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Allergic reactions after vaccination: translating guidelines into clinical practice

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Summary
Vaccination represents one of the most powerful medical interventions on global health. Despite being safe, sustainable, and effective against infectious and in some cases also non-infectious diseases, it's nowadays facing general opinion's hesitancy because of a false perceived risk of adverse events. Adverse reactions to vaccines are relatively rare, instead, and those recognizing a hypersensitivity mechanism are even rarer.
The purpose of this review is to offer a practical approach to adverse events after vaccination, focusing on immune-mediated reactions with particular regard to their recognition, diagnosis and management.
According to clinical features, we propose an algorythm for allergologic work-up, which helps in confirming hypersensitivity to vaccine, nonetheless ensuring access to vaccination. Finally, a screening questionnaire is included, providing criteria for immunisation in specialized care settings.

Introduction

"Smallpox is dead" stated the magazine of the World Health Organisation (WHO) in 1980. It was the first time that a high contagious and dangerous disease was globally eradicated. And the credit went to an extensive worldwide immunisation campaign, begun in 1967, combined to highly organised prevention and surveillance measures. Once again, the vaccines showed their indisputable efficacy (1,2).
To date, vaccines are considered one of the most powerful public health interventions that have contributed to the drastic reduction of the mortality and the morbidity of several infectious diseases (3). Moreover, vaccines have also demonstrated a primary role in preventing virus-associated malignancies, such as HPV-driven cervical cancer (4).
Vaccination saves between 1 and 3 million lives worldwide every year. According to the World Health Organisation, vaccines will save 25 million more lives in the coming decade (adapted from: 5). One of the primary aims of WHO is giving equitable access to vaccines, collected under the name of Global Vaccine Action Plan (GVAP) (6).
The gain from vaccination is not just about human health, but it is also a matter of financial resources for health systems. It has been calculated that for every dollar spent in vaccines, 16 dollars (US $) are expected to be saved in healthcare costs, loss of productivity and incomes (7). Even before, several studies had assessed the cost-benefits of immunisation (8). A loss of more than 60 billion dollars (direct and indirect costs) has been quantified in a hypothetical unvaccinated cohort of three million children in the US (9). Recently, after the introduction of new expensive vaccines and the global financial crisis, a better standardization in the scientific works regarding the topic "cost-benefits" has been advocated, since most of them differ in adopted methodologies (10,11).
In 2018 the European Commission, in agreement with WHO, has reiterated the importance of reaching and maintaining high level of coverage rate of vaccination. The pillars of its proposal included a better financial sustainability and a "tackling vaccine hesitancy" strategy (12,13). Vaccine hesitancy is a recent phenomenon, typical of Western countries, consisting in refusing or delaying an available vaccination (14), that has led to an alarming reduction in coverage rate. The

case of measles best resumes the consequence of the "vaccine hesitancy". In the European Vaccine Action Plan for 2015-2020, WHO aimed at eradicating measles (15). However, the gradual decrease of vaccination against this infection resulted in a resurgence of measles with several outbreaks, 14,600 cases and 37 deaths in the European area in 2017. The highest incidence was observed in children aged < 4 years, especially ≤ 1 year, and most of the cases occurred in unvaccinated subjects, reaching a rate of 96% in children aged ≤ 1 year (16). In December 2018 the European Centre for Disease Prevention and Control (ECDC) reported 34 fatalities due to measles in 2018 (17). Hence, the goal of the European Commission regarding measles has now turned into "reaching at least a coverage rate of 95%".

Although vaccine hesitancy is a multi-layered phenomenon, safety of vaccination is one of its most relevant cofactors. Injecting a potentially dangerous organism in a healthy subject is intuitively experienced as a danger (14); that encourages distrust towards vaccines, especially if false or real claims of adverse reactions are widespread. A striking example was that of Wakefield. His fraudaulent study supporting the association between measles-mumps-rubella vaccine and autism was definitively disproved with heavy consequences for the author, but nevertheless this misinformation still troubles those skeptical towards vaccines (18). Moreover, unlike their parents and grandparents, new generations in Western countries have no confidence with epidemics and their potential consequences. And it has been demonstrated that perceiving more the risks than the benefits of immunisation favours the reluctance against vaccines (19).

Hence, it is necessary to reduce this false perception of unsafety and even uselessness regarding the immunisation. Also, the introduction of new vaccines has required better tools to analyse their real impact on subject's health, as mentioned above. Scientific societies, drug agencies and major health organisations have created several active vaccine-pharmacovigilance entities and working groups. The Vaccine Adverse Event Reporting System (VAERS) in the US (20) or pharmacovigilance section of EMA, for example, regularly collect reports and cooperate with governments in the field of vaccination. The "unmet needs" regarding vaccination is the field of interest of the Working Group on Vaccine Safety (WG), a subgroup of the Council for International Organizations of Medical Sciences (CIOMS), an organization established by the WHO and UNESCO in 1949 (21). There are several working groups dedicated to support research, produce guidelines, organise trainings and offer precise information (for example, the Vaccines Working Party, VWP) (22).

This has led to a better knowledge of the pathogenic mechanisms of the AEFI (Adverse Events Following Immunisation) until the discover of immune-mediated reactions, including allergic ones. Over the time, more and more knowledge has been collected, and to date allergological diagnostic tests and even desensitization protocols are available. Notwithstanding the rarity of allergic events following vaccination, they can be harmful for at least two reasons: first, severe allergic reactions such as anaphylaxis could be life threatening; second, a real, presumed or even feared allergy to vaccines limits or delays the accessibility to a regular immunisation program.

Here we propose a practical approach to AEFI from an allergological point of view aimed at identifying risk factors, diagnosing allergies and providing a framework to ensure vaccination, according to the subjects' risks, either in a standard care or in specialized centres. A better selection of patients requiring an allergological in case of AEFI is necessary to properly address health resources.

Definitions

Adverse event following immunization (AEFI) is "any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine" (23). The Working Group on Vaccine Safety has classified AEFI in 5 groups:

- vaccine product-related reactions: caused or precipitated by a vaccine due to one or more of the inherent properties of the vaccine product;
- vaccine quality defect-related reactions: caused or precipitated by a vaccine due to one or more quality defects of the vaccine product, including the administration device, as provided by the manufacturer;
- immunization error-related reactions: caused by inappropriate vaccine handling, prescribing or administration and that therefore, by its nature, is preventable;
- anxiety-related reactions: arising from anxiety about the immunization;
- coincidental events: caused by something other than the vaccine product, immunization error or immunization anxiety.

Vaccine product- and quality defect-related reactions are those potentially involving immune system, summarized in **table I** according to the latest document of CIOMS.

Classification in systemic and local could help the physician in a faster differential diagnosis between allergic and non-allergic reactions in daily-clinical practice (**table II**). The term "allergy" encompasses all 4 types of reactions according to Gell and Coombs. (25).

Classifying reactions according to timing is also extremely important to better understand their nature: i) immediate type occurs within minutes and usually no more than after 4 hours; ii) delayed type occurs hours to days (up to 2-3 weeks) after vaccination (26).

Table I *- Vaccine product and quality defect related.*

Reactions associated with the route and/or site of administration of the vaccine
Bell's palsy (due to intranasal administration of an influenza vaccine). Pain at the time of injection.
Immune-mediated vaccine reactions
Local reactions, with involvement of the injection site - non-granulomatous inflammation ± regional lymphadenitis: extensive limb swelling, mild, moderate or severe local inflammation; - granulomatous inflammation at the injection site ± regional lymphadenitis.
Multisystem (generalized) reactions: - systemic inflammatory response (e.g. fever); - mast cell degranulation: IgE mediated hypersensitivity (anaphylaxis), non-IgE mediated hypersensitivity (anaphylactoid reactions); - disseminated granulomatous reaction; - immune complex mediated reaction (serum sickness reaction).
Organ-specific reactions: - auto-immune or undefined mechanism: central nervous system (e.g. demyelinising syndromes), blood (e.g. thrombocytopenia), skin (e.g. rashes).
Reactions as a consequence of replication of vaccine-associated microbial agent(s)
For example: - an attenuated vaccine agent; - a wild-type vaccine agent due to insufficient inactivation during the manufacturing process; - a contaminant introduced into vaccine during the manufacturing process.

Direct toxic effect of a vaccine component or contaminant e.g. Quality defect.
Adapted from (24).

Table II *- Clinical classification of AEFI.*

Local Reactions	**Potential Mechanism**
Mild local reactions: Pain, redness, and/or swelling at injection site	Non specific inflammation
Large local reactions	Most non allergic[a]; sometimes Arthus reactions.
Extensive limb swelling	Not allergic
Subcutaneous nodules	Allergic (type 4) and not allergic
Local eczema lesions	Allergic (type 4)
Systemic Reactions	
Fever, irritability, malaise, diarrhea, headache, muscle pains	Not allergic
Syncope, vasovagal reaction, anxiety disorders	Not allergic
Anaphyloctoid reactions	Not allergic
Anaphylaxis	Allergic
Serum sickness reaction	Allergic
Organ specific:	
- Blood: Trombocytopenia, anemia, leucopenia	Usually allergic
- Skin: non specific rashes, immediate and delayed angioedema/urticarial, macopapular rash, systemic eczema, SCAR	Non specific rash usually not allergic; the other usually allergic
- Nervous system: Guillian Barrè, demyelinising syndromes	Allergic
Reactions depending on microbial activity	
e.g Varicella vaccine-strain viral reactivation	Not allergic

SCAR, severe cutaneous adverse reactions; [a]Risk factors, HBV; Pneumococcal and Haemophilus influenzae; high concentration of toxoids (tetanus, diphtheria, Bordetella pertussis) and aluminium hydroxide (27,28,29,30).

Allergic reactions

Epidemiology of allergic reactions

Allergic reactions to vaccines are extremely rare. In a recent review, the World Allergy Organisation reports an estimated rate of allergic reactions from 1 in 50,000 to 1 in 1,000,000 and of anaphylaxis of 1 in 100,000 to 1 in 1,000,000 (31).
Each vaccine shows different incidences of hypersensitivity reactions, including anaphylaxis (**table III**).
In 2009 an unusual higher incidence of anaphylaxis and allergic reactions with H1N1 vaccines was reported, with a rate of incidence 3.3 greater than the previous immunisation campaign. The responsible was an adjuvant (AS03) added to the last vaccine slot (32).

Aetiology

Immediate IgE mediated allergic reactions to vaccines are rare and occur less frequently than delayed reactions (see below). Anyway, detection of IgE responses after vaccination is very common, and involves more than 90% of infants after a booster or vaccine. (42). Atopic children show a higher tendency towards this phenomenon (43) even if a higher incidence of anaphylaxis has not been demonstrated in these subjects (44,45).

Table III *- Anaphylaxis incidence among different vaccines.*

Vaccine	Anaphylaxis incidence
DTaP	0.95 million doses[33] 0.36/100.000 doses[34] 2.07/ million doses[35]
Influenza	7 over 3.3 million doses (IIV)[36] 0 among 232.406 doses (LAIV)[36]
MMR	0.06/100,000 doses[37] 5.14/ million doses[35]
Varicella	0/1.3 million doses[38]
YF	0.42-1.8/100.000 doses[39]
Men ACWY	7 suspected anaphylaxes among 8.2 million doses[40]
HPV	2.6/100.000 doses[41]

DTaP, Diphtheria, Tetanus, acellular Pertussis; YF, yellow fever; MMR, measles, mumps, rubella; HPV, Human papilloma Virus; IIV, Inactivated Influenza vaccine; LAIV, live attenuated influenza vaccine; LAMV, live attenuated monovalent influenza vaccine (LAMV); Men ACWY, meningococcal vaccine groups A, C, W-135, Y.
Except for notes (40) and (41), notes from (33) to (39) are adapted from notes (28) and (31).

Several components of a vaccine may elicit hypersensitivity reactions, although with different incidence and clinical features (28,29,31).
Microbial antigens:
- toxoids (tetanus and diphtheria). After the introduction of highly purified toxoids, the incidence of anaphylaxis has significantly decreased (46,47). Recently, the discovery of traces of cow's milk in diphtheria and tetanus vaccines has suggested milk allergy involvement in the cases of anaphylaxis (see below) (48);
- the mutant, non-toxic form of diphtheria toxin (CRM (197) is a component of some conjugated vaccines, and has been implicated in two cases of allergic reactions: one with prevenar-13® (45) and the other with haemophilus influenzae B (49);
- virus-like particles of HPV could favor anaphylaxis (50), triggered by polysorbate 80 (stabilizer of quadrivalent vaccine) (51).

Stabilizers:
- porcine and bovine gelatin, traceable in vaccines against measles/mumps/rubella (MMR, old brands), varicella, influenza and tick borne encephalitis (28). In the past decades, MMR vaccines contained higher quantities of gelatin and episodes of anaphylaxis were much more frequent (31);
- Dextran, which has been withdrawn from the market.

Adjuvants and preservatives:
- thimerosal, formaldehyde, phenoxyethanol, aluminium hydroxide an aluminium phosphate are the most well known. They are usually associated to delayed cutaneous reactions (28). To date the use of thimerosal has dropped due to its mercury content (52);
- new adjuvants have been introduced such as polysorbate 80 (HPV) (53). Cases of suspected IgE-mediated reactions have been documented in H1N1 influenza vaccines due to squalene adjuvant AS03 (32).

Residual contaminants (of the culture medium):
- ovalbumin from hen's egg in yellow fever vaccine reaches potentially risky concentrations. Other vaccines containing ovalbumin are influenza, MMR, tick-borne encephalitis, some rabies vaccines (28);
- yeast proteins from Saccharomyces cerevisiae have been reported in quadrivalent human papilloma virus vaccine (HPV) (54), potentially in Hepatitis B (54), in PCV13 and in some meningococcal and oral typhoid vaccines (55);
- cow's milk proteins, in some brands of diphteria, tetanus and pertussis vaccines, oral polio vaccine (56);
- antibiotics such as neomycin B (57), polymyxin B, gentamycin, streptomycin (29).

Latex:
- from vaccine vials (e.g. HBV) or syringe plungers (28).

Alpha gal:
- contained in porcine gelatin or cow's milk residual, has been recently implicated in a case of anaphylaxis after zoster vaccine in a patient with known red meat allergy (58).

Clinical features

Allergic reactions can be local and systemic and are summarized in **table I** and **II**.
Types of local allergic reactions are:
- Arthus reaction: is a large local reaction depending on the injection of a vaccine whose antigens encounter their specific IgG in a subject with pre-existing immunisation (59);
- local eczema lesions: especially in those patients sensitized to contact allergens such as aluminium salts, thimerosal, formaldehyde, neomycin;

Systemic allergic reactions:
- IgE mediated: Anaphylaxis (see **figure 1** for the definition of anaphylaxis);
- Delayed type reactions, with involvement of different organs and systems.

Clinical management of reactions

Immunisation should be performed by health care professionals, certified in cardiopulmonary resuscitation (CPR), provided and familiar with an onsite emergency protocol. Low risk immunisation procedures are conducted at general practitioners or pediatricians' office or at vaccination centers, where expertise and equipment such as adrenaline, antihistamines, oral steroids, beta2-inhalers, oxygen and devices in case of emergency must be assured. High risk patients should under-

go immunisation in a *controlled setting* where expert personnel are available to manage anaphylactic reactions providing advanced life support. Observation time after immunization, usually 15 minutes, should be prolonged according to individual risk (61-63;28).

