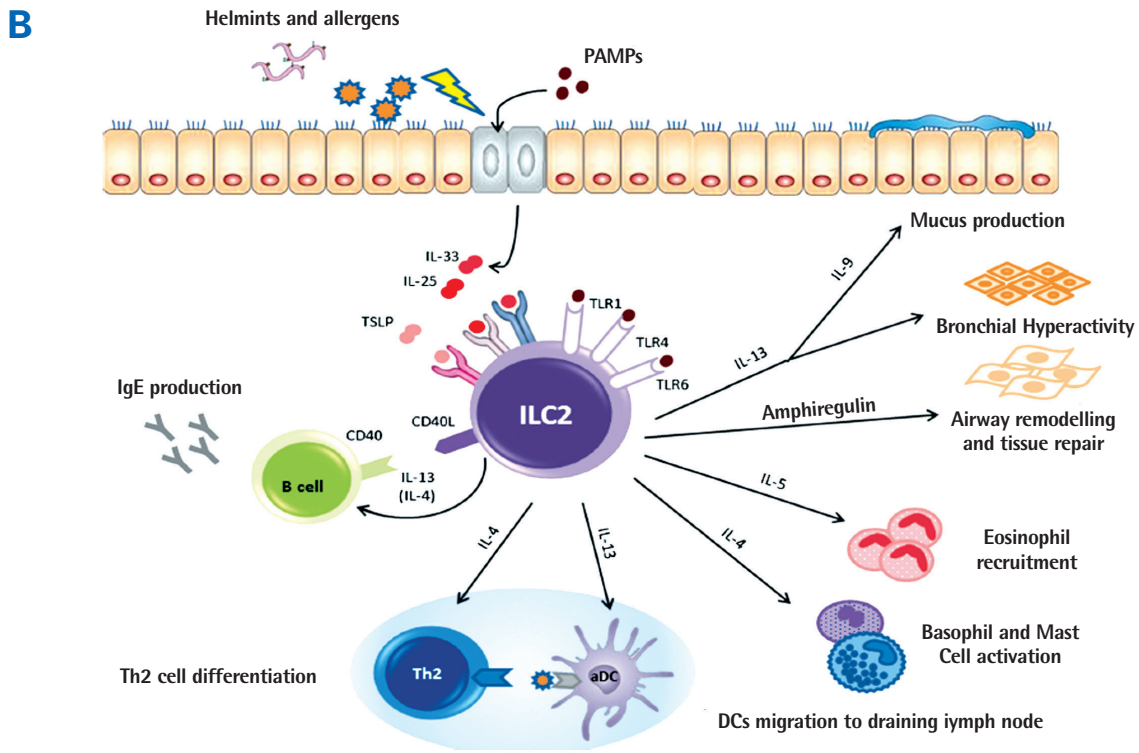
Figure 1



Images taken from articles (2) and (10).

munity. Based on the similarity of the effector cytokines produced with those of the "helper" T lymphocytes, three groups of ILCs can be distinguished: ILC1s, which produce cytokines similar to their Th1 counterparts, ILC2s, which produce cytokines similar to those of Th2 and ILC3s, which produce cytokines similar to those of Th17 (4). ILC2s are present in the mucosa of the respiratory tract and gastro-intestinal tract below the epithelial barrier, in the skin, and in adipose tissue, while they are rarer in peripheral blood. They are characterised by the expression of high levels of the transcription factor GATA-3 (also expressed by Th2 lymphocytes) and membrane receptors that allow them to respond to activation and proliferation stimuli. These include receptors for the proliferative cytokines IL-2R, IL-4R, IL-7R and IL-9R, but also receptors for cytokines of epithelial origin such as IL-33R, IL-25R, TSLPR. ILC2s also express the prostaglandin D2 receptor, CRTH2, the cys-leukotriene receptor and the

Role of Th2 lymphocytes (a) and ILC2 cells (b) in the initiation and maintenance of type 2 inflammation



CD161 molecule (4-5). In recent years, the pathogenetic role of ILC2s in allergic inflammation has been clarified. This cell subset is able to be activated in response to numerous exogenous stimuli, including the aforementioned epithelial cytokines (IL-33, IL-25, TSLP). The submucosal localisation of ILC2s, in close proximity to epithelia, makes these cells the earliest subset of type 2 inflammation activated in response to exogenous stimuli (Figure 1). Overall, the data in literature support a possible pathogenetic role of ILC2s in both the initiation and maintenance of allergic inflammation, and thus nominate this cell subset as a possible therapeutic target in these diseases (6-10).

2. Allergen-specific immunotherapy

AIT was introduced in the treatment of allergic diseases over 110 years ago, when IgE had not yet been identified, nor had it been assumed that there were serum factors capable of mediating the clinical manifestations of allergy (11). AIT encapsulates the basic principles of

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precision and personalised medicine, in that on the one hand it aims to modulate the specific response to the allergen (precision), and on the other hand it provides for the choice of allergen and type of immunotherapy according to the characteristics of the individual patient (personalisation). In addition to all this, AIT represents the only therapy to date that can interfere with the natural history of the disease. However, Noon's pioneering work has not been followed by an equally rapid development of knowledge in the field, and this has probably been a limitation for the spread of this treatment. Added to this is the fact that, in the past, AIT carried out with non-standardised preparations was not free of side effects, even serious ones (12), which were also due to unaware choices of patients eligible for this treatment. The scepticism that has accompanied AIT throughout its long history has also led to an underestimation of its clinical efficacy, which is often neglected and unjustly overlooked. Today we know that, when the patient is correctly selected, AIT is effective in most cases, with rare and generally non-serious side effects, and above all with a modulation of the response to the allergen that lasts over time, unlike that obtained with all other types of drugs used in respiratory allergic diseases, whether biological or not.

Figure 2 Importance of registers in Real World Evidence

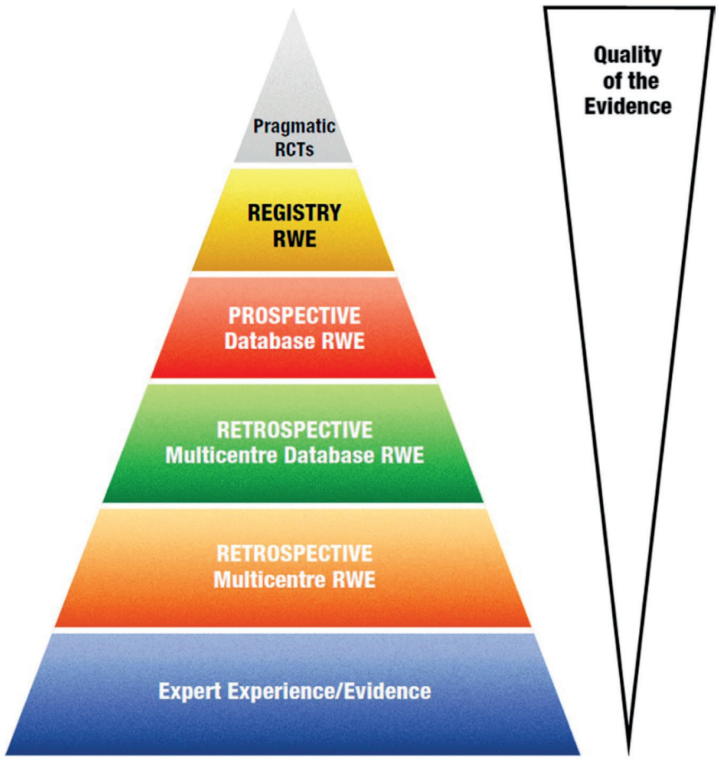

Image taken from article (38).