Management of reactions

Local reactions can be limited to injection site or extended to the limb and most frequently develop as delayed painful, swelling lesions with erythema or eczema, sometimes as subcutaneous nodules. Patients or caregivers should be advised to apply a cold cloth at the injection site and use paracetamol as pain killer up to 15 mg/kg every 6-8 hours (27). Subcutaneous granulomas are benign itchy erythematous waxing and waning masses, secondary to hypersensitivity reactions to alum-adjuvanted vaccines, which can be treated with oral antihistamines and topical steroids (64). As for *systemic reactions*, it is important for health workers to be able to distinguish between panic, vasovagal and hypotonic hyporesponsive reactions and anaphylaxis (see **table IV** Differentiation of anaphylaxis and vasovagal reaction in EAACI position paper, (28)). Anaphylaxis is defined and diagnosed according to Sampson's criteria (**figure 1**) and acute management requires immediate intramuscolar epinephrine administration and ABCDE assessment, as stated in EAACI anaphylaxis guidelines (60). Although the Joint Task Force on Practice Parameters (JTFPP) guidelines identify a 4-hour cut-off for allergic-like events after immunization (ALE), proper anaphylactic reactions rarely occur more than one hour after vaccine administration, so it should be safe enough to restrict subsequent thorough allergological work up to patients with onset of symptoms up to one hour or anaphylaxis, according to the clinical approach proposed by Zafack et al. (65-67). Individual cases of AEFI must be reported to regulatory authorities in each country, working in the framework of international Centers for Disease Control and other stakeholders.

***Table IV** - Useful information for the management of vaccine allergy.*

A complete list of allergens and where they can be found is available on http://www.vaccinesafety.edu/components-Allergens.htm
A list of vaccine potentially at risk for latex allergy is available on http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/latex-table.pdf
Traceable excipients and media in vaccines are listed on https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/excipient-table-2.pdf

Accessed in January 2019.

Patients with suspected hypersensitivity reactions

In patients reporting a previous reaction to a vaccine, case history should be collected in order to assess symptoms, time intervals and treatment needed for resolution. Medical history should focus on specific questions like the presence of previous documented allergy to foods, contact allergy, latex allergy and/or previous reactions to vaccines. A causality checklist has been developed by the Global Advisory Committee for Vaccine Safety (GACVS) of World Health Organization (68) as a tool to establish a causal relationship between the clinical event and immunisation. Immediate reactions with timing and characteristics of allergic symptoms are generally easier to be attributed to vaccine hypersensitivity.

Risk of recurrence of serious adverse events has not been thoroughly studied in high risk patients for ethical reasons, but pa-

***Figure 1** - Anaphylaxis definition.*

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus, or flushing, swollen lips-tongue-uvula) and at least one of the following:
   - respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia);
   - reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence).
2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
   - involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula);
   - respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia);
   - reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence);
   - persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting).
3. Reduced BP after exposure to known allergen for that patient (minutes to several hours):
   - infants and children: low systolic BP (age-specific) or > 30% decrease in systolic BP[a];
   - adults: systolic BP of < 90 mm Hg or > 30% decrease from that person's baseline.

PEF, Peak expiratory flow; BP, blood pressure. [a]Low systolic blood pressure for children is defined as < 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2× age]) from 1 to 10 years, and < 90 mm Hg from 11 to 17 years.
From: Hugh Sampson, officially cited in EAACI position paper (60)

tients who experienced an ALE after immunization can generally be safely reimmunised (65,69). High-risk patients are those who have experienced a severe allergic reaction following immunisation. Anaphylaxis after vaccination is the only contraindication for vaccination in a standard care setting, but allergological investigation may provide alternative approaches for vaccine administration if the benefit of immunisation overweights the risks. Patients who reported a reaction to constituents of the vaccine or idiopathic anaphylaxis may also be at risk of AEFI, and therefore require an allergologist's evaluation to decide for subsequent vaccination schedule and setting. In patients with mastocytosis it is suggested to perform vaccination with single products and to extend observation time to 30 minutes at least, but a controlled setting is not usually required (28,70).

Specific IgE antibodies to vaccine antigens are useless in the case of suspect hypersensitivity to the vaccine as they are produced in the normal immune response to immunisation, as mentioned above (43). On the other hand, serum tryptase level should be measured within 2 hours after a systemic severe vaccine reaction as a marker of anaphylaxis (28).

When suspecting an IgE-driven adverse reaction after vaccination, the allergological workup should first verify whether a sensitization to the vaccine and/or its component occurred. **Table V** summarizes vaccines' most important constituents and provides a quick guide for the clinician. Allergy testing is recommended regardless of the need for further immunizations (71).

Several factors influence the sensitivity of in vivo tests. Besides individual features, time interval between the reaction and the allergological evaluation plays an important role. It may be advisable to perform skin tests at least 3 weeks after the reaction and no more than one year after the suspected IgE-mediated reaction. Of note, positive and negative predictive value of skin tests to vaccines has not been established yet.

Skin tests are performed on the volar surface of forearm and start with prick test with undiluted vaccine (or 1:10 dilution in case of reported anaphylactic reactions); if negative, intradermal tests (IDT) should be carried out with 0.02 ml of 1:100 dilution and then, eventually, with 1:10 dilution. Positive (histamine) and negative (saline) controls have to be included. False positive irritant results may occur in 1:100 dilutions but they have mainly been described with IDT at 1:10 with influenza, MMR, varicella vaccines, and even more frequently with undiluted IDT, so that the latter is not recommended (75,28). According to the patient's history and to the culprit vaccine, it is advisable to analyze even the vaccine components such as egg, gelatin and alpha-gal, latex and yeast through skin tests and/or specific IgE.

When suspecting a type 4 mechanism in case of delayed local reactions after vaccination, diagnostic tests are not mandatory, because of the low risk of recurrence at revaccination, without contraindications for future immunization. However, patch test are easily-available, non-invasive tests which can confirm a diagnosis of hypersensitivity to preservatives/stabilizers (e.g. thiomersal, phenoxyethanol, formaldehyde), adjuvants (aluminum) or antibiotics (e.g. aminoglycosides). Patch tests for phenoxyethanol or formaldehyde are standardised. Aluminum can be tested as metallic aluminium (using an empty Finn Chamber) or as aluminium chloride hexahydrate 2% in vaseline (using a plastic chamber). Late readings are needed after 3 or 4 days and after 7 days (76,28). Unconventional approaches to diagnosis may still be useful in cases of severe anaphylaxis with negativity of in vivo tests and also when other concomitant therapies were ongoing at the time of reaction: Herreros et al. described the crucial role of BAT in determining the offending antigen (77).

Revaccination of patients with suspected hypersensitivity reactions

As a general rule, if the benefit of protection against pathogens outweighs the potential risk of reaction following immunization, the patient should undergo vaccine administration with a modified protocol or procedure. For example, in order to limit the incidence of local reactions, deeper injections and thigh instead of arm site of injections are preferable (78).

In the case of systemic and more severe reactions to vaccines requiring booster doses, administration should be preceded by blood tests aimed at evaluating if a protective IgG title has already been reached and it is stable. When there is no evidence of protection and a booster is needed, immunization with an alternative vaccine (not containing the allergenic component) may be performed. If an alternative product is unavailable, vaccination can proceed in a hospital setting with a i.v. line placed as follows: a) if skin tests result negative, a *two-phase graded dose challenge* can be injected (e.g. 10% and 90%, 30 minutes interval between the doses adopted from Kelso et al. and EAACI position paper); b) if prick or IDT confirm IgE hypersensitivity, a *transient desensitization* can allow administration of increasing doses of vaccine every 15 minutes (e.g. 0.05 ml of 1:10 dilution, then undiluted solution starting from 0.05 ml, then 0.1 ml, 0.15 ml, 0.2 ml and for some vaccines 0.5 ml). Of note, the latest procedure cannot provide a permanent tolerance, therefore if other doses are required, desensitization should be carried out every time. In both cases patients should be observed for at least 60 minutes (65,28). An alternative graded challenge was described by Seitz et al in 2009 (10%, 30% and 60% of the normal vaccination dose) (66).

Conclusions

Allergic reactions after vaccinations are rare events. Since they can delay or even interrupt a regular vaccination plan, an allergological workup is required when a suspected immune-mediated AEFI occurs. The aims of the allergological evaluation are i) identifying or excluding hypersensitivity to vaccines, ii) se-

Table V *- Vaccine content and advice for ALE management.*

	(Bovine, calf, fetal bovine) Serum Albumin (72)	Egg (ovalbumin)	Yeast	Protamine	Gelatine alpha-gal[1]	Amphotericin B	Chlortetracycline	Gentamicin	Latex	Neomycin	Polymyxin	Streptomycin	Thimerosal	Aluminum	Formaldehyde	Glutaraldehyde	Polysorbate-80
DTaP, Td, Tdap[2]	▲		▲		4				▲	▲	▲		●	●	●	●	▲
Hepatitis B[2]			▲						▲	▲				●	●		▲
Hepatitis A			▲						▲	▲				●	●		
HPV			▲											●			▲
Influenza[2,5]		●			🏥			▲	▲	▲	▲		●		●		▲
JEV	▲			▲	🏥									●	●		
Meningococcal [2,3]			▲						▲				●	●	●		▲
MMR[2]					🏥					▲							
PCV13			▲											●			▲
Polio	▲									▲	▲	▲			●		
Rabies[2]	▲	▲			🏥	▲	▲			▲							
Rotavirus[2]	▲								▲								▲
Typhoid fever[2]			▲		🏥										●		
Yellow fever		▲			🏥												
TBE				▲													
Varicella/Zoster[2]	▲				🏥					▲							▲

[1] Caution should be used in parenteral administration of gelatin-containing products in patients with known alpha-gal hypersensitivity (73);
[2] Different brands, compositions and associations;
[3] Some contain DT as a carrier > contraindication in the case of a previous severe reaction to DtaP/DT/Td;
[4] Gelatin content prior to 1997 (ICON, 2016) (31)
[5] According to AAP/COID guidelines egg allergy of any severity is not a contraindication to receive an influenza vaccine (including IIV) in a standard care setting (74).
JEV, japanese encephalitis virus

▲	general precautions unless a previous anaphylaxis to the component was demonstrated
●	general precautions (GP, P or vaccination center)
🏥	controlled setting (hospital, Allergy Unit)

Data from: https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/b/excipient-table-2.pdf

http://www.vaccinesafety.edu/components-Allergens.htm

https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/appendices/B/latex-table.pdf

lecting those subjects who require immunisation in specialized care settings and iii) ensuring access to vaccination. We propose a flow chart (**figure 2**) to assess an AEFI and particularly an ALE, providing specific clinical and laboratory tests, according to the onset and type of reaction and subsequent vaccination protocols. This work flow also comprises the management of patients with severe reactions to vaccine components and with idiopathic anaphylaxis. Finally, a screening questionnaire (**table VI**) might help the physician to decide whether an allergological workup and eventually a vaccination in a specialised care setting are advised.

Figure 2

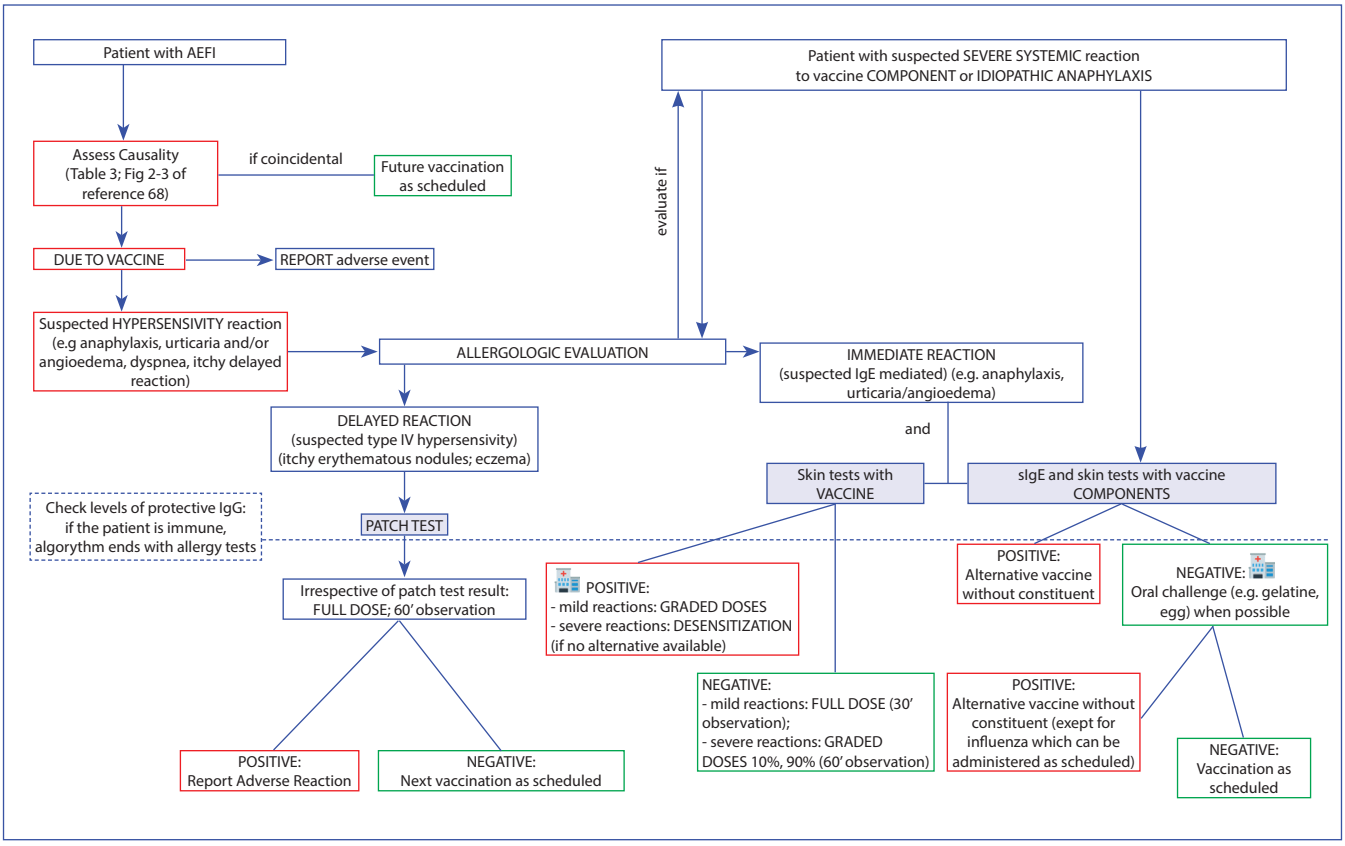

Figure 3 *- Screening questionnaire for allergological evaluation in case of AEFI.*

PATIENT NAME:	DATE OF BIRTH:
Vaccine name:	
Indications for allergological evaluation before vaccines and for vaccination in specialised care setting:	
Previous vaccine reactions: ☐ yes > vaccine name: ☐ no	date:
Previous idiopathic anaphylaxis: ☐ yes > date: ☐ no	
Previous anaphylaxis associated to meat, gelatin or egg ingestion: ☐ yes > allergological workup: ☐ yes ☐ no ☐ no	

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Cross-elicitation responses to 2-methoxymethyl-p-phenylenediamine in p-phenylenediamine highly allergic volunteers using allergy alert test: the Italian experience

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Summary

Background. *Allergic contact dermatitis after exposure to p-phenylenediamine (PPD)-containing hair dye products is a common and important clinical problem. Because there is a high rate of cross-elicitation of allergic contact dermatitis to other important hair dye products (such as p-toluene diamine [PTD] and other aminophenol hair dyes) in PPD allergic patients, safer alternative dyes with excellent hair coloring options are needed. We studied 2-methoxy methyl-PPD (Me-PPD), a chemical derivative of PPD for tolerance versus cross-elicitation in a cohort of eight PPD-allergic volunteers.* ***Objective.*** *To study tolerance to Me-PPD in a PPD highly allergic Italian cohort.* ***Methods.*** *Eight volunteers with a history of contact dermatitis to hair dyes or other PPD-containing chemicals and positive patch tests to 1% PPD in petrolatum, were recruited to study their immediate and delayed skin reactivity to PPD, vehicle control and 2-methoxy-methyl-PPD (Me-PPD), using the allergy alert test (simulating hair dyeing conditions) on volar forearm skin. This is a short-contact open patch test.* ***Results****. All eight volunteers reacted to PPD allergy alert test (100%); none reacted to vehicle (0%), and seven of eight reacted to Me-PPD allergy alert test (88%). However, in those seven volunteers who exhibited cross-elicitation to Me-PPD, their aggregate skin test reactivity to Me-PPD was significantly less than that of PPD (****figure 3****, p < 0.0062, highly significant, paired two-tailed, students t test).* ***Conclusions.*** *Me-PPD may offer a safer alternative for PPD-allergic patients with an absent or reduced elicitation response in the allergy alert test simulating hair dye use conditions. Even patients with strong patch test reactions, with appropriate selection by allergy alert test and counselling, may be able to tolerate hair dyeing with Me-PPD containing products.*

Introduction

Para-phenylenediamine (PPD) is a component of permanent hair dyes, and may be used to dye textiles and fur. Allergic contact dermatitis to PPD has increased significantly in the general population over the last ten years. The prevalence of positive patch-test results to PPD has been in the range of 4% to 5% in a large series of dermatitis patients (1-4).

PPD and the structurally related compound p-toluene-diamine (PTD) are considered the most important allergens associated with allergic contact dermatitis related to the use of hair dyes. There is a positive relationship between an elicitation response

to PPD and concomitant reactions to other chemically related components of oxidative hair colors, such as para-substituted benzene. This phenomenon has been termed "cross-elicitation", whereby sensitization to PPD or PTD elicits patch test reactivity to the other compound, even though there has not been a prior exposure. This phenomenon occurs because of the chemical similarities between the two compounds.

Clinically, there may be a severe acute dermatitis involving the face, eyelids and neck with only minimal scalp involvement in PPD allergic individuals that use PPD-containing hair dyes. Recently, a PPD derivative called 2-methoxymethyl-p-phenylenediamine (Me-PPD) has been developed by the introduction of a methoxymethyl side chain into PPD parent molecule. This molecule is a hair dye precursor with excellent hair coloring performance, and exhibits significantly reduced skin sensitizing properties compared to PPD or PTD. Therefore, Me-PPD has favorable properties, that include reduced propensity to allergy induction with excellent color results when used as a hair dye (5). Herein, we investigated eight PPD-allergic patients to evaluate the risk of develop cross-elicitation responses to Me-PPD under conditions that simulate the hair-dyeing process. We selected a cohort of patients with strong PPD allergy (by clinical symptoms, and with confirmation by strong patch test reactivity (PPD 2-3+ reactivity).

Materials and methods

All volunteers were recruited from the Department of Dermatology at the University of Rome Sapienza, Sant'Andrea Hospital. Eight female volunteers aged between 32 and 47, with a documented history of signs and symptoms consistent with PPD allergy were enrolled. All the patients exhibited a positive standard patch test result to PPD (1% PPD in petrolatum [100 µl]). Patch testing and allergy alert testing was scored, according to the International Contact Dermatitis Research Group (ICDRG) classification system, between 2+ and 3+ (6 patients were scored as 2+, 4 patients were scored as 3+). There were no volunteers that exhibited 1+ reactivity or weaker. All of the volunteers had experienced allergic contact dermatitis related to the use of hair dyes in the past. **Table I** summarizes the eight volunteers enrolled in our study.

We excluded PPD-allergic patients with a history suggestive of severe allergic reactions, such as anaphylaxis or contact urticaria. Volunteers were not permitted to use topical corticosteroids or oral antihistamines one month prior to Visit 1, or systemic corticosteroids or immunosuppressive agents three months prior to Visit 1. Other exclusion criteria were the history of drug or alcohol abuse within the past 6 months, as determined by the medical record or patient interview. Any scarring, infection, or skin disease in the area being patch tested (ventral forearms) were also exclusion criteria. Other exclusion criteria included an inability to make study visits or anticipated poor compliance, pregnant females or nursing mothers, any history or evidence of severe illness or any other condition that would render the volunteer unsuitable for the study.

Eligible women of reproductive age were required to have a negative urine pregnancy test at screening. Eight volunteers enrolled. The following compounds were used in this study:

i) PPD (1% PPD in petrolatum under a FINN chamber, the standard diagnostic patch test). A positive patch test to PPD (along with a history of intolerance to PPD-containing hair dye) was the major criterion for enrollment in this study;
ii) Me-PPD hair dye tint: vehicle (Koleston Perfect formula without fragrance), hair dye precursor (4% Me-PPD, free base), couplers (3.6% 2-methylresorcinol and 1.9% 2-methyl-5-hydroxyethylaminophenol). This chemical derivative of PPD was the unknown in this study, which was evaluated for cross-elicitation or tolerance in volunteers with known PPD allergy related to prior use of hair dyes containing PPD;
iii) PPD hair dye tint: vehicle (Koleston Perfect formula without fragrance), hair dye precursor (4% PPD, free base), cou-

Table I *- Summary of characteristics of volunteers enrolled in study.*

Patient no.	Skin type	Ethnicity	Age/gender	PPD patch test	Route of exposure
1	II	caucasian	31/female	3+	hair dye
2	II	caucasian	51/female	3+	hair dresser (hair dye)
3	II	caucasian	39/female	3+	hair dye
4	II	caucasian	60/female	3+	hair dye
5	II	caucasian	33/female	3+	hair dye
6	II	caucasian	35/female	2+	street tattoo
7	II	caucasian	32/female	2+	cosmetics
8	II	caucasian	34/female	3+	hair dye

plers (3.6% 2-methylresorcinol and 1.9% 2-methyl-5-hydroxyethylaminophenol), PPD served as a positive control for the allergy alert test;

iv) vehicle control (Koleston Perfect formula without fragrance). The vehicle (containing no PPD) served as a negative control, to prove that PPD was responsible for the positive allergy alert test (in addition to the standard patch test described above).

Immediately prior application to the volunteers' forearms, the hair dye tints (either PPD or Me-PPD) and the vehicle control were mixed with an equal volume of hydrogen peroxide solution (6% [w/w] Welloxon) at a 1:1 ratio with the hair dye tints, resulting in a final concentration of 2% Me-PPD and PPD in the allergy alert test, respectively. This is the exact methodology used in a prior study (8).

The PPD (1% in petrolatum) patch test was used under a FINN chamber occlusion, with removal of the patch test after 48 hours and readings performed at 48 and 72 hours. This standard patch test had been performed prior to enrollment in this study.

Me-PPD and PPD (formulations with couplers and hydrogen peroxide solution) and vehicle alone were tested using a modified protocol: after 30 minutes following placement of a 1 cm^2 area of an open application to the ventral forearm, the Me-PPD, PPD, and vehicle formulations were gently rinsed off with a hypoallergenic soap and water. This simulates hair dyeing conditions (allergy alert test).

Skin evaluation readings for reactivity to PPD, Me-PPD and vehicle control (allergy alert test) were performed at 30 minutes, 48 hours and 72 hours.

The International Contact Dermatitis Research Group (ICDRG) scoring system was used to grade patch testing results (6). Scores range from 0 to 3+: 0 (-) = negative reaction; ?+ = doubtful reaction / erythema only; 1+ (+) = weak (non-vesicular) positive allergic reaction (erythema, infiltration, and possible papules); 2+ (++) = strong (vesicular) positive reaction (erythema, infiltration, papules, and vesicles); 3+ (+++) = extreme positive allergic reaction; bullous reaction.

Statistical analysis

To compare the allergy alert test results of the eight volunteers against PPD and Me-PPD, we used the Graph Pad Prism Software Program (Graph Pad Software, Inc., La Jolla, CA.). Because we compared PPD and Me-PPD reactivity within each of the eight volunteers, we used the paired, two-tailed student's t-test. P values < 0.05 were considered significant.

Results

We evaluated the potential of Me-PPD to cross-elicit allergic contact dermatitis on the ventral forearm skin of eight female volunteers with patch-test proven PPD-allergy and a history of clinical hair dye intolerance (a standard definition of PPD allergy related to the use of PPD containing hair dye). To assess the immediate reactivity of the skin, we performed readings 30 minutes after the removal of Me-PPD, PPD (formulations with couplers and hydrogen peroxide solution) and vehicle. At the 30-minute reading, none of the eight volunteers exhibited a positive skin test. This was interpreted as negative immediate skin reactivity to PPD, Me-PPD and vehicle (data not shown).

Table II summarizes the delayed reactivity to PPD, vehicle and Me-PPD on allergy alert test in the context of their diagnostic PPD patch test. All eight volunteers reacted to PPD allergy alert test (100%); none reacted to vehicle (0%), and seven of eight reacted to Me-PPD allergy alert test (88%).

Figure 1 summarizes the cross-elicitation of the eight volunteers using the allergy alert test for Me-PPD at the final (72 hour) read. Of the six volunteers with 3+ (extreme) reactivity to PPD standard patch tests, five exhibited cross-elicitation to Me-PPD on allergy alert. Of the two volunteers with 2+ (strong) reactivity to PPD standard patch tests, both (100%) exhibited cross-elicitation to Me-PPD.

Figure 2 summarizes tolerance (non-reactivity) to Me-PPD in our eight allergic-PPD volunteers. Of the two volunteers with 2+ PPD reactivity on standard patch testing, none (0/2, 0%) was tolerant. Of the six volunteers with 3+ PPD reactivity on standard patch testing, one (1/6, 16%) was tolerant.

We compared the strength of the reactivity of Me-PPD to PPD in those seven volunteers who reacted to both compounds. In five of seven volunteers, the reaction to PPD allergy alert test was stronger than that of Me-PPD (not shown). In two of seven volunteers, the reaction to PPD allergy alert test was the same as Me-PPD (not shown). None of the seven volunteers exhibited a stronger allergy alert test to Me-PPD compared to

Table II *- Summary of allergy alert test for PPD, Vehicle and Me-PPD (number positive/number tested).*

Strength of PPD patch test	PPD allergy alert	Vehicle allergy alert	Me-PPD allergy alert
+++	6/6[1]	0/6	5/6
++	2/2	0/2	2/2
+	n/a[2]	n/a	n/a
% positive	100%	0%	88%

[1]Number of positive on allergy alert test (1+ to 3+/number tested);
[2]n/a = not applicable (there were no volunteers with moderate reactivity to PPD on patch testing).

PPD (not shown). In aggregate, the mean reactivity to PPD was stronger than Me-PPD on allergy alert test (**figure 3a**). This difference was highly significant, despite the small sample size ($p < 0.0062$, two tailed, paired t-test with eight paired values). The results of the 72-hour reading of allergy alert test in one of the volunteers enrolled in this study are depicted. This includes PPD, vehicle and Me-PDD, see **figure 3b**, revealing a stronger reaction to PPD (2+) than to Me-PPD (1+).

Figure 1. *Final (72 h reading) scores of Me-PPD allergy alert test in the context of PPD patch test results in eight volunteers enrolled in the present study. Volunteers are grouped by their diagnostic patch test to 1% PPD, either 2+ or 3+ (there were no volunteers who exhibited 1+ reactivity).*

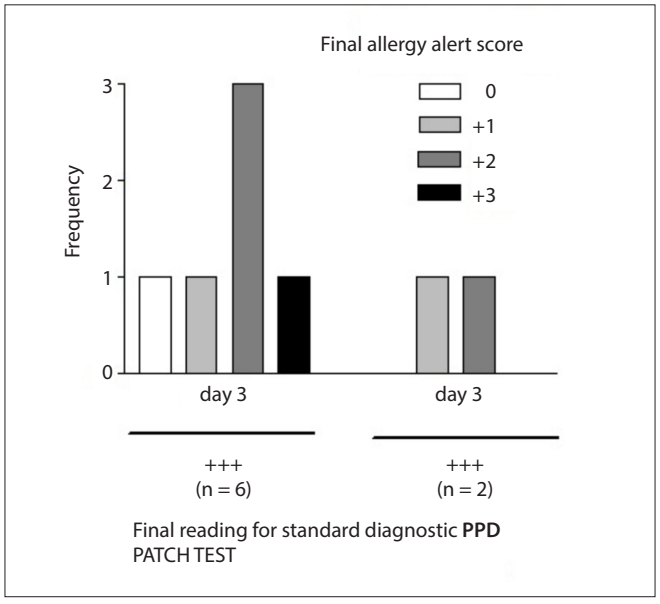


Figure 2. *Tolerance to Me-PPD allergy alert test in context of PPD patch test results. Of the eight volunteers in this study, two exhibited moderate (2+) patch test reactivity to 1% PPD diagnostic patch test, and none of these two subjects were tolerant to Me-PPD (0%). The remaining six subjects exhibited extreme (3+) reactivity to 1% diagnostic patch test, and one of six (16%) were tolerant of Me-PPD. The overall rate of tolerance in this cohort of highly reactive PPD volunteers was 12% (one of eight).*