3. Allergen-specific immunotherapy in respiratory allergic diseases

AIT is currently approved for clinical use in the treatment of oculorhinitis and mild to moderate allergic asthma, and allergy to hymenoptera venom. Allergic diseases, and severe asthma in particular, have benefited over the last decade and more from a number of new drugs that have enabled better disease control. These are the so-called biological drugs, i.e. humanised monoclonal antibodies that are able to interfere with key molecules of the type 2 inflammatory process, such as IgE, IL-5, IL-4 and IL-13. Obviously, the correct use of these new drugs cannot disregard knowledge of the clinical phenotype and inflammatory endotype that characterises each individual patient. With this in mind, the concept of precision medicine and personalised medicine has emerged in recent years to emphasise how indispensable patient characterisation is for the choice

of the most suitable biological drug for disease management. Frequently, when one speaks of biological drugs in allergic diseases, one refers only to monoclonal antibodies used for the treatment of severe asthma, atopic dermatitis, CRSwNP, and chronic idiopathic urticaria, neglecting AIT, which constitutes, as already pointed out, a paradigmatic example of precision medicine and personalised medicine.

Focusing on asthma sustained by type 2 inflammation, AIT should not be considered as an alternative drug to mAbs, but rather as a valuable resource to treat the patient with a disease-modifying approach. As is well known, monoclonal antibodies are approved in the treatment of severe asthma, whereas AIT is indicated in mild-moderate forms. One can therefore imagine a pathway of biological therapies for the severe asthmatic patient who, once controlled with mAbs, becomes eligible for treatment with AIT (13). With this in mind, there are already some data indicating a potential synergy of AIT with certain mAbs, in those patients who use mAbs for diseases other than asthma (CRSwNP, atopic dermatitis, chronic idiopathic urticaria), and AIT for rhinitis or mild-to-moderate asthma (14).

Among the most recent advances that have enabled the use of AIT in asthma are the use of SLIT as a safer mode of administration, and the advent of molecular diagnostics. In addition to these two crucial steps, there is an increasingly detailed understanding of the biological mechanisms by which the clinical benefits, induced by AIT, are generated.

4. Personalising AIT: the role of SLIT and molecular allergy diagnostics

As already pointed out, among the reasons that have hindered the use of AIT in asthma there are the side effects, which in the past have also caused rare fatal events (12). Severe asthma and uncontrolled asthma are indeed contraindications to the implementation of AIT, but at the same time it is universally accepted that SLIT is a safer treatment than SCIT (15), and it is precisely considering the "safety" aspect that SLIT has progressively replaced SCIT in the treatment of respiratory allergies; in fact, as far as clinical effects are concerned, the two types of administration have comparable efficacy, and according to some authors SCIT still retains a slight superiority (16). The first step along the pathway that allowed SLIT to rise to the status of a possible treatment for mild-to-moderate allergic asthma was its recognition as an effective therapy in modulating the allergen-specific response (17). Subsequently, SLIT's ability to reduce the dosage of inhaled corticosteroids (ICS) required for asthma control was demonstrated, as well as its ability to reduce the frequency of exacerbations (18). All of this makes SLIT a candidate as a potential approach to induce asthma remission even after treatment is discontinued (19).

Another equally important step forward has been made thanks to the advent of molecular allergy diagnostics, which is indispensable for the correct selection of patients eligible for AIT. This approach has in fact made it possible to unmask those false polysensitisations by panallergens, which are in fact a brake on the prescription of AIT. At the same time, the study of the molecular profile of sensitisations makes it possible to identify patients who are not candidadable for certain types of AIT, and thus to avoid treatment with a low probability of efficacy (20-21).

5. AIT-induced immunological modulation

Although it has been used for over a hundred years with excellent results, the biological mechanisms by which AIT exerts its clinical effects are still not fully known. Nevertheless, multiple aspects of the allergen-specific response undergo substantial changes during AIT and, more importantly, these changes correlate with efficacy (22).

The earliest effect of AIT is probably the modulation of the allergen-specific antibody response, in terms of antibody isotypes. An increase in allergen-specific IgG4 levels occurs already within the first few weeks after the start of SCIT treatment. It was recently described how SLIT preferentially induces the production of allergen-specific IgA, which can also be found in nasal secretions, whereas SCIT predominantly induces IgG4 production (23). Similarly, the ability of mite-SCIT to induce IgD as well as IgG4 and decrease IgE has been described, while the ability to increase IgG2 is also reported for SLIT (24-25). All of these IgG isotypes or subclasses have the ability to compete with IgE for binding to the allergen, and thus lead to a reduction in the activation level of mast cells and basophil granulocytes. Another im-

portant biological effect of AIT is, partly related to the previous one, the capability of inducing a decrease in the degranulation potential of mast cells and basophil granulocytes, which is maintained even once AIT is discontinued (26).

As far as specific immunity is concerned, the two main effects of AIT are its ability to induce tolerance and modulation of the response towards the allergen. The first of these effects occurs as a consequence of chronic exposure to the allergen, which induces the expansion of regulatory T lymphocytes. A multitude of factors contribute to the achievement of the state of tolerance, and particularly important among these is the effect of the regulatory cytokine IL-10, secreted by activated regulatory T lymphocytes, whose levels increase during repeated exposure to the allergen. The second important effect is its ability to modulate the type 2 response to the allergen. Indeed, it is known that the acquisition of a type 1 phenotype is associated with a decrease in inflammatory potential and a decrease in IgE production. These two mechanisms, far from being mutually exclusive, can coexist, and the prevalence of one or the other in the individual patient probably depends on individual genetic factors and the type of immunotherapy used (27-28).

Similar to T lymphocytes, B cells are also a target of AIT. Regulatory B lymphocytes (B reg) are able to control the excessive inflammatory response by producing IL-10, which inhibits the secretion of pro-inflammatory cytokines and facilitates differentiation into the regulatory component of T lymphocytes. B lymphocytes specific for phospholipase A2 (PLA2), a major allergen in bee venom, have been isolated from beekeepers with tolerance to these antigens (29).

6. ILC2s as a therapeutic target in allergen-specific immunotherapy

In addition to the effects on lymphocytes of specific immunity, it has emerged in recent years that ILC2s are also modulated during AIT (30). ILC2s contribute to the protective response against parasitic infestations, and at the same time play a major role in triggering and maintaining inflammation during allergic diseases (31). It has recently been shown that ILC2s are modulated by microenvironmental factors, and that they possess a functional plasticity very similar to that previously described for T helper lymphocytes (32). The observation that circulating ILC2 cells increase in frequency after allergen challenge in subjects allergic to cat epidermal derivatives (33), and during the pollen season in patients allergic to grasses (34), confirms that this subset is implicated in allergen-induced inflammation and thus candidates it as a potential therapeutic target. It has recently been shown that during AIT there is an expansion in peripheral blood of an ILC2s subset secreting IL-10 and expressing CTLA-4, the presence of which correlates with clinical improvement in treated patients (35). The AIT-induced production of IL-10 by ILC2 cells is associated with a decreased ability of this modulated subset to produce IL-13, a cytokine whose pro-phlogistic role in type 2 inflammation is well documented (36). ILC2 cells producing IL-10 constitute a subpopulation that is functionally distinct from the classical phenotype and have therefore been termed regulatory ILC2s. Regulatory ILC2s and "pro-allergic" ILC2s originate from a common immature precursor but evolve to different functional types based on exposure to different modulating agents. Regulatory ILC2s are less represented in the peripheral blood of grass-allergic patients, but their level is restored by AIT, and this is probably the most intriguing aspect of these new findings (37). The functional modulation of ILC2s is absolutely superimposable to that described for CD4 lymphocytes during AIT, and this suggests that the concept of functional plasticity of the immune response is not exclusive to specific immunity but should be extended to the innate component.