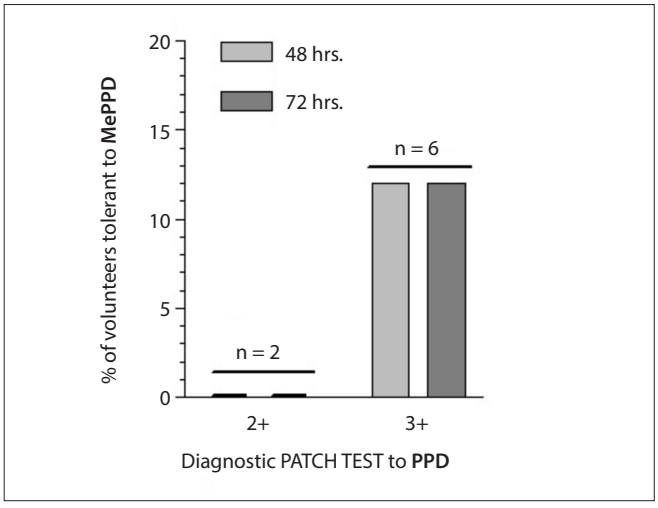
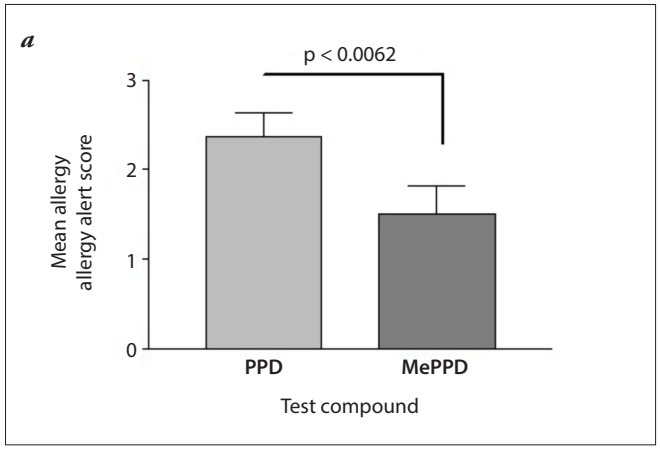
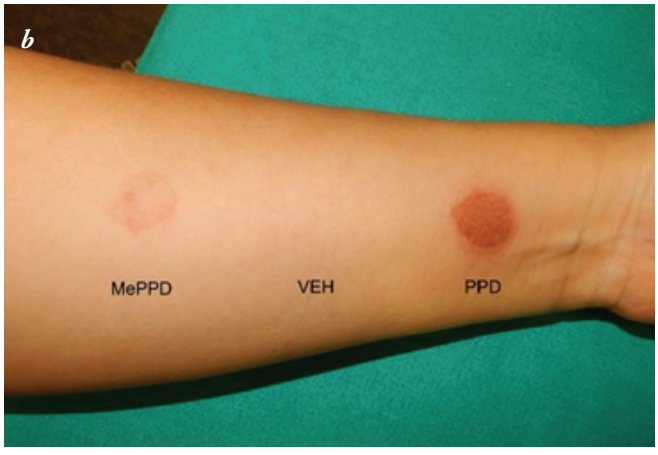


Figure 3. *Comparison of PPD and Me-PPD allergy alert test results in eight volunteers.* ***a*** *- the aggregate (mean +/- SD) allergy alert scores for PPD and Me-PPD were compared. The mean reactivity of PPD was stronger than that of Me-PPD. The reactivity to Me-PPD was significantly less than that of PPD (highly significant, $p < 0.0062$, two tailed, paired t-test, with eight pairs);* ***b*** *- allergy alert test at 72 hours in volunteer who was not tolerant of Me-PPD. Her reaction to PPD was stronger than that of Me-PPD. There was no reaction to the vehicle control.*





Discussion

PPD is widely used as a permanent hair dye, but it may also been found in textile or fur dyes, temporary tattoos, photographic developer and lithography plates, photocopying and printing inks, black rubber, oils, greases and gasoline. Despite its presence in a variety of products, exposure to PPD-containing hair dyes is the main cause of PPD allergy (7).

The introduction of a methoxymethyl side chain into PPD created a hair dye precursor with excellent hair coloring performance. The Me-PPD has been studied in pre-clinical predictive assays to determine its relative sensitization capacity compared to the parent compound, PPD. Using the local lymph node assay (a standard in vivo mouse sensitization assay), the effective concentration of Me-PPD necessary to induce an immune response 3-fold above vehicle control (EC3 value) in the local lymph node assay (LLNA) was 4.3%, indicating a moderate skin sensitizing potency compared to values of 0.1% and 0.17% for PPD and PTD. Both PPD and PTD are considered to be strong sensitizers, and thus induce a response in the local lymph node assay at much lower concentrations compared to Me-PPD. These pre-clinical data indicate that Me-PPD has significantly reduced skin sensitizing properties compared to PPD or PTD (8).

Blomeke et al. (9) studied the sensitivity of the allergy alert test in a cohort of patch test-proven PPD allergic volunteers. She demonstrated that under stimulated hair coloring conditions (that is, the skin allergy alert test, a short contact open patch test, see methods), the rate of cross-elicitation to Me-PPD (30%) was lower than to PPD (84%). On this basis, we have conducted a study to investigate if patients with a documented history of allergic contact dermatitis to PPD assessed by positive patch test results, develop a cross-elicitation response to Me-PPD under conditions mimicking hair dyeing. We enrolled eight female patients who had experienced hair allergic contact dermatitis to PPD-containing hair dye (confirmed by standard diagnostic patch testing).

In past studies, tolerance to cross-elicitation to Me-PPD by allergy alert testing in PPD allergic volunteers was inversely proportional to PPD patch test reactivity (9). That is, tolerance to Me-PPD under hair-dyeing conditions was between 50-100% in volunteers with 1+ PPD patch test reactivity; 40-85% in volunteers with 2+ PPD patch test reactivity, and 33-50% in volunteers with 3+ PPD reactivity (5,8-10). In the present study, we enrolled only volunteers with a positive standard patch test to PPD scored between +2 and +3, according to the International Conctact Dermatitis Research Group (ICDRG) classification system. The overall rate of tolerance to Me-PPD in our highly PPD-reactive cohort was 12%. The lower rate of tolerance to Me-PPD is due to the small population size, that was comprised only of strong and very strong PPD reactors. The previous three studies had significant numbers (53%, 35% and 9%) of volunteers with moderate reactivity to PPD on patch testing. In our study, there were no moderate PPD reactors (1+), only strong (2+) and extreme reactors (3+).

Interestingly, our only Me-PPD tolerant volunteer was an extreme PPD reactor (3+ on PPD patch testing), which also occurred in previous studies (33 to 50% of volunteers with extreme patch test [3+] results to PPD). Since allergic contact dermatitis to PPD is T-lymphocyte mediated (11-14), it is likely that the mechanism for the cutaneous tolerance involves T-lymphocyte tolerance. The immunology of tolerance to Me-PPD in the PPD highly allergic individual warrants further study, as it may be an example of powerful suppressor mechanisms. Most likely, this involves suppressor mechanism(s) such as T-regulatory cells (15,16), which control potent T-effector/T-memory mechanisms in such highly PPD-allergic individuals.

Lastly, in those PPD-allergic volunteers who were not tolerant of Me-PPD, their relative reactivity to Me-PPD on allergy alert testing was, in most cases, less than PPD on allergy alert testing (**figure 3a**). These data are in line with the results from a recent study from the USA (8).

These data confirm that it may be possible to safely use Me-PPD even in those individuals who are highly allergic to PPD (i.e., complete tolerance to Me-PPD, as demonstrated by one of eight volunteers in our study). This may only be possible with careful patient selection with allergy alert test and counseling being key components to assure consumer safety. Those subjects who exhibit any reactivity to Me-PPD on allergy alert test (even if this reactivity is less than that of PPD on allergy alert test) should *not* use a Me-PPD containing hair dye.

Limitations of this study are the small sample size (n = 8). Further long-term studies are needed, as this study was only a single exposure.

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Statement of ethics

Subjects (or their parents or guardians) have given their written informed consent. The study protocol has been approved by the research institute's committee on human research.

Conflict of Interest

Dr. Carsten Goebel is an employee of Coty, Inc.

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Cost-effectiveness of the SQ HDM SLIT-tablet for the treatment of allergic asthma in three Eastern European Countries

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[4]R. Babela was an employee of ALK-Abelló at the time when this research was conducted.

Key words
cost-effectiveness; allergic asthma; allergy immunotherapy; sublingual immunotherapy; SQ HDM SLIT-tablet

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Summary

Background. *The SQ® house dust mite (HDM) sublingual immunotherapy (SLIT)-tablet ACARIZAX®, ALK-Abelló A/S, Hørsholm, Denmark) is an allergy immunotherapy tablet for people with allergic respiratory disease. This analysis aims to assess the cost-effectiveness of the SQ HDM SLIT-tablet from the perspective of three Eastern European countries: Czech Republic, Poland and Slovakia.* ***Methods****. A cost-utility model per country was developed, which compared the SQ HDM SLIT-tablet as add-on to pharmacotherapy with pharmacotherapy alone in patients with HDM allergic asthma (AA) over a five year time horizon. The effectiveness of the two interventions was based on the results from a large-scale randomised controlled trial. In the models, annual costs and quality-adjusted life year (QALY) scores from the trial were extrapolated over a five year period, and the incremental cost-effectiveness ratios (ICERs) were estimated. One-way deterministic sensitivity and scenario analyses were undertaken.* ***Results.*** *The SQ HDM SLIT-tablet is cost-effective in all three markets over the five year time horizon (ICERs of less than € 10,000 per additional QALY). Treatment with the SQ HDM SLIT-tablet improves patient outcomes, with QALY gains of 0.35, versus pharmacotherapy only. In all three countries, the SQ HDM SLIT-tablet also incurs increased costs compared to pharmacotherapy treatment only. The sensitivity analysis identified utility values from the clinical trial as the main driver of the model results.* ***Conclusion.*** *The SQ HDM SLIT-tablet is a cost-effective treatment option for people with HDM AA in three different health care settings in Eastern Europe.*

Introduction

Asthma is a global health problem affecting 300 million people worldwide, a number expected to rise to 400 million people by 2025 (1). In Poland, Czech Republic and Slovakia, it is estimated that 5 to 8% of the population have asthma (2,3). Allergic Asthma (AA), usually defined by the presence of sensitisation to environmental allergens, accounts for approximately 50% of all asthma (4). House dust mites (HDM) are a significant factor underlying AA, with sensitisation to HDM present in 50% to 85% of people with asthma (5,6). In most cases of HDM AA, the disease is accompanied by allergic rhinitis (AR) (7). Close to 30% of people are sensitized to HDM in Poland and Slovakia (8,9).

The symptoms of asthma include breathlessness, chest tightness, wheezing and obstruction of airflow. The ever-present risk of severe exacerbations of symptoms, which may require emergency treatment and/or hospitalisation, can have a significant detrimental influence on daily quality of life. Quality of life can also be affected by limitation of daily activities, emotional functioning and lack of sleep (10). Overall, poor asthma control has been shown to reduce quality of life (11,12). A global survey among people with asthma reported poorer symptom control in Central and Eastern Europe, with 74% of people reporting

daytime symptoms compared to 56% in Western Europe (13). According to a Polish study, 47% of the population with asthma reported their symptoms to be partly controlled and 32% reported that their asthma was uncontrolled. A similar study in Czech Republic reported that 32% have partly controlled and 57% have uncontrolled asthma (12,14).

Asthma is associated not only with poor quality of life but also with significant health resource utilisation. In Europe the annual costs for an adult with asthma are estimated at € 1,583 and these costs increase with reduced asthma control (15). The cost of a single asthma exacerbation was estimated to range from € 737 to € 1,074, depending on asthma severity (16). Hospitalisation and medications have been identified as the most significant drivers in regards to direct costs. A systematic literature review reported that 52% to 86% of direct asthma-related costs come from in-patient hospitalisation (17). Absence from work or school are also significant contributors to indirect costs. Previous research indicates that 23% of adults in Central and Eastern Europe lost workdays due to asthma, as reported in the worldwide survey on asthma severity and control, compared to 17% in Western Europe (13).

For certain patients, symptoms of asthma can be controlled and relieved by allergen avoidance and controller medications, such as inhaled corticosteroids (ICS) or long-acting beta agonists (LABA), as well as asthma relievers such as short-acting beta agonists (SABA). For more severe asthma, IgE anti-bodies or Il-5 receptor agonists are add-on treatment options. Allergy immunotherapy (AIT) is the only treatment option for allergic diseases which aims to have a disease-modifying effect to limit disease progression and facilitate a long-term reduction in symptoms of the disease. The Global Initiative for Asthma (GINA) has included treatment with HDM sublingual immunotherapy (SLIT) in their latest strategy for asthma management (18). The SQ® HDM SLIT-tablet (ACARIZAX®, ALK-Abelló A/S, Hørsholm, Denmark) is a sublingual AIT that contains a 1:1 mixture of allergen extract from the 2 major mite species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae.* It is indicated for people with HDM AA whose symptoms are not well controlled despite the use of ICS. The results from the MITRA trial (MT-04; NCT01433523), a large-scale (n = 834) phase III double-blind, randomised controlled trial, which assessed two doses for the HDM SLIT-tablet (6 SQ-HDM and 12 SQ-HDM) indicate that the 12 SQ HDM SLIT tablet significantly reduces the risk of moderate to severe asthma exacerbation compared with placebo (hazard ratio 0.69; 95% CI 0.50 to 0.96) (19). Based on the findings from this trial it has previously been shown that the 12 SQ HDM SLIT-tablet is a cost-effective treatment for AA in the German setting (20).

The SQ HDM SLIT-tablet is now available as a treatment option for people whose asthma is not well controlled in Czech Republic, Poland and Slovakia. The aim of the analysis was, therefore, to assess the cost-effectiveness of the 12 SQ HDM SLIT-tablet plus pharmacotherapy versus pharmacotherapy alone in the treatment of AA from the perspective of these three Eastern European countries.

Methods

Three cost-utility models were developed, to compare the costs and outcomes associated with patients with AA treated with SQ HDM SLIT-tablet plus pharmacotherapy versus patients treated with pharmacotherapy only over a five-year time horizon in the 3 countries of interest. The decision-tree model structure was based on a modelling approach described previously (20). The same model structure was applied for all three countries, with certain input parameters changed to reflect local variations (e.g. distinct unit costs for healthcare resources). For the model it was assumed that patients treated with the SQ HDM SLIT-tablet stay on treatment for 3 years, as per recommendations, and afterwards pharmacotherapy could be continued to be used as needed for the remaining time horizon. For patients using pharmacotherapy only, it was assumed that there were no changes in treatment throughout the time horizon of the model. The following cost inputs were included in the models: the cost of the SQ HDM SLIT-tablet (treatment arm only), specialist/general physician visits, emergency room visits, ICS use and SABA use (**table I**). The total usage of these resources was based on data recorded in the MT-04 trial per treatment arm and extrapolated over the full time horizon. The total annual costs were estimated by combining the resource use with country specific cost data and prices (21-26). To reflect the local health care setting and treatment practice, some adjustments had to be made to the different models. For Poland, emergency room visits are not applicable in the model as hospitals are paid a flat-fee for emergency treatment and not per patient or per visit, i.e. additional emergency room visits do not incur measurable extra cost to the health care system. Based on local guidelines and expert input the number of physician visits required for patients receiving the SQ HDM SLIT-tablet was adjusted by country (**table II**). For example, local experts suggest that in Poland and Slovakia patients should see their physician twice a year while treated with a SLIT-tablet, while this is not standard of care in Czech Republic. For all the countries, an extra visit for the first administration of the SQ HDM SLIT-tablet, which is required by the product label, was also added in the first year of treatment. Finally, for the Czech and Polish analyses the costs were converted from local currencies into Euros, using exchange rates of 25.61 and 4.25, respectively (valid on 06/11/17), to ensure consistent reporting across the three country settings.

For the cost of the SQ HDM SLIT-tablet the prices in the Czech Republic and Slovakia are defined by reference prices that are updated biannually. Prices relevant to 2017 have been ad-

Table I *- Annual resource use and cost per country.*

Resource	Cost per unit[1]	Annual resource use		Cost per year	
		SQ HDM SLIT-tablet	pharmaco-therapy	SQ HDM SLIT-tablet	pharmaco-therapy
SQ tablet (unit value: per tablet)					
Czech Republic	€ 2.63	365 tablets	0 tablets	€ 959.95	€ 0.00
Poland	€ 2.63	365 tablets	0 tablets	€ 923.45	€ 0.00
Slovakia	€ 2.63	365 tablets	0 tablets	€ 959.95	€ 0.00
Physician visits (unit value: per visit)					
Czech Republic	€ 7.89	1.0 visits	1.0 visits	€ 7.89	€ 7.89
Poland	€ 8.39	0.17 visits	0.1 visits	€ 1.47	€ 0.88
Slovakia	€ 60.48	0.17 visits	0.1 visits	€ 10.57	€ 6.33
Emergency room visits (unit value: per visit)					
Czech Republic	€ 21.63	0.01 visits	0.03 visits	€ 0.22	€ 0.55
Poland	N/A	N/A	N/A	N/A	N/A
Slovakia	€ 54.00	0.01 visits	0.03 visits	€ 0.54	€ 1.36
ICS daily dose (unit value: see below)					
Czech Republic (40,000 µg)	€ 20.00	205.5 mg	202.6 mg	€ 101.58	€ 100.14
Poland (10,000 µg)	€ 5.18	205.5 mg	202.6 mg	€ 106.50	€ 104.99
Slovakia (3,375 µg)	€ 8.00	205.5 mg	202.6 mg	€ 164.40	€ 162.06
SABA intake (unit value: see below)					
Czech Republic (200 doses)	€ 8.60	266 doses	297 doses	€ 11.43	€ 12.75
Poland (600 doses)	€ 8.81	266 doses	297 doses	€ 3.90	€ 4.36
Slovakia (25 doses)	€ 59.28	266 doses	297 doses	€ 26.28	€ 29.32

[1]All unit costs were based on local 2017 prices.

Table II *- Extra physician visits per treatment year for patients treated with the SQ HDM SLIT-tablet.*

Country	Unit cost	Resource use			Total cost
		year 1	year 2	year 3	
Czech Republic	€ 7.89	1.00	0.00	0.00	€ 7.89
Poland	€ 8.39	3.00	2.00	2.00	€ 58.76
Slovakia	€ 60.48	3.00	2.00	2.00	€ 423.36

opted but are liable to change in the future. Further, at the time of the analysis the SQ HDM SLIT-tablet was not reimbursed nationally in Poland and therefore the same price as the other two countries was applied.

The effectiveness of the two interventions is captured via the impact on patients' health-related quality of life (HRQoL), as measured by utility. Utility is a measurement of patient wellbeing on a scale of zero to one and can be combined with time to estimate quality-adjusted life year (QALY) scores.

The utility values applied in the models are based on data recorded during the MT-04 trial (**table III**). The values were obtained by calculating the change from baseline to end of the maintenance period in the trial per treatment arm. Although there was a significant change from baseline to end of trial, the

Table III *- Utility values from MT-04 (Change from baseline and end of maintenance period values).*

	Placebo	SQ HDM SLIT-tablet
baseline utility for full sample	0.736	0.736
change in utility (p = 0.0318)	0.0059	0.0315
final utility for analysis	0.742	0.768

values from the end of the maintenance period are more reflective of a real-world setting than the following period. This is because after the maintenance period in the trial ICS was removed by 50% for 3 months and completely withdrawn for the last 3 months (19). Between the two treatment arms the difference in utility change from baseline to end of maintenance period was 0.026 (p = 0.0318). Further, to account for baseline differences in utility between the treatment arms, the average baseline utility for the full trial sample was calculated and the change from baseline to the end of the maintenance period for the two treatment arms were applied to obtain the utility values used in the cost-effectiveness analysis.

The utility values from MT-04 were used for the first year of the analysis. For the remaining four years of the time horizon the utilities were extrapolated based on the following assumptions:

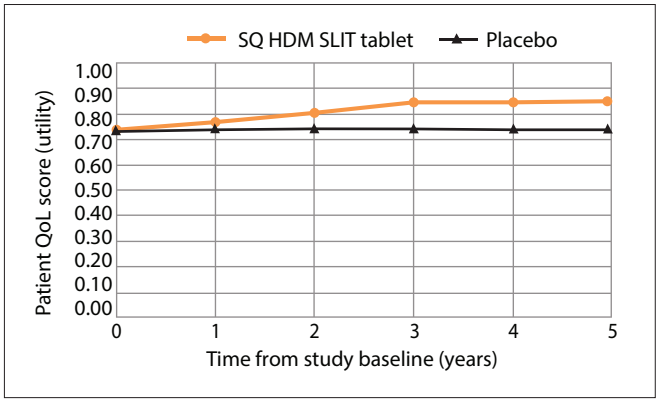
- in year 2-3 there will be an increased treatment effect and therefore further increase in utilities of 5% in the treatment arm;
- during year 4-5 this effect will be sustained due to the disease modifying effect.

These assumptions are based on the disease modifying effect of AIT, which has previously been evidenced when using AIT for respiratory allergies (27-30). For pharmacotherapy patients it has been conservatively assumed that the utility gains achieved in the trial remained throughout the time horizon, such that there is no change in utility from years one to five. The change in utility predicted over the course of the model time horizon for the two interventions is summarised in **figure 1**.

Cost-effectiveness was established by the estimation of the incremental cost-effectiveness ratio (ICER). The ICER is a standard measurement used in economic evaluations that facilitates a comparison of two interventions, taking into account the returns that could be achieved by spending the budget elsewhere in the healthcare system (i.e. the opportunity cost). The ICER equation is as follows:

$$ICER = \frac{Cost_{Treatment} - Cost_{Comparator}}{QALY_{Treatment} - QALY_{Comparator}} = \frac{\Delta Cost}{\Delta QALY}$$

Figure 1 *- Summary of utility changes over time.*



The interpretation of an ICER requires a cost-effectiveness threshold, which takes into account societies willingness to pay for new interventions, and thus formally quantifies whether the benefits achieved by an intervention is adequate given the cost consequences of that intervention and opportunity costs. To reflect local willingness-to-pay, cost-effectiveness thresholds based on local reimbursement guidelines were applied in the models. Costs as well as the QALYs estimated within each model were discounted based on local payer requirements. The cost-effectiveness thresholds, and discount rates, applied for each country setting are summarised in **table IV**.

To investigate first order uncertainty, one-way deterministic sensitivity analysis was undertaken by altering the values applied for individual model parameters to examine the impact on results. For all parameters a range of approximately +/-30% of the base case value was applied, based on guidelines for Slovakia and applied equally across the three countries to ensure consistency (31). The parameters tested were: unit cost of the SQ HDM

Table IV *- Summary of cost-effectiveness threshold and discount rates, by country.*

Parameter	Czech Republic	Poland	Slovakia
cost-effectiveness threshold (2017)[1]	€ 49,721	€ 30,626	€ 21,192
discount rate, costs	3.00%	5.00%	5.00%
discount rate, QALYs	3.00%	3.50%	5.00%
reference	(32)	(33)	(31)

[1]Cost-effectiveness threshold values for the Czech and Polish analyses were converted from local currencies into Euros, using exchange rates of 25.61 and 4.25, respectively.

SLIT-tablet, ICS dose, SABA intake and utility value. Emergency room visits and physician visits were not incorporated in the sensitivity analysis because the values obtained from the MT-04 trial were very low and, therefore, changes to these parameters were not expected to have a meaningful impact on the results unless unrealistic variations were tested.

To test the assumptions behind the extrapolation of utility data from the MT-04 trial to the five-year time horizon, alternative values were tested within the models via two scenarios. In the first scenario, a smaller improvement in utility for SQ HDM SLIT-tablet patients of 2% during years two and three was applied, whilst for the second scenario it was assumed there was a 0% change in utility for patients on active treatment during years two and three.

Results

The results of the economic analysis in the three countries are presented in **table V**. These results indicate that the SQ HDM SLIT-tablet is a cost-effective treatment for HDM allergic asthma in Czech Republic, Poland and Slovakia, as shown by the ICERs of less than € 10,000 per additional QALY in all three countries. Over the five-year time horizon, the SQ HDM SLIT-tablet is associated with higher overall costs of approximately € 2,500 to € 3,000, but also improves patient outcomes via QALY gains of approximately 0.35.

The results of the deterministic sensitivity analysis show that the results of the model are most sensitive to changes in utility for both intervention arms. Changes in utilities within the ranges examined, changed the direction of the results enough for the ICERs to be above the threshold in the three countries.

The results from the analyses, assessing different assumptions around the extrapolation of utilities over the time horizon, are presented in **table VI**. The results show that the ICERs increase as the utilities in year 2 and 3 are decreased. In both tested scenarios, the ICERs stay within the local cost-effectiveness thresholds, except for the Slovakian ICER in the second scenario.

Table V *- Results of the cost-effectiveness analysis (costs, QALYs and ICERs).*

	SQ HDM SLIT-tablet	Pharmaco-therapy	Difference
Czech Republic			
cost per patient	€ 3,283	€ 561	€ 2,722
QALYs per patient	3.76	3.40	0.37
cost-effectiveness threshold	-	-	€ 49,721
ICER	-	-	€ 7,455
Poland			
cost per patient	€ 3,152	€ 477	€ 2,675
QALYs per patient	3.71	3.35	0.36
cost-effectiveness threshold	-	-	€ 30,626
ICER	-	-	€ 7,492
Slovakia			
cost per patient	€ 3,875	€ 862	€ 3,013
QALYs per patient	3.55	3.21	0.34
cost-effectiveness threshold	-	-	€ 21,192
ICER	-	-	€ 8,814

Discussion

The results of the five-year analysis indicate that the SQ HDM SLIT-tablet plus pharmacotherapy is a cost-effective treatment option versus pharmacotherapy alone for people with allergic asthma in Czech Republic, Poland and Slovakia. All three analyses resulted in an ICER below € 10,000, which is substantially lower than the cost-effectiveness thresholds for each individual country (€ 49,721, € 30,626 and € 21,192 for Czech Republic, Poland and Slovakia, respectively). Consistent results were obtained despite the three country settings, with differences in local clinical practice, costs and payer requirements. Nevertheless, there were small variances in the results estimated for the three countries, driven mainly by different requirements for health economic analyses. In particular, the discount rate for QALYs was 5% in Slovakia, which was higher than the rates of 3% for the Czech Republic and 3.5% for Poland, and this reduced the QALY gains achieved by the SQ HDM SLIT-tablet in Slovakia. At the same time, the cost-effectiveness threshold was substantially lower for Slovakia compared to the other markets, meaning the values placed on the QALY gains are lower in this country. Overall, the results are in line with a previously published cost-effectiveness analysis for the SQ HDM SLIT-tablet in the treatment of AA in Germany (20).

Table VI *- Results of sensitivity analyses of long-term effect.*

Country	Base case ICER	Scenario 1 ICER	Scenario 2 ICER
Czech Republic	€ 7,455	€14,191	€22,861
Poland	€ 7,449	€14,164	€22,787
Slovakia	€ 8,814	€16,706	€26,766

The results of the sensitivity and scenario analyses indicate that the model is most sensitive to changes in the utility values in the model, including how utility changes during years 2 and 3 following treatment with the SQ HDM SLIT-tablet. The utility data applied in the model were taken from a large-scale, double blind randomised controlled trial. Given the robust trial design, this source should ensure that values adopted are valid and accurate reflections of patient HRQoL and should also provide reliable estimates of efficacy for both the SQ HDM SLIT-tablet and pharmacotherapy.

To estimate the long-term impact of the SQ HDM SLIT-tablet it was necessary to make a small number of assumptions regarding HRQoL change over five years. These assumptions were based on the disease-modifying properties of AIT, which address the underlying disease and induce tolerance to the allergen in question. Evidence shows that the effect of AIT improves throughout a full three-year course of treatment and that this effect can last for up to 7 years after finalizing treatment (27-30). Therefore, the SQ HDM SLIT-tablet may continue to benefit patients after the five-year time horizon considered here. For pharmacotherapy patients it has been conservatively assumed that the utility gains achieved in the trial remained throughout the time horizon, such that there is no change in utility from years one to five. However, the improvements measured in the trial may have occurred due to the placebo effect, in which case the long-term difference in patient HRQoL between the SQ HDM SLIT-tablet and placebo patients would be greater than modelled here.

One limitation of the model is that the resource use values applied in order to estimate the total cost burden for patients are based on the values reported in the MT-04 trial, which are protocol driven and may not reflect healthcare utilisation rates in real clinical settings. This approach was necessary due to a paucity of relevant local data and may lead to an underestimation of the cost difference between SQ HDM SLIT-tablet and pharmacotherapy patients. For example, if the SQ HDM SLIT-tablet leads to greater disease control, then this is likely to reduce the risk of hospital inpatient admissions, which are associated with large costs to the healthcare system. Besides from health care utilization, asthma and allergic rhinitis are also known to cause an indirect cost burden to society due to absenteeism and presenteeism, which is known to be particularly large in Eastern Europe (13). The impact of treatment on these societal costs was not captured in this model, due to a lack of specific local data.

Allergic asthma is a transient condition and disease control can vary on a day-by-day basis, sometimes resulting in asthma exacerbations which are costly and have a detrimental impact on HRQoL. While the MT-04 trial showed that the risk of experiencing such exacerbations is reduced by 34% when patients were treated with the SQ HDM SLIT-tablet, it did not report exacerbation rates which would be required to include exacerbations in a health economic model (19). Therefore, this was not captured in the model, potentially underestimating HRQoL and cost benefits in these markets. Data to support the assumption of reduced exacerbations should be considered for future clinical and health economic research.

Conclusion

SQ HDM SLIT-tablet is a cost-effective treatment for patients with HDM AA not well controlled by pharmacotherapy in Czech Republic, Poland and Slovakia. It can therefore be considered a relevant treatment option, addressing an unmet need for improved asthma control and HRQoL in these countries.

Conflict of Interests

William Green and Jessica McMaster work at York Health Economics Consortium (YHEC). YHEC received funding from ALK-Abelló to complete the manuscript. At the time when research was conducted, Robert Babela was a salaried employee of ALK-Abelló. He has also received salary from the St. Elizabeth University, Bratislava, Slovakia, as lecturer. Sarah Buchs is a salaried employee at ALK-Abelló.

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Allergic Bronchopulmonary Mycosis due to fungi other than Aspergillus

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Summary

Allergic bronchopulmonary mycosis (ABPM) is a clinical syndrome associated with immune sensitivity to various fungi that colonize the airways. Early diagnosis and treatment with systemic corticosteroids is the key in preventing the progression of the disease to irreversible lung fibrosis. Although Aspergillus has progressively gained recognition as a causative agent in past few decades, other fungi, that have been reported to cause ABPM, are not yet widely evaluated.