7. AIT: the importance of patient registers

The complex mechanisms by which the therapeutic effect of AIT is realised are many and varied. As we have seen, they depend both on the mode of administration of AIT (SCIT vs. SLIT) and on the type of preparation used, but there are also factors related to the individual patient that account for the different biological response in each individual. One methodological approach, which is certainly useful in understanding this sort of "pleiotrophic" effect of AIT, is to create registers of patients treated with AIT. Such registers will in fact make it possible to assess the actual extent of the use of this therapy and will give us im-

portant information on the biological changes induced by the different AITs. In addition to reporting when and how AIT is performed, the registers will also be a source of data on those biomarkers whose modulation is associated with AIT. Given the great strides made by AIT over the last few years in terms of safety, identifying the "responder" patient, and understanding biological mechanisms, the creation of the registers represents a fundamental building block, since the advancement of scientific knowledge is based on the collection and analysis of data. This will enable AIT to make that much hoped-for transition from empirical treatment to therapy supported by RWE (Real World Evidence) (38) (Figure 2).

8. Conclusions

Allergic diseases are underpinned by a complex inflammatory network known as type 2 inflammation, in which Th2 lymphocytes certainly play the leading role, but which involves other players, including B lymphocytes, eosinophils, ILC2s, mast cells and also tissue cells, such as epithelial cells, smooth muscle cells and fibroblasts. AIT is able to interfere with this inflammatory circuit via multiple mechanisms that are not yet fully known. The different formulations of AIT probably lead to different biological effects in treated individuals. In this regard, we must emphasise how we often approach AIT in a global manner, without considering that the different allergenic extracts may behave differently. This is well known with regard to the evaluation of safety and efficacy, but the same type of reasoning is also valid with regard to mechanisms of action. A more accurate understanding of the biological mechanisms by which the clinical benefits induced by AIT are generated will certainly be facilitated by the creation of registries where efficacy data can be obtained on an international scale to understand which immunotherapies are able to modulate which biomarkers and in which patients.

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Anaphylaxis: 10 steps to recognise and manage this emergency

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SUMMARY

Keywords
• Adrenaline • Anaphylaxis • Corticosteroid • IgE • Mast cell
• Adverse reaction • Treatment

Anaphylaxis is a severe allergic reaction, which can potentially be life-threatening. Although its prevalence and incidence are low, it is underdiagnosed due to a lack of consensus on its definition and its often poor recognition. Moreover, the therapeutic management of the reaction, once it occurs, is not always adequate, delaying the administration of the treatment of choice (adrenaline) and, thus, the prognosis. The following article addresses, in 10 simple steps, the main elements to know about anaphylaxis in order to arrive at a correct identification, diagnosis and treatment.

1. What is it?

Anaphylaxis is defined as 'a systemic, rapid-onset and potentially fatal hypersensitivity reaction' (1). It is an acute reaction in which several organs/systems are affected simultaneously, and which can be potentially severe. This is why it is of particular importance to know its mechanism of action, its main triggers, how it manifests itself and how to manage it.

2. How many people does it affect?

The overall prevalence of anaphylaxis in the general population varies depending on the series, but ranges between 0.3 and 5% (1). The described incidence also fluctuates in a wide range from 6 to 110 episodes per 100,000 people/year (2). These figures may be even higher in the paediatric population. The wide differences between one study and another are due to different causes, but among the main ones is the lack of consensus on its definition and selection criteria, as well as the underdiagnosis of the event, mainly due to the lack of recognition of its symptoms by those who work in emergency departments and receive patients during acute episodes.

3. Why does this happen?

The symptoms of anaphylaxis are due to the release of mediators by various cell types. Traditionally, this release has been attributed mainly to the activation of mast cells and basophils, although the involvement of other cells such as macrophages, neutrophils and myeloid cells has also been described (3).

Among the mechanisms of mast cell activation, the main and best-known comes from the interaction between IgE and their high-affinity receptor (FcεRI). This process also occurs, to a lesser extent, in basophils. There are also mechanisms other than the IgE-mediated ones, among which the IgG-mediated one with the FcR receptor (which leads to the release of platelet aggregation factor to a greater extent than histamine) and the complement-mediated one, mainly by the active fragments C3a and C5a, which are considered anaphylatoxins, should be highlighted. Less well known, but also described, is secondary activation due to genetic alterations or by transcription factors (3). In addition to these immunological mechanisms

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described above, there are also mechanisms that are considered 'non-immunological', including drugs such as opioids or mast cell receptor polymorphisms (MRGPRX2) that can activate the cell, causing it to degranulate.

4. What are the triggering factors?

The main causes of anaphylaxis are drugs, food and insect bites (4). However, there is wide variability in the prevalence of reactions to these triggers between age groups, as repeated exposure to potential allergens proportionally influences the frequency of reactions. Thus, the main cause of anaphylaxis in the paediatric population is food, whereas drugs are the main cause of anaphylaxis in adults. Similarly, geographical location and consumption habits mean that the trigger may vary even within the same group. In adults, the foods most commonly involved in anaphylaxis episodes are nuts, shellfish, fish and fruit, whereas in children it is eggs, milk, nuts, fish and shellfish (5,6).

The main causes of anaphylaxis (globally) are shown in figure 1.

5. Are there any risk factors?

The onset (and severity) of anaphylaxis does not depend solely on the triggering allergen. It appears to be related to multiple factors, including individual susceptibility. In general, the severity of anaphylaxis may be related to factors depending on the patient, the reaction itself or the affected organ (5,7):

Patient-related factors:

- Atopy;
- Age;
- Sex;
- Presence of respiratory and/or cardiovascular disease;
- Treatment with angiotensin-converting enzyme inhibitors and/or beta-blockers;
- Mastocytosis or mast cell activation syndromes;
- Hereditary alpha-tryptasemia.

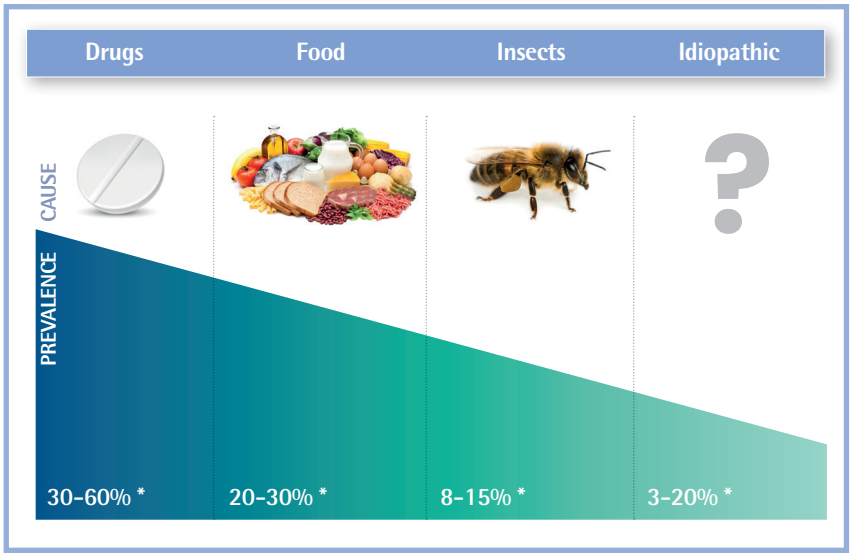
Response-dependent factors:

- Route of entry;
- Allergen;
- Presence of cofactors (menstruation, alcohol, exercise, NSAIDs, fever, infection, stress, sleep deprivation).