We studied hundred and two patients with asthma for occurrence of ABPM. Patients were tested for cutaneous hypersensitivity and serum precipitin to 12 common fungal antigens. The positive cases were further evaluated for ABPM using standard criteria. Out of 102 asthma patients screened, 18 patients had either skin prick test (SPT) and/or serum precipitin positive. While 14 patients were SPT positive for one or more fungal antigen, two patients were serum precipitin positive for one or more fungi. Two patients had both serum precipitin positive as well as SPT positive. Six (5.8%) patients were diagnosed as ABPM as they fulfilled the criteria. Three of these were because of Aspergillus sp. Two were because of fungi other than Aspergillus namely Schizophyllum and Curvularia. One patient had ABPM because of both Aspergillus and Curvularia. In our study absolute eosinophil count (AEC), total IgE, serum precipitin and SPT had sensitivity of 100%, 100% 50% and 83.3% respectively for diagnosing ABPM. The specificity of these tests was 44.79%, 64.58% 98.96% and 88.54% respectively. Specfic IgE was positive in 50% of patients with either serum precipitin or SPT positivity. SPT or serum precipitin followed by specific IgE had sensitivity of 100% and specificity of 96.88% for diagnosing ABPM. SPT alone followed by Specific IgE had a sensitivity of 83.33% and specificity of 96.88% for diagnosing ABPM.

We found that fungi other than Aspergillus such as schizophyllum, and curvularia, can be implicated in ABPM. Multiple fungal agents may be responsible for ABPM in an individual. There is a subset of patients of BA who have fungal sensitization but do not fulfil the criteria for ABPM. SPT was the single most sensitive and specific test, AEC >350 and total IgE more than 417IU were most sensitive tests and SPT followed by specific IgE was most effective strategy for diagnosing ABPM.

Introduction

Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity mediated disease of the lower airways caused by environmental fungi, the most common being Aspergillus fumigatus. The other etiologic agents include Candida albicans, Schizophyllum commune, species of Alternaria, Bipolaris, Cladosporium, Curvularia, Fusarium, Penicillium, Pseudallescheria, Rhizopus, Saccharomyces, Stemphylium and Trichosporon (1). While allergic bronchopulmonary aspergillosis (ABPA) has been extensively studied worldwide, there is paucity of information on ABPM due to other fungi. The most commonly accepted criteria for diagnosing ABPA are those proposed by Rosenberg et al. (1977) and Patterson et al (2,3). Early recog-

nition and treatment with corticosteroids prevents progression to fibrotic lung disease (4). The term severe asthma with fungal sensitisation (SAFS) has been proposed for those patients who have persistent severe or brittle asthma (despite standard treatment) and evidence of fungal sensitisation, as defined by positive prick testing, or fungal antigen-specific blood IgE testing, and do not meet the criteria for ABPA (5).

The paucity of information on sensitization by variety of fungal allergens other than Aspergillus spp. in patient of asthma and their clinical profile prompted us to undertake the present study. The present study is aimed to observe the occurrence of ABPM in bronchial asthma.

Material and Methods

This was a cross sectional observational study of 102 subjects (age >18 years) of asthma attending Dr. RML Hospital from Nov 2014 to March 2016, who were diagnosed as per GINA guidelines. Patients were subjected to Skin Prick Test (SPT) with 12 common fungal allergens (Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Curvularia lunata, Scedosporium apiospermum, Schizophyllum commune, Bipolaris hawaiiensis, Cladosporium, Rhizopus species and Candida). Serum precipitin for these fungal agents was done for all patients. Those found positive on SPT or those with serum precipitin positive for a specific fungus were further investigated for specific IgE. Total IgE, Absolute eosinophil count (AEC) and X-ray Chest PA view were done for all the patients. CECT/HRCT chest was done wherever indicated.

Pregnant ladies, patients who were on systemic steroids any time during last 2 weeks, patients who were on leukotriene inhibitors or antihistaminics any time during last 6 weeks were excluded from the study. Patients with pulmonary tuberculosis were also excluded.

Approval was obtained from the ethics committee of our institute and written informed consent was taken from all patients.

The skin prick test was performed in accordance with guidelines (6). A reaction was graded as a negative (equal to negative control), 1 + (<3mm), 2+(3-5mm), 3+ (5-7mm), 4+ (7-9mm). A response equal to or greater than that of positive control was considered significantly positive for that antigen.

Specific IgE in the patie55nts serum was determined by Enzyme Linked Immunosorbant Assay (ELISA). Serum precipitins (specific IgG) were detected using 'Ouchterlony's immunodiffusion. Serum total IgE was measured by the Calbiotech IgE ELISA Kit according to manufacturer's instruction.

Spirometry was performed using SpiroPro v2.0 spirometry machine. Readings were taken before and 15 min after the inhalation of salbutamol and was interpreted in accordance with the American Thoracic Society guidelines.

Those patients who fulfilled the modified Patterson-Greenberger criteria were labelled as ABPM (4).

Results

SPT was done for 12 fungal antigen in 102 patients in which 15.7% (16 out of 102) patients showed positive type I hypersensitivity reaction to at least one fungal antigen. Serum precipitins were positive in 4 patients. Six (5.8%) patients fulfilled the modified Patterson Greenberger criteria (4) and were diagnosed ABPM. One of our patients fulfilled 7 criteria, one had 6 while the other four fulfilled 5 criteria. All patients had Bronchial Asthma and fulfilled at least 5 criteria for diagnosing ABPM as suggested in a recent review by Gupta et al (7). Details of diagnostic criteria for 18 patients with Skin prick test and/or serum precipitin positive are given in **Table I**. Clinical characteristics and asthma control is given in **Table II**.

In 16 patients, a total of 35 positive skin reactions were observed, of which 11 were positive for A flavus and 6 were positive for A fumigatus, 4 were positive for A niger and A terreus respectively and 2 were positive for Schizophyllum commune, Rhizopus spp, Candida spp, Cladosporium spp, each. Only 1 SPT was positive for Curvularia spp and Bipolaris hawaiiensis. Correlation of skin prick test positivity with specific IgE is as per **Table III**.

In 4 patients Serum precipitins were positive in seven instances. One patient had serum precipitin positive for all four aspergillus species. The same patient had three SPT positive for Aspergillus spp. and specific IgE was positive for Aspergillus fumigatus only. One patient each had serum precipitin positive for Curvularia lunata, Scedosporium apiospermum and Schizophyllum commune. Only 7 out of 102 patients had radiological abnormality. Out of these 7 only 3 fulfilled ABPM criteria. One patient had central bronchiectasis with mucus plug. In other 2 cases one had fibrosis in left lung and other had paraseptal emphysema and fibrosis. Total IgE was >417in 39.2% (40 out of 102) and </=417 in 60.8 %(62 out of 102) patients. Specfic IgE was done in eighteen patients who had SPT and/or Serum precipitin positive. It was positive in 9 (50%) and negative in 9 (50%). In our study AEC was most sensitive test. It had sensitivity of 100%. Serum precipitin was most specific test. It had specificity of 98.8% SPT was single most sensitive and specific (83 and 89%) test for diagnosing ABPM. SPT followed by specific IgE was most effective strategy (83% sensitivity and 97% specificity) for diagnosing ABPM (**Table IV**).

Discussion

This study was done to see the occurrence of ABPM in cases of bronchial asthma irrespective of the severity of asthma. In our study we found prevalence of ABPM in BA was 5.8%. This was

Table I - *Detailed diagnostic profile of asthmatic patients with serum precipitin and/or SPT positive.*

S No	Serum Precipitins	Skin Prick Test	AEC	Total IgE	Specific IgE	CT Chest	Fulfilling ABPM criteria
1.	A f, An, Afl, At	Af, Afl, At	900	6128	A f	CB	Yes (Af)
2.	Schizo	Schizo, Cand	12000	6400	Schizo	ORF	Yes (Schizo.)
3.	Scedo	-	930	359	-	-	No
4.	Curvularia	-	420	836	Curv.	-	Yes (Curv.)
5.		An, At, Cand	789	1675	-	-	No
6.		A fl	483	1258	-	-	No
7.		Af	760	1125	-	-	No
8.		Af, An,Afl	1024	1014	An	-	Yes (An)
9.		Af, An, Afl, At	828	1256	Af, An, At	ORF	Yes (Af, An, At)
10.		Rhizo	868	1631	-	-	No
11.		An, Afl, Clado	560	75	Afl	-	No
12.		Afl, Clado	1200	2480	-	-	No
13.		Af,	16	740	-	-	No
14.		Afl	800	42	Afl	-	No
15.		Afl	1716	191	-	-	No
16.		Afl, Bipolaaris, schizo	360	44	Bipolaris, Schizo	-	No
17.		Afl, At, Curvularia, Rhizo	480	864	At, Curv.	-	Yes (At,Curv.)
18.		Af, Afl	790	1272	-	-	No

Abbreviations- AEC (absolute eosinophil count), Af (aspergillus fumigates), An (aspergillus niger), Afl(aspergillus flavus), At (aspergillus terreus), Schizo(Schizophyllum communae), cand(candida), Scedo (Scedosporium apiospermum), Curv. (Curvularia lunata), Rhizo (rhizopus oryzae), Clado (cladosporium spp.), CB (central Bronchiectasis), ORF (other radiological features)

more than a study done in Saudi Arabia which showed period prevalence close to 3% for ABPM (8). In this study all patients were screened for ABPM with skin prick test (SPT) using a panel of fungal antigens. Panel included Aspergillus fumigatus, A. niger, A.versicolor, A. clavatus, A. repens, Alternaria, Cladosporium, Rhizopus, Penicillium, Mucor, Trichophyton,Candida, Herbarum, Phoma, Fusarium. A study done in Ireland over four years showed period prevalence of ABPM was a little above one percent (9). In this study patients were checked for only A fumigatus and candida species. A study similar to our study was done in a tertiary care hospital of Kolkata. Antigens for the following fungi namely Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Alterneria alternata, Cladosporum herbarum, Curvularia lunata, Penicillium sp., Fusirium solari, Rhizopus nigricans, Candida albicans, Phoma tropicallis were used in this study. They found prevalence of ABPM to be 7.9 % which is similar to our study but more than the studies done outside India (10). However, they found that in their 10 cases of ABPM, 9 cases were of ABPA and only1 case was of ABPM because of fungi other than Aspergillus (Penicillium spp). In our study out of 6 cases 2 cases were of ABPM due to fungi other than Aspergillus and one patient fulfilled criteria both for Aspergillus and Curvularia while 3 patients had ABPA. Out of these one patient had SPT and specific IgE positive for three aspergillus species namely, A. Niger, A. terreus and A. fumigatus. Two other cases had SPT and specific IgE positive for A.fumigatus and A. niger respectively. Two cases that had ABPM because of fungi other than Aspergillus, the fungi implicated were Schizophyllum and Curvularia lunata respectively. There was a subset of cases that had low total IgE but SPT and specific IgE were positive for fungal antigens. It was either because the disease was not in active stage at the time of study or they were cases of fungal sensitization only.

One case each of Schizophyllum and Aspergillus fumigatus fulfilled all the seven criteria of diagnosis and both AEC and total IgE were very high. Similarly other cases also fulfilled 5 criteria

Table II *- Comparison of Clinical characteristics and control in ABPM and Non ABPM group.*

Category	ABPM(6)	Non ABPM(96)
Day time symptoms		
None	3	56
Once a month	2	20
Once a week	0	4
Twice a week	1	7
>Twice a week	0	9
Night time awakening		
None	3	60
Once a month	1	10
Once a week	1	10
Twice a week	1	10
>Twice a week	0	6
Previous hospitalization		
None	4	91
1-2	2	3
2-5	0	1
>5	0	1
Acute episodes		
None	3	79
1-2	2	9
2-5	1	7
>5	0	1
Fev1		
>80	1	43
60-79	5	46
50-59	0	5
<50	0	2
Control		
Controlled	3	56
Partially controlled	2	23
Uncontrolled	1	17

Table III - *Correlation between skin prick test and specific IgE positivity.*

Fungal antigen	Skin Prick Test positivity	Specific IgE positivity
A flavus	11	2
A fumigates	6	2
A terreus	4	2
A niger	4	2
Schizophyllum	2	2
Curvularia	1	1
Bipolaris	1	1
Rhizopus	2	0
Candida	2	0
Cladosporium	2	0

Table IV *- Sensitivity and specificity of Different Criteria for diagnosing ABPM.*

Criteria	Sensitivity	Specificity
Absolute eosinophil count>350	100%	45%
Total IgE >417	100%	65%
Skin Prick Test	83%	89%
Serum Preciptin	50%	99%
SPT/Serum Preciptin followed by Specfic IgE	100%	97%
SPT followed by Specfic IgE	83%	97%

at least4. These results are similar to study by Ishiguro et al (11) who showed that presence of 6 or more than 6 diagnostic criteria had a sensitivity of 97.6% and specificity of 98.3%. However if only more than 6 criteria were taken as diagnostic criteria the sensitivity dropped to 57%. However Ishiguro et al included patients without asthma in their analysis as 33.3 % of their patient did not have asthma. Analysing for ABPA in asthmatic patients only they found that 96.5% of patients showed positive result for specific IgE or SPT. As discussed in a review by Gupta et al, we considered patients with at least 5 criteria positive for the diagnosis of ABPM(7).

In our study we came across one patient with ABPA because of 3 Aspergillus fungal antigens, and one case of ABPM because of Aspergillus and a non-aspergillus fungi. Our study suggests that ABPM may be caused in an individual because of multiple antigens. In our literature review we came across only few cases of ABPM where more than one fungal antigen was implicated. Multiple causative fungi have been seen in a retrospective study by Ishiguro et al (11). In their study one patient had multiple Aspergillus species as cause and another patient had ABPM because of A.fumigatus and Schizophyllum commune. Whether the

identification of multiple agents in our study could have been caused by cross-reaction among antigens or other similar factors, is a subject of further investigation and needs a larger study.

Chaudhary et al (1) have done review of globally reported cases of ABPM and they found India accounted for 47% of globally reported cases which is much higher than any country in the world, although number of fungi tested in the previous studies were limited to few. In our study we have investigated for fungi like Schizophyllum, Curvularia and Bipolaris also and we found cases of ABPM or fungal sensitization because of these fungi. In previous studies these fungi have not been evaluated. This could account for the high prevalence of ABPM in our study compared to other studies done outside and similar to study done in India (10).

In our study, only 2 out of 102 patients had FEV1 less than 50%. Eighteen patients had uncontrolled asthma, 1 in ABPM group and 17 in non-ABPM group. The relation between ABPM and severity or control of asthma would require a larger number of subjects with ABPM.

The diagnostic criteria for allergic fungal diseases are evolving (12). However, since the focus of our study was ABPM because of fungi other than Aspergillus, we used the standard criteria established for ABPA.

Conclusion

Fungi other than Aspergillus such as schizophyllum, bipolaris and curvularia, can be implicated in ABPM. Multiple fungal agents may be responsible for ABPM in an individual. There is a subset of patients of BA who have fungal sensitization but do not fulfil the criteria for ABPM. SPT was the single most sensitive and specific test, AEC >350 and total IgE more than 417 IU were most sensitive tests and SPT followed by specific IgE was most effective strategy for diagnosing ABPM.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Lipid transfer protein sensitization in an apple-allergic patient: a case report from Northern Europe

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Summary
We describe a case of a woman who developed three separate episodes of urticaria and anaphylaxis during exercise after consuming an apple, with immunological evidence that nonspecific lipid transfer proteins (LTP) may have been responsible for these reactions. LTP sensitivity can cause life-threatening allergies and anaphylaxis. LTP sensitization is common in Mediterranean countries. The knowledge is growing with the frequency of diagnoses in Northern Europe. Despite the geographic differences, LTP allergy should be kept on sight when facing severe anaphylaxis after consuming LTP-containing food.

Introduction

Nonspecific lipid transfer proteins (LTPs) are common in plants, widely distributed throughout different species, and they are one major cause of food allergy, especially in the Mediterranean region (1). The most clinically important LTP in this region is found especially in peach (Pru p 3) (2). Outside of Mediterranean areas, Pru p 3 associated with mugworth allergy was reported as a major allergen (3). Although reports on LTPs as the causative agents for food allergy in Northern and Eastern Europe are rare, observations in clinical practice to this regard are increasing in number. In these areas, predominantly birch pollen (Bet v 1) homologous allergens induced mild oropharyngeal reactions and individual cases of LTP sensitization have been described, for example, hazelnut (Cor a 8), apple (Mal d 3), peanut (Ara h 9), wheat (Tri a 14), cherry (Pru av 3), kiwi (Act d 10) and celery (Api g 6) (4). However, a very rare case of anaphylactic reaction to dragon fruit LTP was reported in recent years from Northern Europe (5).

Nonspecific LTPs are small and basic proteins with four disulfide bonds found in plants, (pollen and plant-derived foods), and some fungi. The disulfide bonds play a protective role and provide resistance to heat and digestion in the gastrointestinal tract (6). Therefore, they often induce primary gastrointestinal sensitization.

Exercise-induced anaphylaxis is a potentially fatal disease in which an immunological condition (immediate type allergy) is triggered by mild to heavy exercise. When food is identified as the causative source of allergen, the respective clinical condition is referred to as food-dependent exercise-induced anaphylaxis (FDEIA) (7). The pathomechanism of FDEIA is currently not fully understood. One interesting theory suggests that changes in mucosal permeability induced by cofactors such as non-steroidal anti-inflammatory drugs, alcohol consumption, exercise, or a combination thereof can enhance allergen absorption via the mucous membranes, resulting in increased exposure of the mast cells to allergens (8). Other mechanisms proposed to explain this syndrome include increased skeletal muscle and

splanchnic blood flow and increased gastrin-induced mediator release in the postprandial phase (9)**.**

We describe a case of a woman who developed three separate episodes of urticaria and anaphylaxis during exercise after consuming an apple, with immunological evidence that nonspecific lipid transfer proteins (LTP) may have been responsible for these reactions.

Case report

A 40-year-old woman presented to the emergency department with generalized urticaria, pruritus, sweating, and facial angioedema of the lips and tongue. She had no significant past medical history (except depression) or allergies, and was taking only venlafaxine as a regular antidepressant medication for 6 years. She presented to the emergency department a second time after developing head tingling accompanied by dizziness, swelling of the face, and sweating while jogging in the forest. The previous evening, the patient had drunken beer and eaten goulash with beef. The next morning, she had eaten an apple and buttermilk, and started exercise. The symptoms developed approximately 120 minutes after consuming the apple during exercise. Finally, she presented to the emergency department a third time after developing generalized urticaria and mild angioedema: The patient had gotten up, eaten breakfast (dark bread with cheese) and apple. After that, she went running and developed an allergic reaction.

Diagnostic allergy testing: serum total immunoglobulin E (IgE) was normal (67.1 kU/L). Skin prick testing revealed sensitization to all components of apple with a reaction diameter of 9 mm. She was also sensitized to walnut (5 mm), celery (4 mm), anise (4 mm), kiwi fruit (4 mm) and chamomile (4 mm), but did not have symptoms of allergic rhinitis or oral allergy syndrome in her history. The positive control (histamine) was 7 mm. While she was waiting in the outpatient clinic, the patient had eaten an apple in a resting state before the prick test was performed, because she did not at all assume apple to be the causative, and this accidental "open food challenge test" was tolerated well without exercise.

Investigation of specific IgE-antibodies to allergen sources and single allergens using ImmunoCAP (Immuno Solid-phase Allergen Chip; Phadia, Uppsala, Sweden) revealed a moderate sensitization to nonspecific LTPs from apple (Mal d 3) and peach (Pru p 3) as well as a low sensitization to peanut (Ara h 9), hazelnut (Cor a 8), and wheat (Tri a 14). She was not sensitized to birch (Bet v 1), or any of the storage proteins, profilins, or PR-10 proteins included as potentially causative allergens for severe allergic reactions. In addition, we searched and found no sensitization to Gal-alpha-1.3-Gal Thyroglobulin. The positive and negative results are shown in **table I**. The provocation under exercise was not performed due to high risk of anaphylaxis. The synopsis of the patient's history, in vivo- and in vitro-tests led to the diagnosis of a FDEIA to apple due to the LTP sensitization. The patient was advised to avoid the consumption of fruits of the *Rosaceae* family (peach, apple, apricot, plum, cherry, and pear). We also recommended to especially observe the consumption of food in connection with physical exertion and alcohol consumption, as well as the intake of non-steroidal anti-inflammatory drugs. An adrenaline auto-injector, oral cetirizine, and prednisolone were prescribed, and the patient was provided with an anaphylaxis action plan. Since she was avoiding the consumption of apples, there was no re-presentation to the emergency department.

Comparison of the protein sequences of LTP from apple (Malus domesticus, Mal d 3) with the sequences of LTPs from other food allergen sources

We used www.allergen.org and the NCBI Database to compare the protein sequences in the identified allergens. The protein sequence of apple LTP (Mal d 3) showed 80.22% to 86.81% similarity with LTPs from other *Rosaceae* fruits. The protein sequences of nut LTPs showed only 61.54% to 68.13% similarity

Table I *- In vitro allergy diagnostic test: specific IgE-antibody detection results (ImmunoCAP).*

allergen source	allergen component	IgE-concentration (kU/l)	RAST-class
apple	apple extract	2.80	2
apple (*Malus domesticus*) (NsLTP)	rMal d 3	13.10	3
peach (*Prunus persica*) (NsLTP)	rPru p 3	6.25	3
peanut (*Arachis hypogaea*) (NsLTP)	rAra h 9	0.67	1
hazelnut (*Corylis avellana*)(NsLTP)	rCor a 8	0.53	1
wheat (*Triticum aestivum*) (NsLTP)	rTri a 14	0.36	1

Negative results to the following allergen components: Bet v 1 (the major birch pollen allergen); Gal-alpha-1.3-Gal thyroglobulin (red meat allergen); rAra h 1, rAra h 2, rAra h 3 (the storage proteins of peanut); rTri a 19 (wheat allergen); Cor a 9 (the storage protein of hazelnut); nGly m 5 (the storage proteins of soybean); Api g 1 (PR-10 protein of celery); alpha lactalbumin, beta lactoglobulin and casein (milk); rye; sesame scrap; rice, mustard.

with Mal d 3. The longest peptide in the protein sequences of the fruits that was similar between several fruit LTPs was between 20 and 31 amino acids (GGAVPPACCNGI). We consider that this protein segment may play an important role in the cross-reactions of fruits (**table II**).

Discussion

LTP sensitization with FDEIA is a rare disorder in which urticaria or anaphylaxis occurs during or after exercise and consumption of foods (mostly *Rosaceae* fruits). The symptoms may include erythema, rash, itching, dyspnea, nausea, flushing, diarrhea, and abdominal cramps. The symptoms may vary from mild to severe life-threatening anaphylactic reactions if the physical activity continues, including facial angioedema, laryngeal edema, sudden hypotension, and, as a result, cardiovascular collapse. Discontinuation of physical activity usually causes rapid improvement of the symptoms. Further external triggers include alcohol consumption, hot or cold temperatures, drugs (e.g., non-steroidal anti-inflammatory drugs such as aspirin), humidity, seasonal changes, lack of sleep, familial background, psychological stress, and certain phases of the menstrual cycle (10,11). The prognosis and long-term follow-up of FDEIA have not been well described.

In our case, apple-dependent exercise-induced anaphylaxis was demonstrated. Our patient showed IgE-mediated moderate sensitization to apple and peach, and low sensitization to peanut, hazelnut, and wheat non-specific LTPs; however, there was no clinical relevance or history of allergic reactions except to apple. The observed apple-dependent, non-specific LTP-mediated, immediate-hypersensitivity-type reaction would be subthreshold at rest; however, because of the influence of exercise on mast cell releasability, it became clinically overt. We consider that, in our case, the established cofactors (exercise and alcohol) played an important role in the development of urticaria and anaphylaxis. However, environmental factors such as cold temperature and other non-immunologic factors may have also contributed to the increased mediator release.

Table II - *Results of a sequence alignment of LTP sequences from different sources.*

LTP	0-10	11-20	21-30[1]	31-40	41-50
apple (Mal d 3)	ITCGQVTSSL	APCIGYVRS**G**	**GAVPPACCNG**	**I**RTINGLART	TADRQTACNC
apricot (Pru ar 3)	ITCGQVSSSL	APCIGYVRG**G**	**GAVPPACCNG**	**I**RNVNNLART	TPDRRTACNC
pear (Pyr c 3)	ITCSQVSANL	APCINYVRS**G**	**GAVPPACCNG**	**I**KTINGLAKT	TPDRQAACNC
plum (Pru d 3)	ITCGQVSSNL	APCINYVKG**G**	**GAVPPACCNG**	**I**RNVNNLART	TADRRAACNC
cherry (Pru av 3)	LTCGQVSSNL	APCIAYVRG**G**	**GAVPPACCNG**	**I**RNINNLAKT	TADRQTACNC
peach (Pru p 3)	ITCGQVSSAL	APCIPYVRG**G**	**GAVPPACCNG**	**I**RNVNNLART	TPDRQAACNC
peanut (Ara h 9)	ISCGQVNSAL	APCIPFLTKG	GAPPPACCSG	VRGLLGALRT	TADRQAACNC
walnut (Jug r 3)	ITCGQVASSV	GSCIGYLRGT	VPTVPPSCCN	GVKSLNKAAA	TTADRQAACE
hazelnut (Cor a 8)	LTCPQIKGNL	TPCVLYLKNG	GVLPPSCCKG	VRAVNDASRT	TSDRQSACNC

LTP	51-60	61-70	71-80	81-91	% identity
apple (Mal d 3)	LKNLAGSISG	VNPNNAAGLP	GKCGVNVPYK	ISTSTNCATVK	100
apricot (Pru ar 3)	LKQLSGSISG	VNPNNAAALP	GKCGVNIPYK	ISASTNCATVK	86.81
pear (Pyr c 3)	LKNLAGSVSG	VNPGNAESLP	GKCGVNVPYK	ISTSTNCATVK	85.71
plum (Pru d 3)	LKQLSGSIPG	VNPNNAAALP	GKCGVNVPYK	ISASTNCATVK	83.52
cherry (Pru av 3)	LKQLSASVPG	VNANNAAALP	GKCGVNVPYK	ISPSTNCATVK	82.42
peach (Pru p 3)	LKQLSASVPG	VNPNNAAALP	GKCGVHIPYK	ISASTNCATVK	80.22
peanut (Ara h 9)	LKAAAGSLRG	LNQGNAAALP	GRCGVSIPYK	ISTSTNCATIKK	68.13
walnut (Jug r 3)	CLKKTSGSIP	GLNPGLAAGLP	GKCGVSVPYK	ISTSTNCKAVK	68.13
hazelnut (Cor a 8)	LKDTAKGIAG	LNPNLAAGLP	GKCGVNIPYK	ISPSTNCNNVK	61.54

[1]The longest peptide in the protein sequences of the fruits that was similar between several fruit LTPs was between 20 and 31 amino acids (GGAVPPACCNGI).

In a study published by Pascal and colleagues in 2012, no correlation was found between LTP-specific IgE levels and the severity of an allergic reaction. In their research, the main suspected foods reported by LTP allergic patients were peach, lettuce, walnut, hazelnut, peanut, and green beans. In 40% of patients, cofactors were necessary to induce symptoms (2). The co-factors for our patient were alcohol and exercise. In another study conducted by Asero et al. in 2014, the higher level of IgE to peach LTP (Pru p 3) was found to be associated with the cross-reactions of other plant-derived LTPs (12). In our patient, a cross-reaction was observed with other food LTPs, such as peach, hazelnut, peanut and wheat, but it was clinically insignificant. Therefore, it was thought that cross-sensitization did not fully reflect the clinical condition, but can be helpful to determine a diagnosis. In addition, similar sequential epitopes of LTPs may play an important role to the cross-sensitization.

Moreover, some cases in the literature developed allergic symptoms in the following period only by intake of foods containing heated apple, without exercise (13). In some countries such as Spain, sublingual immunotherapies are currently available for severely allergic patients, with the aim to increase the provocation threshold (6). A large number of foods have already partially been described on a molecular level, defining major allergens and the respective protein families, and the list is still growing. Although, the reason for the observed geographical distribution and differences in LTP sensitivity is not fully understood, the nutrition habits, a genetic predisposition, and differences of pollen exposure may play an important role (14).

Conclusion

LTP sensitivity can cause life-threatening allergies and anaphylaxis. Although LTP allergy is common mainly in Mediterranean countries, the number of cases is increasing in Northern Europe. Despite the geographic differences, LTP allergy should be considered when facing severe anaphylaxis after consuming LTP-containing food.

Conflict of Interest

The authors declare that they have no conflict of interest.

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LTP Allergy: a pragmatic and reasonable approach in clinical practice

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To the Editor

We read with great interest the article by Asero and colleagues concerning LTP allergy (1). We have to thank these authors for investigating a topic that is very intriguing and clinically relevant in our Country (2). The proposed pragmatic approach, based on encouraging LTP-sensitized patients to go on eating all foods containing LTP that they had tolerated until the first visit, is, in our opinion, very reasonable and represents a correct management of these patients. Consistently, we fully agree with the authors about the concept that going on eating well tolerated foods is a correct practice from an immunological point of view, particularly in children. Indeed, as suggested by Asero and colleagues, to continue eating the tolerated food constitutes a physiological sort of "natural, attenuated oral immunotherapy" (1). On the contrary, avoiding a tolerated food could promote true clinical allergy onset because of impaired immunological tolerance as a consequence of failed allergen exposure.

However, another crucial question is how to behave with subjects showing mere sensitization to LTP molecules. This eventuality happens more and more in allergy clinics, especially when using molecular allergy diagnostics. Actually, the most frequent mistake, made also by some allergist, is to confuse mere sensitization with true allergy. The consequence is the prescription of restriction diets that are useless or even dangerous as defined above. Another regrettable approach is to *a priori* recommend strict avoidance of all LTP-containing foods in the belief of the risk of potential anaphylaxis. One further common (mal)practice is the prescription of self-administered epinephrine even in the presence of a mere sensitization as a consequence of defensive medicine.

We are deeply convinced that allergy approach towards LTP-sensitized patients should be based on a rational and logical attitude based on immunological knowledge. In other words, LTP-sensitized subjects (such as without clinical reaction) could ingest all tolerated foods, at least until evident symptoms appears. A correct medical approach should aim at improving patient's "engagement" on his real clinical condition to distinguish between tolerance and symptoms. Information and education should always be part of the medical visit.