Dependent factors of the affected organ:

- Respiratory route (hypoxia);
- Cardiovascular system (hypotension);
- Neurological involvement.

Figure 1 Main causes of anaphylaxis. Adapted from (5). *In the Spanish and Latin American population.



6. How is the diagnosis made?

The symptoms of anaphylaxis are those produced, to a greater extent, by the action of mast cell mediators released during their activation. These can be very varied and herein lies the main difficulty in diagnosing anaphylaxis: there are no pathognomonic signs or symptoms in this event. More than 80% of allergic reactions have skin involvement, so a correct diagnosis will be made in ¾ of anaphylaxes with skin and/or mucous membrane involvement. The difficulty lies in identifying

Figure 2 Diagnostic criteria for anaphylaxis

CRITERIA IMPLEMENTED (8)	WAO PROPOSAL (1)
1. Acute onset of the syndrome affecting the skin and/or mucous membranes together with at least one of the following: a) Respiratory impairment b) Decreased BP or symptoms of organic dysfunction	**1. Acute onset of the syndrome affecting the skin and/or mucous membranes together with at least one of the following:** a) Respiratory impairment b) Decreased BP or symptoms of organic dysfunction c) Persistent gastrointestinal symptoms
2. Rapid onset of ≥ 2 of the following symptoms following exposure to a potential allergen a) Involvement of the skin and/or mucous membranes b) Respiratory impairment c) Decreased BP or symptoms of organic dysfunction d) Persistent gastrointestinal symptoms	**2. Decreased BP or bronchospasm or laryngeal involvement minutes to a few hours after exposure to an allergen known to the patient:** a) Infants and children: low PS or >30% drop in systolic BP* b) Adults: systolic BP < 90 mmHg or drop > 30% from baseline BP
3. Decrease in BP within a few minutes/some hours after exposure to an allergen known to the patient: a) Infants and children: low PS or >30% drop in systolic BP* b) Adults: systolic BP < 90 mmHg or >30% drop from baseline BP	

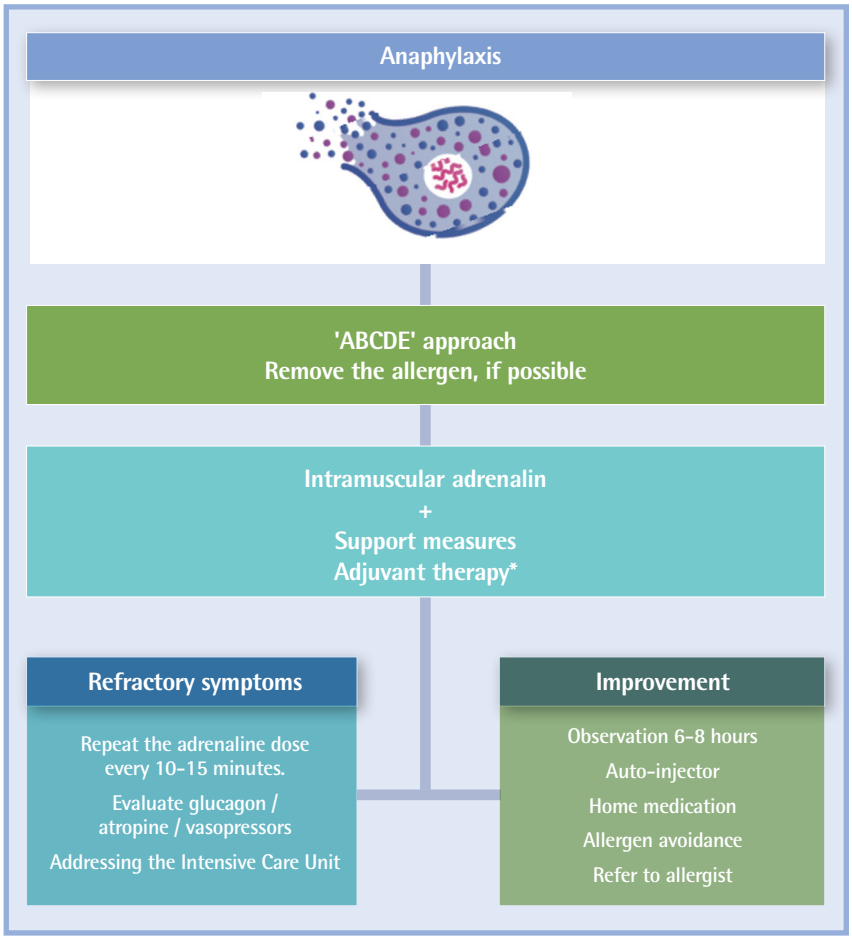
BP, *blood pressure *Low systolic blood pressure in childhood: less than 70 mmHg from 1 month to 1 year, less than [70 mmHg + (2 x age)] from 1 to 10 years and less than 90 mmHg from 11 to 17 years.*

the 20% of anaphylaxes that do not involve skin damage. The diagnosis is based on suspicion regarding three criteria (8). Recently the World Allergy Organisation proposed to reduce them to two (1). Both proposals are described in figure 2.

In addition to the clinical aspects of the patient's reaction, we can also use laboratory tests to help us arrive at a diagnosis. The main diagnostic tool in the case of clinical suspicion of being faced with a reaction secondary to the release of mast cell mediators is the quantification of the level of some of them - mainly tryptase - during an episode suspected to be the consequence of mast cell activation. If the tryptase level at the acute time is >20% of the basal value + 2 ng/mL, our suspicion will be confirmed. Example: If the basal tryptase is 6 ng/mL: (6 x 0.2) + 2 = 3.2. An increase in acute tryptase ≥ 6 ng/mL + 3.2 ng/mL (≥ 9.2 ng/mL), suggests mast cell activation. It has recently been proposed that an acute tryptase/base ratio ≥ 1.6 suggests anaphylaxis (9).

The severity of anaphylaxis is classified into levels ranging from I to V according to increasing vital impairment. Since the form in which it manifests itself can vary depending on the trigger of the reaction, there are different classifications depending on whether the anaphylaxis is secondary to food, drugs

Figure 3 Action algorithm for anaphylaxis in healthcare. Adapted from (5)



ABCDE; *A (Airway), B (Breathing), C (Circulation), D (Disability), E (Exposure).*
**Treatment according to the symptomatology presented by the patient.*

or hymenoptera. It would be ideal to unify the criteria and have a single classification that allows a better comparison of reactions and/or triggers.

7. Anaphylaxis ... and something else?

When faced with an episode of anaphylaxis, it is necessary to consider - in addition to a correct differential diagnosis - the possibility that the patient has an underlying pathology whose main manifestation is anaphylaxis. Those most relevant to our speciality are:

a) Mast cell activation syndromes (MCAS): this term encompasses a group of diseases in which all three of the following requirements are present (10): **1)** involvement of two or more organs due to the release of mast cell mediators, **2)** basal increase of mast cell mediators during the episode and **3)** response to treatment with conventional anti-mediator drugs. In turn, MCAS can be classified into (10): **(I)** primary (clonal), characterised by the presence of abnormal MCs, which can be further subdivided into patients with mastocytosis or clonal MCAS, **(II)** secondary, mainly due to the presence of IgE-mediated allergy, and **(III)** idiopathic, in which no known etiology is identified.