Another interesting issue is the protective role exerted by co-sensitization to some pan-allergenic molecules, i.e. PR-10 or

profilins. In this regard, local geographic factors may have a relevant impact on the sensitization profile and the natural history of food allergy onset. Actually, the Genoa area model may be paradigmatic. Even though Genoa is placed in a birch-free geographic area, *Betulaceae* pollens sensitization (mainly to hazelnut tree and hornbeam [*Ostrya carpinifolia*]) is very common and its prevalence is increasing (3). Consequently, co-sensitization to Bet v 1 and LTP molecules is quite common (4). Notably, it has been recently reported that a group of adult patients with allergic rhinitis to Parietaria allergy (i.e. an LTP-molecule) did not report any severe adverse reactions to LTP-containing foods (5). Interestingly, 44% of these patients were co-sensitized to PR-10 allergen molecules and 16% to profilin ones. However, age may be another critical factor involved in the progression from sensitization to allergy. In this regard, we recently reported that severe LTP allergy may occur in children with allergic rhinitis due to Parietaria pollen and with Pru p 3 sensitization (manuscript submitted). On the other hand, a large quote of Pru p 3-sensitized children had no clinical allergy to LTP molecules. It is well known that the age has a relevant impact on IgE production, both concerning pollen and food allergens (6,7). A final point should be considered: the level of serum allergen-specific IgE; it is usually considered a valuable biomarker for defining allergy diagnosis (8), symptom severity (9), and responsiveness prediction to allergen-specific immunotherapy (10). Unfortunately, as reported by the authors themselves and anecdotally, the serum level of IgE to LTP molecules does not predict the risk of the evolution toward clinical reaction to LTP foods (1).

In conclusion, we believe, in agreement with Asero and colleagues, that LTP sensitization and allergy should be correctly managed on an individual basis both in adult and paediatric patients to improve wellness and quality of life. Moreover, some variables should be carefully addressed, including age, area of residence, co-sensitization, co-morbidity, and sports practice after eating.

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Pediatric food allergy prevention, "Much ado about nothing"?

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Keywords
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To the editor

In the early eighties, when I was a young paediatrician, I usually spent my afternoons with Dr. Alvaro Cimaglia who was, at the time, the head of a paediatric hospital, in Naples.

Dr Cimaglia was a clever and serious paediatrician who loved his job and working with children. He taught me numerous practical aspects of paediatrics, including the introduction of solid food in the infancy. Thanks to his lessons, I started baby weaning at the beginning of the fifth month of life. Wheat, rice, vegetables soup, parmesan cheese, olive oil, apple, pear, were the first foods to be introduced in the diet. In the next days, lamb, calf, chicken, turkey followed. At the beginning of the sixth month, fish (sole or cod) and boiled egg yolk were added. At ten months of age, the egg white was finally introduced. This was Dr Cimaglia's (and mine) way of weaning our patients. This was equally applied for both normal babies and at-atopic-risk babies (i.e. those with a family history of atopy): same foods, same timing.

Since the late nineties it seemed reasonable to delay the introduction of many foods to the second year of life to try and reduce food allergy in the infancy (1). This strategy was believed to reduce the risk of food sensitizations in children. Dr Cimaglia, who was eighty years-old, had a medical weekly newspaper translated into Italian. In these occasions, we often discussed about the new paediatric food allergy prevention trends. I introduced him to the new concepts, but he didn't change his mind and kept doing what he used to do. "Tolerance is a specific immunological strategy" he said. "How is it possible to tolerate an allergen without having met it?"

Things change. "New concepts" were being proposed by the UK, Israel and Australia: some studies suggested that a "window" period around the fifth month could have been the best choice for weaning (2), while others indicated that the early contact with peanuts could have a protective effect for the development of peanut allergy. Dr Cimaglia was still working hard everyday, until late, in his office, filled with newborns. Even if he was getting older, he could not stop working. I met him at a paediatric allergy congress in Naples, when he asked me if he had to modify his behaviours, at the light of the new studies. I reassured him he was right, as he had always been.

These last years have witnessed many important trials published on this topic: the GINI study (3), the LEAP study (4) and the EAT study (5). The questions addressed were i)

whether a partial or extensive hydrolyzed cow milk formula could do better than a normal formula in cow milk allergy prevention, and ii) whether the early introduction of peanuts in at-risk infants or several foods in a normal population may, at least partially, prevent the appearance of food allergies.

Bottom line, the answer to these questions is *No*. Methodologists and evidence-based medicine experts may find the specific reasons why significant results have not been reached. The only appreciable result is that early peanut introduction may have a positive impact in highly selected infants with a very severe atopic status (not many indeed, in real life) for what concerns the risk of peanut allergy. Even if this result may be considered extremely relevant for United States paediatricians in their clinical practice, it is much less so in countries where peanuts are not likely to be administered in the first years of life.

As of today, paediatricians may relax and act as they have always done: the right age to start solid food introduction in infants is the fifth month, no matter whether they are at risk of atopy or not.

Dr Cimaglia has unfortunately passed away few years ago. He would have probably asked: "How can you assume that thousands of infants, taken together just because they have an allergic parent, may represent a valuable population? Children are so different from each other! Wouldn't it be better to study a sample of few, well defined, well characterized infants?"

I have no answer. I don't know. We'll see. But I'm sure that Dr Cimaglia, wherever he is now, is smiling.

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Evaluating the management of anaphylaxis in emergency departments: a survey in two French regions

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To the editor

Anaphylaxis is defined by the EAACI as a severe, life-threatening generalised or systemic hypersensitivity, characterised by its rapid onset with life-threatening airway, breathing and/or circulatory problems (1). Epinephrine represents the treatment of choice in anaphylaxis, with intra-muscular injection being recommended upon reaching grade 2 of the Ring and Messmer severity scale (1-3). To assess both the diagnosis and management of anaphylaxis in emergency departments (EDs), we performed a study from November 2016 to May 2017 via an electronic survey. This two-region survey consisted of 19 questions (demographic data, knowledge and management of anaphylaxis, and management after acute episodes).

The questionnaire was returned by 18% (44) of physicians, fifty percent of whom were female physicians. Most respondents had less than 5 years' experience, and about one third with more than eleven years; 77.3% of respondents worked in general hospitals, 70.5% in ED, 59.1% in prehospital and 40.9% in an emergency call centre.

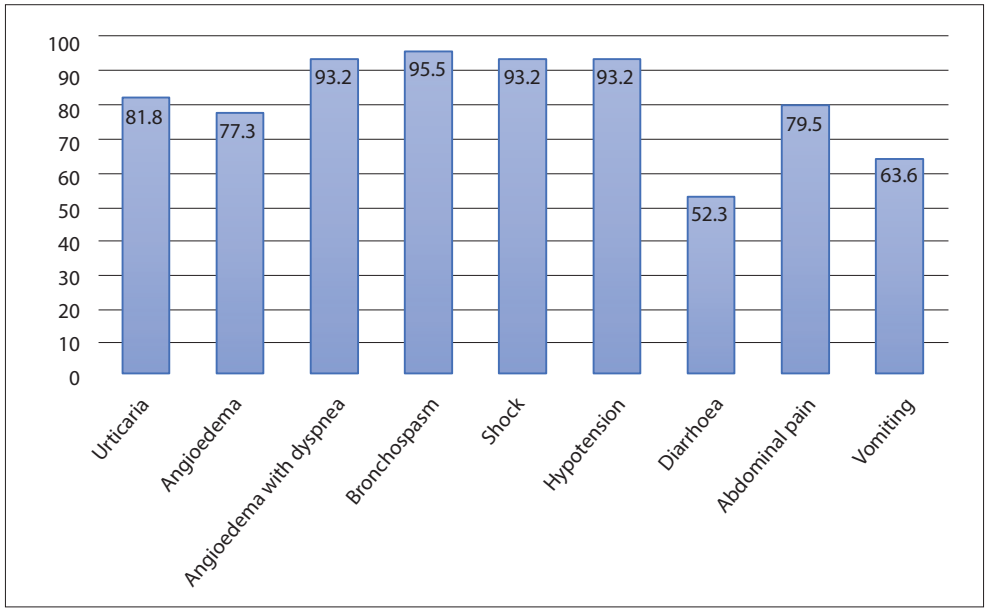
Among the respondents, 84.1% knew the recommendations from the French Society for Anaesthesia and Resuscitation (4). The European or American recommendations were less known (**figure 1**). A total of 47.7% (21) of physicians knew the classification of Ring and Messmer, while 15.9% of respondents were not knowledgeable in any classification.

The recognition of symptoms of anaphylaxis are summarised in **figure 1**. This recognition was overall good for both respiratory and cutaneous symptoms, whereas the rate of recognition was lower for abdominal symptoms and especially for diarrhoea and vomiting. A standard protocol was available for only 33.3% (14) of physicians.

When considering biological markers, measurement of tryptase and histamine rates was performed immediately in respectively 69.2% and 73.3% of patients, within the first hour in respectively 15.4% and 13.3%, and along similar proportions beyond the first hour. Tryptase measurement was performed more than once by 12.2% (5) of physicians.

Regarding first-line treatment, cutaneous-mucosal symptoms were treated by oral antihistamines by 95.3% of physicians,

Figure 1. *Percentage of clinical symptoms identified as anaphylaxis symptoms.*



and by intravenous antihistamines or corticosteroids in 2.3%. When symptoms were of grade 2 according to Ring and Messmer, intra-muscular epinephrine injection was administered by a mean 11.2% of physicians as first-line treatment, intravenous epinephrine injection by 8.4%, antihistamines (oral route or IV) by 16.4% and corticosteroids (oral route or IV) by 17.8%. For grade 3 symptoms, intramuscular epinephrine injection was performed by a mean 12.4% of physicians as first-line treatment, intravenous epinephrine injection by 64.3%, intravenous antihistamines by 6.2% and corticosteroids (oral or intravenous route) by 16.6%. First-line treatments for all grades are summarised in **figure 2**.

When considering management after an acute anaphylaxis episode, 2.3% of physicians provided for an allergy assessment, 86.4% never and 11.4% occasionally. For grade 2 reactions, 22.7% of physicians prescribed epinephrine self-injectors, and 52.2% for grade 3. Over 88% of physicians reported not providing discharge documents to the patients while 86.4% did not refer patients to an allergy specialist.

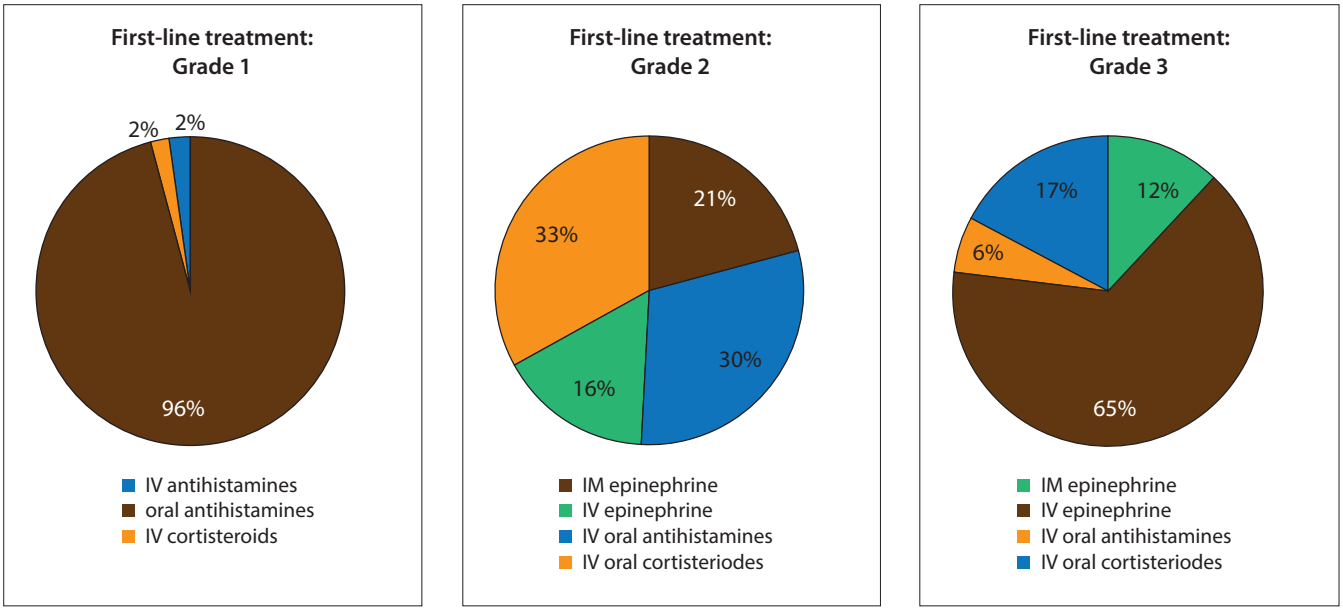
The recognition of symptoms was good in the present study even for abdominal symptoms. However, several studies have shown that at least 50% of anaphylaxis episodes are misdiagnosed in the ED when current guidelines are not used (5,6). This good recognition of symptoms contrasts with the fact that epinephrine was not given as first-line treatment and especially for grade 2 symptoms. Indeed, only 19.6% of physicians responded administering epinephrine as first-line treatment, regardless of route. This rate increased to 76.7% for grade 3 symptoms, although 6.2% of respondents answered using antihistamines as first-line treatment to counter these severe reactions. Another gap possibly reflecting the lack of standard protocols was that a standard protocol was only available for one-third of respondents. The lack of epinephrine use may also represent a low awareness of guideline recommendations or mistaken concerns regarding the efficacy and safety of intramuscular epinephrine (7,8), of which several studies show similar results (9).

A recent retrospective study conducted by Corriger et al. in EDs in Lorraine, France, revealed interesting albeit somewhat discordant results with those of the present study (10). In their study, epinephrine use was reported in 7.5% of grade 2 and 32.4% of grade 3 reactions. The use of epinephrine by 76.7% of physicians in patients with grade 3 observed in our study was very high compared to the above study conducted in the same French region, although the discrepancy may be linked to differences in data collection methods. In Corriger et al., the data was collected by analysing the medical records selected by their ICM-10 code, with no specific code for anaphylaxis (10).

Measurement of tryptase rates within the first hour was performed by about two-thirds of our respondents. Despite recommendations, only 12.2% of respondents repeated this measurement more than once. This is likely related to the fact that these measurements are not useful for either acute diagnosis or management.

With regard to discharge recommendations, both provisions of written information and prescription of an epinephrine self-in-

Figure 2. *First-line treatment according to the Ring and Messmer severity scale.*



jector appeared inconsistent. Furthermore, the referral to an allergy specialist was rarely done. This may be indicative of certain difficulties in the collaboration with allergy centres, in order to complete diagnosis and treatment indications for prevention in subjects at risk.

In the study of Corriger et al. (10), tryptase measurement was performed in only 12.7% of patients, their data being similar to ours. Furthermore, the above study con-firmed the lack of epinephrine self-injector device prescriptions (17.3%) and of referral for an allergy consultation (52.7%) as demonstrated herein.

In conclusion, to the best of our knowledge, this is the first survey to assess the concordance between the management of anaphylaxis in EDs and guideline recommendations in a French region. The outcomes confirm both the lack of epinephrine use in the ED as well as discharge indications.

As described in the literature, this poor use of epinephrine is likely the result of a lack of standard definition of anaphylaxis and of diagnostic difficulties.

The present data further underscore the need to strengthen collaboration between EDs and allergy centres. Lastly, this survey highlights the necessity for graduate and postgraduate courses on anaphylaxis in order to improve its management.

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