While secondary and idiopathic MCAS correspond to patients who fulfil the criteria for anaphylaxis, mastocytosis and clonal MCAS may have different forms of manifestation and do not always start with severe release reactions. However, the frequency of anaphylaxis in these patients is much higher than that reported in the general population and can be up to 10 times higher (11), and anaphylaxis is often the episode that leads to the suspicion of such a disease.

b) Hereditary alpha-tryptasemia (HaT): this event, which consists of an increase in the copy number of the TPSAB1 gene responsible for encoding alpha-

tryptase (an isotype of tryptase, also considered as immature, constitutive or inactive tryptase), has recently been described. Patients with this genetic trait have higher than normal basal tryptase levels and may also be associated with a higher frequency of anaphylaxis (12).
The prevalence of this entity in the general population is estimated to be around 5% (13-16). However, the presence of HaT has been reported in approximately 10% of patients with anaphylaxis and in 20% of patients with clonal mast cells (16). The reason for this association, or why some carriers of this genetic trait present symptoms and others do not, is currently unknown.

8. What do I need to know about treatment?

Successful management of this emergency is based on recognising the event, removing the allergen involved when possible, and starting treatment early.
As in any other medical emergency, after identifying the state of severity, it is important to seek help, if necessary, based on the environment, personnel, equipment and drugs available, and to assess the 'ABCDE' approach (A - Airway; B - Breathing; C - Circulation; D – Disability/neurological dysfunction; E - Exposure of the body).
When starting treatment, it should be borne in mind that adrenaline is the most effective drug and that its early administration improves survival (1,4,17). The intramuscular route, on the anterolateral aspect of the thigh, is the preferred route initially. It has a rapid onset of action, a short duration of action and fewer side effects than the intravenous route. The recommended dose is 0.01 mg per kilogram of body weight, up to a maximum of 0.5 mg; administration may be repeated every 10-15 minutes depending on the patient's progress and severity. There are no contraindications to its administration, although the elderly, pregnant women or persons with cardiovascular disease and/or hyperthyroidism may be at greater risk of unwanted effects. Adrenalin has an agonist action on α1, β1 and β2 adrenergic receptors. For this reason, patients treated with β-blockers may be resistant to adrenalin treatment and require glucagon administration because its inotropic and chronotropic action is not mediated by β-adrenergic receptors (4).
In early form, supportive measures, such as fluid resuscitation and/or oxygen administration, should be provided in addition to adrenaline if necessary. Short-acting β-agonist adrenergic bronchodilators are indicated if the airways are compromised. Antihistamines are the second line of treatment and are used to control the cutaneous symptoms of the reaction. As for corticosteroids, they can be useful to prevent or reduce prolonged reactions and can be used as a third line of treatment for asthma or shock (18). The action algorithm is detailed in Figure 3.

9. Can I prevent it?

After resolution of an anaphylaxis episode, all patients should be evaluated by an allergy specialist to identify the cause of the episode, if possible, and to establish an action plan based on future risk, including measures to prevent recurrence and/or to manage the episode.
Individuals at risk of a new episode of anaphylaxis should be instructed in the use of adrenaline and should carry an auto-injector. As to whether one or two devices should be recommended, there are arguments for and against (4,18,19). However, training in the use of the auto-injector is undoubtedly necessary in order to be able to use it in the event of a new reaction.

10. ...recommendations after the acute episode?

Education about anaphylaxis after an acute episode must cover several levels:

- From the patient's point of view. After the evaluation in the allergy clinic, in addition to a personalised, written action plan tailored to the patient, there should be regular follow-up to assess any inconveniences (20). In addition, patient associations can act as a crucial support to help patients better manage their daily living with the diagnosis.
- From a social point of view. It is increasingly common for nurseries and educational centres, in the case of children, or workplaces, in the case of adults, to have protocols for action plans. It is estimated that 10-20% of reactions occur at school (21) or when eating out. Along these lines, catering establishments are obliged to follow the European Regulation (E.R.) 1169/2011 (on the provision

of food information to consumers of 13/12/2014), which provides consumers with information on the 14 allergens considered to be of mandatory declaration.

Just as there is a great global awareness of the importance of having a basic knowledge of first aid to initiate basic resuscitation in the event of a cardiorespiratory arrest, the creation of PIT (Prevention-Information-Training) action plans in recreational centres is also currently being promoted in order to have 'protected' spaces.

Knowing these steps will help to better identify and manage a condition that can sometimes be unpredictable and potentially lethal.

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An unusual case of contact allergy to gold

Contact allergy to gold from a coffee cup: An unusual source of sensitisation

Brazão C. et al. Contact Dermatitis. 2023, 89(2):132-134. DOI: 10.1111/cod.14337.

Gold is a precious, malleable and corrosion-resistant metal; it is considered safe in contact with skin and mucous membranes, and is widely used in medicine and dentistry. With respect to allergies, metallic gold is considered safe in contact with the skin, but in ionised form it is a potential sensitising agent. Allergic reactions to gold are quite rare and generally manifest themselves as allergic contact dermatitis, or with oral symptoms (e.g. stomatitis) in the case of exposure to gold in dental implants and fillings.

Epicutaneous tests (*patch tests*) with *gold sodium thiosulphate* (GST) can be conducted for the diagnosis of a possible gold allergy. Positive reactions to this substance are quite common and may develop late (e.g. after one week), but the clinical relevance is limited or difficult to define and is a matter of debate. These reasons contributed to the *American Contact Dermatitis Society's* decision to nominate gold as 'Allergen of the Year' in 2001.

A rather unusual case of contact allergy to gold recently appeared in the journal *Contact Dermatitis*. It concerned a young woman (27 years old) who had been suffering from burning, itching and swelling of the upper lip for about a year. The woman used no orthodontic appliances or prostheses, no cosmetics, no perfume, and wore no jewelry.

Suspecting allergic contact dermatitis, dermatologists performed *patch tests* in occlusion for 48 h with IQ-Ultra Chambers and with three series of allergens: the basic series from the Portuguese group for the study of contact dermatitis, a *dental screening series*, and a series consisting of substances contained in foodstuffs, especially bakery products (*bakery series*), from the Chemotechnique Diagnostics company (Sweden). The readings conducted at day 2 (D2) and D4, as indicated by the *International Contact Dermatitis Research Group* (ICDRG), showed no reaction to the substances tested.

However, after one week, the woman noticed the appearance of an itchy red papule on her back, right at the *patch test* site. The tests were then repeated, one month later, including delayed readings. Positive reactions to gold (+++; GST 2% pet.) and cobalt (+; cobalt chloride 1% pet.) were observed at D7, then again visible at D28.

Further investigation revealed that the patient, a software engineer, had been working in the *home-office for a* year due to the COVID-19 pandemic and was in the habit of drinking two coffees a day using the same cup. The mug, manufactured by a historical Portuguese company (*Vista Alegre*), was made of porcelain with decorations in shades of blue on the outside and a golden rim on the inside (Figure 1). The colours used for these decorations were cobalt and gold, respectively, as later confirmed by the manufacturers. By avoiding the use of the cup, the symptoms resolved and no relapses were observed six months later.

Based on the evidence gathered (symptom assessment, *patch test* results, daily exposure to gold on the cup, and resolution of the problem once use of the object had

Figure 1. Porcelain cup with gold trim

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stopped), the doctors diagnosed allergic contact dermatitis to gold. Sensitisation to cobalt, on the other hand, did not seem to have a clear relevance, considering the moderate positivity in the patch test and the presence of the symptoms on the upper lip, which was in contact with the inner gold part while the blue decorations were on the outside when using the cup.
This work emphasises the importance of also taking into account the possibility of a gold allergy, even if rare, of conducting a careful history and, above all, of performing patch test readings even at longer intervals if a metal allergy is suspected.

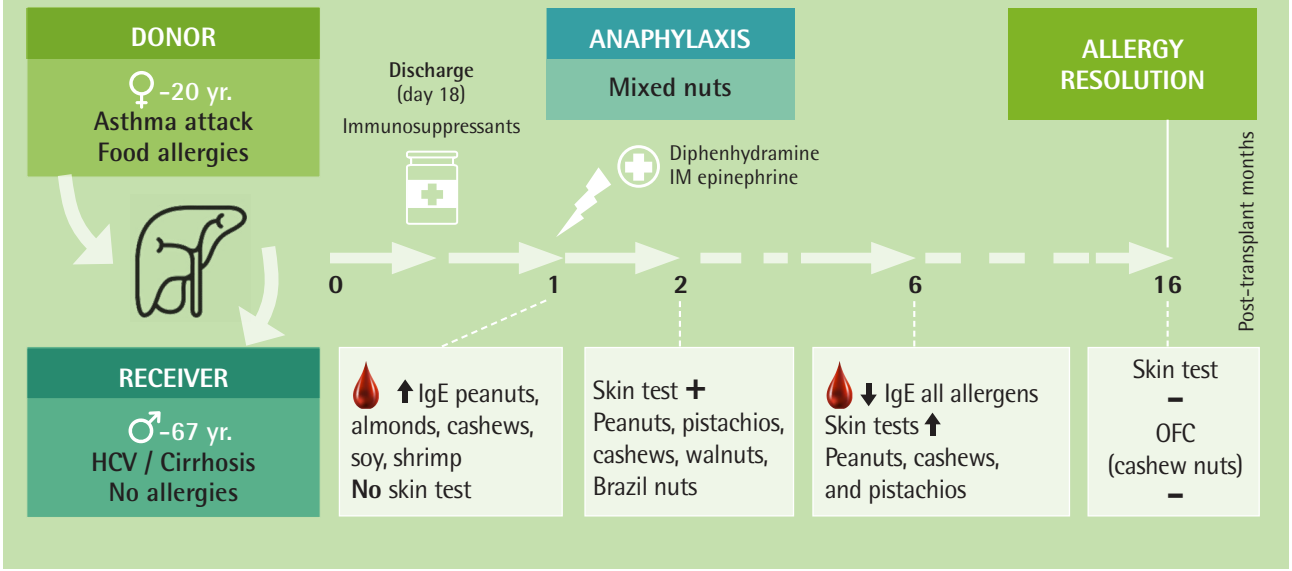
Development of food allergy after liver transplantation

Tough Nut to Crack: Transplant-acquired Food Allergy in an Adult Liver Recipient

Horwich B.H. et al. Transplant Direct. 2023 Oct 16;9(11):e1552. DOI: 10.1097/TXD.0000000000001552.

In this paper, Horwich and colleagues describe the case of a man who developed a *de novo* food allergy after a liver transplant. The phenomenon, which is known in English as *transplant-acquired food allergy* (TAFA), is more common in paediatric patients, usually manifests itself within 6 months after transplantation and often resolves within 2 years, and affects liver transplant recipients more severely. In the case of TAFA described here (Figure 1), the patient (67 years old) had undergone liver transplantation due to cirrhosis associated with hepatitis C. The organ came from a young woman (20 years old) who had died following a severe asthma attack. The transplant was successfully performed, there were some post-operative difficulties but no rejection episodes, and the patient was discharged on day 18 after the operation with immunosuppressant therapy (Tacrolimus, Mycophenolate Mofetil and Prednisone).
The allergic episode occurred one month after the transplant, when the man consumed mixed nuts (cashews, almonds and hazelnuts) for the first time after the operation. The reaction was manifested by lip angioedema, throat discomfort, diffuse itching and abdominal pain; emergency treatment with diphenhydramine and intramuscular epinephrine led to rapid improvement. Before the transplant, the man regularly consumed peanuts, nuts, eggs, wheat and soy without any problems.
Serological tests detected the presence of IgE for several allergens, in particular peanuts (1.72 kU/L), hazelnuts (0.73 kU/L), pistachios (0.54 kU/L), almonds (0.39 kU/L), cashews (0.20 kU/L), soy (0.41 kU/L) and shrimp (0.96 kU/L). Analyses conducted over the following months showed a progressive reduction in IgE levels for these allergens. One month after the allergic reaction, skin tests were also conducted, which gave positive results for peanuts, pistachios, cashews, walnuts and Brazil nuts. At six months, skin tests showed increased positivity to peanuts, cashews and pistachios, with the test negative at 16 months post-transplant, such that ingestion of the same was tolerated.
The doctors reviewed the girl's medical history, which was positive for peanuts, chocolate, eggs, wheat and shellfish. Unfortunately, there is no information on the development of allergies in patients who have received other organs from the same donor.
Cases of TAFA in adults are quite rare and often, as in the case described here, involve patients receiving a liver transplant. However, the exact pathogenesis of the phenomenon is not fully elucidated and further studies are needed. The authors report some of the proposed hypotheses, including 1) donor liver-related allergen-specific IgE transfer, 2) donor allergen-specific T- and B-cell transfer and 3) preferential selection of Th2 lymphocytes induced by Tacrolimus therapy.
The authors then collected information on the cases in the literature of adults hepatotransplanted with TAFA, highlighting that almost all donors had died of atopy-related causes (e.g. anaphylactic shock and asthma), emphasising that, unlike in paediatric cases, the donor's allergic status

Figure 1 Overview of the case (icons from unwing.com)



HCV hepatitis C virus; **OFC** oral *food challenge* / oral provocation test; **IM** intramuscular

plays a significant role in the development of TAFA in adult liver transplant patients.

At the moment, the authors, while acknowledging that there appear to be no definite strategies to limit the risk, suggest that special caution should be observed when monitoring patients who have received their liver from young donors (< 25 years) who have died of anaphylaxis or an atopy-related condition.

For hepatotransplanted adults diagnosed with TAFA, current indications are to avoid the triggering allergen indefinitely and to carry an epinephrine auto-injector. Horwich and colleagues suggest monitoring IgE levels over time and conducting oral provocation tests in selected patients after one year. They also emphasise that these individuals should be followed by an experienced allergist and appropriately educated in order to avoid exposure to allergens and recognise the symptoms of an allergic reaction.

AD patients: which fabrics to wear?

Fabric Selection in Atopic Dermatitis: An Evidence-Based Review

Jaros J. et al. Am J Clin Dermatol. 2020; 21(4):467-482. DOI: 10.1007/s40257-020-00516-0

Atopic dermatitis (AD) is an inflammatory skin disease with a complex pathogenesis, in which genetic predisposition, immune system imbalances and dysfunction of the skin barrier play an important role. It manifests itself with itching, erythematous and scaling lesions and predisposes to the development of infections, in particular of the *Staphylococcus aureus* bacteria. For patients with AD, it is important to carefully choose the fabrics that come into contact with

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the skin, since depending on their properties (e.g. breathability, occlusive action, absorbency, thermoregulatory action) these can influence the skin microenvironment and have an impact on AD. The properties and characteristics of the ideal fabric in AD are listed in Figure 1 and, in particular, it should limit *transepidermal water loss* (TEWL) and reduce inflammation and itching. The latter is related to the stimulation of nociceptors (sensory receptors) by the fibres of the tissue that protrude from the surface of the garment and is directly related to the diameter of the fibres that make up the tissue. Traditionally, natural fabrics with fine, smooth fibres, such as cotton and silk, are preferred for sensitive skin.

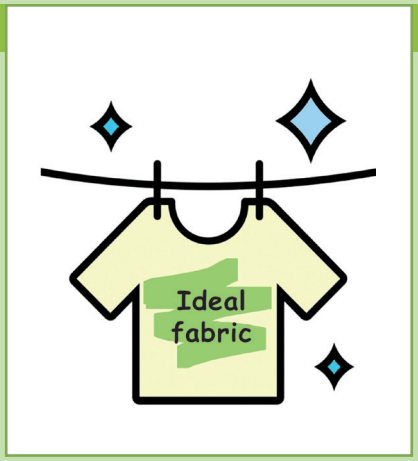
The choice of tissues for AD patients is the subject of this review, published in the *American Journal of Clinical Dermatology*, which presents a critical analysis of the available scientific evidence and also provides some guidance for tissue selection. The authors conducted a systematic search of the bibliographic databases PubMed and EMBASE to identify relevant articles, published between January 1994 and January 2020, using 'atopic dermatitis', 'eczema' or 'dermatitis' as keywords, in combination with various terms referring to textiles and textile fibres (such as 'cloth', 'fabric', 'linen', 'wool', etc.). They only included clinical studies (in adult and paediatric patients) in English, and focused on the effect of textiles on AD. After selection, 27 studies were identified, listed and summarised in Table 2 of the original paper. The scientific evidence gathered and the considerations relevant to AD patients are presented in the text, grouped according to the type of fabric, starting with natural and traditional fabrics and ending with more innovative, synthetic or combined fabrics. These are textiles based on natural or synthetic fibres, which are treated or combined with other materials/components, e.g. zinc oxide or silver, capable of 'functionalising' them, i.e. giving them new properties, such as antimicrobial activity.

Among natural fabrics, cotton is traditionally the preferred fabric for AD patients and is often used as a control fabric in clinical trials on textiles and AD. However, under certain conditions and/or following certain manufacturing processes, cotton may irritate the skin or cause allergic contact dermatitis (e.g. due to chemical dyes). Furthermore, the authors point out that data from extensive clinical studies are rather scarce. Another natural fibre, but of animal origin, is wool, which is characterised by its great capacity to absorb moisture and regulate temperature. The common perception is

Figure 1 Properties and characteristics of ideal tissues for AD patients

PROPERTIES

- ↓ TEWL
- ↑ hydration
- ↑ thermoregulation
- ↑ emollient action
- ↑ antibacterial action
- ↑ topical drug penetration
- ↓ skin inflammation
- ↓ itching
- ↓ sleep disorders



CHARACTERISTICS

- fibres with Ø < 21 µm
- non-occlusive
- hydrophilic
- comfortable
- light
- easy to wash

that wool products are irritating and, indeed, this may be the case for traditional wool with thicker fibres (∅ > 30 µm). Those composed of finer fibres, such as superfine (15 - 18.5 µm) and ultrafine (11.5 - 15 µm) merino wool, do not cause itching and appear to induce some benefits in AD patients, although larger studies are needed to assess the effects on the skin microenvironment. However, the authors advise AD patients who wish to wear woollen garments to choose garments made of superfine or ultrafine merino wool. Lyocell (or Tencel; 10 - 30 µm), on the other hand, is made from cellulose derived from wood pulp (often from eucalyptus trees) treated with a non-toxic, recyclable solvent. It is a fabric that allows good breathability and moisture control and was preferred to cotton in a study of 30 patients; however, it does not appear to significantly improve the clinical picture and symptoms of AD. Silk, with its cylindrical, thin (11 - 12 µm) and smooth fibres is the other fabric traditionally chosen in AD due to its low friction on the skin. In some innovative fabrics such as MICROAIR DermaSilk® and DreamSkin™, silk is combined with quaternary ammonium compounds (quats) that give the fabric antibacterial and anti-odor properties. However, the cost of products made from these fabrics is quite high, while the benefits of their use in AD cases are not fully defined. Further clinical investigations are therefore needed, which also pay attention to the safety profile of quats.

Studies of other synthetic fabrics and combinations show that the most interesting for potential efficacy in reducing AD severity and/or *S. aureus* infection are fabrics with chitosan (with antibacterial activity), cellulose-based fabrics with seaweed and silver ions (such as Sea Cell Active fibers® , SkinDoctor® and Skintoskin®) which showed significant improvement in TEWL, sleep quality and itching sensation, and a reduction in *S. aureus* colonisation. Fabrics with silver-coated fibres also led to a reduction in bacterial load and an improvement in AD severity. However, despite good preliminary data, according to the authors, further investigations are needed to assess the long-term safety (for health and environment) of silver-coated fibre fabric. Several other fabrics are discussed in the review, such as anionic fabrics (polyester-based with tourmaline), with zinc oxide (antibacterial, odour and UV protection properties), treated with citric acid (pH 5.5-6.5; to restore the skin's acid mantle), with borage seed oil (with high gamma linolenic acid content, usually deficient in the skin of AD patients), with fibres derived from ethylene vinyl alcohol (EVOH), and polyurethane bedding fabrics (with occlusive effect to reduce exposure to dust mite allergens). However, the evidence for their application in AD cases is limited and further studies are needed.

Finally, it should be kept in mind that most textiles are composed of mixed fibres and subjected to different types of processing and treatments (including washing) that may alter their properties, and therefore their effect on sensitive and atopic skin deserves further investigation. Given the intimate relationship between fabrics and the skin microenvironment, fabric recommendations should be discussed with patients as part of the AD management strategy.

Dupilumab and risk factors for hypereosinophilia in severe asthma

Efficacy of dupilumab and risk factors for dupilumab-induced hypereosinophilia in severe asthma: a preliminary study from China.

Li Y. et al. Annals of Medicine 2024; 56(1):2311843. DOI: 10.1080/07853890.2024.2311843.

Dupilumab is a biological drug for the treatment of disorders dominated by a type 2 inflammatory response, such as asthma, atopic dermatitis and chronic rhinosinusitis with nasal polyposis. It is a monoclonal antibody that binds to the alpha subunit of the receptor for interleukin-4 (IL-4) and inhibits the IL-4/IL-13 signalling pathway, cytokines that characterise the type 2 inflammatory response. The drug has also been shown to be effective in cases of severe asthma, but it has been observed that 4-25% of patients develop hypereosinophilia (HE), defined as a blood eosinophil count (BEC) of 1.5 × 10^9 cells/L or higher. This is usually tran-

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sient HE that causes no clinical symptoms, although complications such as eosinophilic pneumonia and eosinophilic granulomatosis with polyangiitis may occur in some cases. It is not yet clear which patients are at risk of developing HE related to dupilumab treatment, nor whether the presence of HE affects the efficacy of therapy. In order to clarify these points, Li and colleagues conducted a retrospective, single-centre clinical trial of 20 patients with severe asthma treated with dupilumab for at least 12 months, between January 2019 and May 2022. The initial dose of dupilumab was 600 mg (two subcutaneous injections of 300 mg).

Clinical data and laboratory test results of patients before treatment with dupilumab and after 4 and 12 months from the start of treatment were compared. In addition to BEC, various parameters were analysed, including fractional exhaled nitric oxide (FeNO), total IgE, respiratory function parameters such as FEV1/FVC and asthma control test (ACT) as well as oral corticosteroid therapy (OCS) data.

The mean age of the patients (70% males) was 46.63 years, and all had pre-treatment BEC values < 1.5×10^9 cells/L (in 85% of cases < 1.0×10^9 cells/L). Most of the patients had comorbidities, such as chronic rhinosinusitis with nasal polyposis (55%), atopic dermatitis (25%), eosinophilic otitis media (EOM) (25%) and respiratory disease exacerbated by aspirin (10%). 75% were on OCS therapy (median daily dose = 12.50 mg). 40% of the patients (n = 8) had positive values of specific IgE for food allergens and/or inhalant allergens.

Treatment with dupilumab led to a significant reduction in the number of flare-ups and a reduction or elimination of OCS use, improved lung function and a reduction in biomarkers of type 2 inflammation. However, an increase in BEC was observed, peaking around 4-5 months, then returning to similar levels after 12 months, in line with literature data (1). Based on BEC values, patients were divided into two groups: HE (n = 8) and non-HE (n = 12). The results showed no significant differences in the effect of dupilumab between the two groups; the presence of HE therefore does not seem to alter the therapeutic efficacy of the drug.

In order to identify possible risk factors for HE during dupilumab therapy, the authors conducted a subgroup analysis, based on different clinical characteristics of the patients, comparing changes in BEC from baseline values at 4 and 12 months of treatment between the different subgroups. After 4 months, the median value of the change in BEC was significantly higher in the group with food allergies and the group with EOM than in the control groups (without food allergies and without EOM, respectively), a difference that remained statistically significant even at month 12. In patients with initial FeNO ≥ 60 ppb, the increase in BEC was also significantly higher than in patients with FeNO < 60 ppb, but only at month 4.

In the discussion, the authors also report how they managed two patients who developed HE with BEC values ≥ 3.0×10^9 cells/L, cases in which dupilumab should be discontinued if organ damage is suspected (2). Suspicion of eosinophilic pneumonia in one of the patients, who was asthmatic and had chronic rhinosinusitis with nasal polyposis, led to discontinuation of dupilumab and administration of OCS (20 mg/day). BEC decreased rapidly and remained stable; the patient was then treated again with dupilumab with fluctuations in BEC but no clinical symptoms. In the second patient, after one month since dupilumab administration, the OCS dose was reduced from 40 to 30 mg, and this may have contributed to the increase in BEC; the OCS dose was increased again, leading to a reduction in BEC, and was then slowly reduced at subsequent follow-up visits, without leading to new increases in BEC. Finally, they emphasise the need for larger prospective studies to investigate risk factors for HE in patients with severe asthma treated with dupilumab.

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Provide information, create a profession

Edited by **Franco Frati**

Specialist in Paediatrics, Allergology and Clinical Immunology Director Lofarma Academy

We are pleased to present in the Notiziario Allergologico, in the area reserved for young researchers in allergology of the Lofarma Academy, the contributions of Dr. Richard Borrelli of the University of Turin and Dr. Michele Santoboni of the University of Bologna.

Kind regards,
Dr. Franco Frati, Director Lofarma Academy

In this issue:

- Innovation for immunotherapy according to allergists
- Immunotherapy in pregnant and lactating women

Relationship between allergen immunotherapy and innovation: an international survey

Dr. R. Borrelli
University of Turin

In many fields, a treat-to-target approach (T2T) is nowadays mandatory for the development and the usage of therapies. In their last version, the Allergic Rhinitis and its Impact on Asthma (ARIA) recommendations clearly state the importance of evaluating the specific scenario of patients with allergic rhinitis and, if appliable, administering allergen immunotherapy (AIT) in order to treat the patient with a tailored approach. Nevertheless, despite the data concerning both its effectiveness and safety, as of today there is a lack of sufficient prescriptions.

In an attempt to understand the causes of this occurrence, the University of Turin (under the supervision of Prof. Brussino and Prof. Nicola) and the University 'La Sapienza' of Rome (under the supervision of Prof. Masieri) carried out an international web-based survey to investigate how experts from many third-level centres of allergy and clinical immunology around the world perceived the innovative aspects of AIT.

I hereby report a brief summary of the study that we will present as a scientific contribution at the upcoming EAACI in Valencia (Title: "How allergists around the world perceive innovation in the context of allergen Immunotherapy (AIT): an international survey").

Participants in the questionnaire-based electronic survey ranked 20 items on a creativity scale from 1 to 5. The study was conducted from May to June 2023 through the SurveyMonkey™ system. Fifty-six major allergists participated, representing a variety of age groups and genders, with a majority specializing in allergology and immunology. Factors affecting AIT prescription were assessed, with ratings indicating a need for improvement in accessibility, safety, and innovation, particularly in new administration routes and palatability for children.

The survey highlights the need to address the under-prescription of this effective treatment. Factors such as the availability

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of sublingual formulations and the safety profile of AIT medications received moderate to high ratings, indicating room for enhancement.
Furthermore, the interest in new administration routes and the importance of palatability for paediatric patients underscore the potential for innovation in AIT delivery methods. Addressing these areas of improvement could help increase the adoption of AIT and improve outcomes for patients with allergic diseases.

Use of allergen immunotherapy in pregnancy and lactation

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One of the fundamental aspects of modern pharmacotherapy is related to the analysis of potential effects of individual products. In recent years, this peculiarity has also spread to allergology with the study of potential adverse reactions to allergen immunotherapy (AIT).
I was introduced to the world of pharmacovigilance during my undergraduate studies, and in collaboration with Lofarma's Pharmacovigilance team, I carried out a study on the safety of AIT during pregnancy and lactation. This object of study was the subject of my dissertation.
The results of the study will be presented at the upcoming European Congress of Allergology and Clinical Immunology (EAACI 2024 - Valencia) (Title: "Allergen immunotherapy (AIT) in pregnancy, focus on the use of carbamylated monomeric allergoid").
A brief summary of the work follows.
In industrialized countries asthma and rhinitis affect 18-30% of women in childbearing age and around 20% of pregnant women. Although AIT has been largely used for a long time, current literature data on the safety of AIT in pregnancy are still limited.
Several studies reported that uncontrolled maternal asthma was associated with an increase of perinatal Adverse Events (AEs) and complications due to inadequate drug therapy. AIT Guidelines generally recommend continuing well-tolerated ongoing AIT during pregnancy with caution; therefore, its safety during this period is crucial to a positive outcome.
Pharmacovigilance is mandatory for authorized allergen products only; however, it has been systematically implemented for several years for carbamylated monomeric allergoids, even as Named Patient Product (NPP).
Post-marketing surveillance guarantees the safety records collection on targeted groups of patients, such as pregnant and/or breastfeeding women of different age groups, exposed to AIT in post-marketing.
We report spontaneous safety data on exposure to sublingual monomeric allergoids during the gestational period and breastfeeding (defined according to GVP as cases of special interest) collected in a validated pharmacovigilance database from 1990 to 2023.
Any collected AE, coded through MedDRA, was classified according to its nature, intensity, severity and degree of correlation with the drug taken (as per GVP requirements).
In the period considered of about 30 years a total of 16 cases (exposed to HDM, grass, olive, pellitory pollen and cat) were processed: 13 cases concerning pregnancy and breastfeeding (reported by patients) and 3 cases of exposure during conception (reported by physicians). No adverse drug reactions (ADRs) were recorded. For each case the follow-up included the outcome of the pregnancy up for 4-6 weeks after the birth with notification of any ADR.
Considering the expected rate of continuation of AIT in pregnancy, a large under-reporting is very likely; however, at this time there has been no evidence of risks related to the administration of sublingual monomeric allergoids during conception or in the gestational/breastfeeding period, which might potentially impair the benefit/risk profile of the product administered.
These data confirms that the current approach of continuing the administration of monomeric allergoid during pregnancy is not associated with an increased risk for either the mother or the fetus.

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