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Anaphylaxis (M Sanchez-Borges, Section Editor)

The Use of Molecular Allergy Diagnosis in Anaphylaxis: a Literature Review

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Key Points
- Molecular allergy diagnosis (MAD) seems to be, at present, the most powerful laboratory method to identify allergen sensitization at single component level.
- Despite MAD was introduced more than 10 years ago in allergy practice, its use in patients with anaphylaxis seems to be at present restricted to research more than to clinics.
- However, in many situations, such as in suspected hymenoptera, food or idiopathic anaphylaxis, MAD is probably the only suitable approach.

This article is part of the Topical Collection on *Anaphylaxis*

Keywords Anaphylaxis · Molecular allergy diagnosis · Allergen sources · Molecular components

Abstract

Purpose of the review Anaphylaxis can be caused mainly by drugs, foods, and hymenoptera or it can be idiopathic, when no cause is identified. The identification of the cause(s) of an anaphylactic reaction can be made by using both top-down (i.e., from patients' history to MAD) and bottom-up (from MAD screening to the patients' characterization) strategies.
Recent findings Independently from the strategy, the patients' history, skin prick test,

second, third, and fourth level in vitro serum or cellular assays, and in vivo challenge tests are mandatory. The diagnosis is based on the results of these tests used at best. Third level specific IgE assays, based on the use of molecular allergens, allow a very accurate description of the specific IgE profile of the patient, resulting in a significant support in the identification of the trigger of the anaphylactic reaction.
Summary In recent years, this third level has been empowered by the availability of allergen arrays that allow screening a large number of molecular allergens in a single test. In this paper, we analyze the recent scientific literature on this topic, showing that molecular allergy diagnosis does not seem to be yet a standard procedure in the identification of the cause of anaphylaxis.

Introduction

Anaphylactic reactions represent the most severe clinical picture within the spectrum of allergic reactions and imply a vital risk for the patient. Identification by health professionals is vital both for the immediate and for the long-term management. The diagnosis in the acute phase is only clinical, according to criteria adopted by all major allergy societies [1, 2]. There is no absolute confirmatory test, although a rise in total serum tryptase during the acute event, compared to basal values, supports the diagnosis [3, 4]. If basal values are increased, mast cell disorders should be ruled out. For long-term management, it is crucial to identify the cause of the reaction in order to implement adequate avoidance or immunomodulatory treatments, such as immunotherapy. In the context of a compatible clinical history, allergic sensitization to potential culprit agents has to be carefully considered. This can be assessed by skin tests or in vitro tests. The emergence of component-resolved diagnosis has enabled the identification of non-obvious allergens, relevant and irrelevant cross-reactivities, or provided information on future risks. Therefore, it should be considered an added value for the diagnostic armamentarium in anaphylaxis. An accurate description of the added values of MAD in anaphylaxis has been recently published [5] and for this reason, this review will describe the latest uses of MAD in patients with anaphylaxis.

Molecular allergy diagnosis and anaphylaxis

The identification of the allergens or the molecule responsible for an episode of anaphylaxis may represent a real challenge for the clinician. Indeed, even if in some cases the nature of the allergens is clear, in others, the group of suspected allergens can be very large. The identification of the cause of anaphylaxis is based the patients' history, SPT, and then the second and the third level of serum assays. In some instances, controlled challenges are necessary to rule out or confirm the causality of a specific culprit agent, always carefully assessing the risk/benefit, and provided they are performed according to approved protocols.

It should be considered that some of the allergen molecules maybe poorly represented or absent in allergen extracts used for in vivo and in vitro diagnosis. This is the reason why, at least in the past, both skin prick tests and specific IgE testing were sometimes unsuccessful or only partially indicative. Thus, when recombinant allergens were introduced in diagnostics, it was evident that a new era of allergology had started. This was immediately evident for inhalant, food, and hymenoptera allergy [6•], but it was soon realized that also anaphylaxis could significantly benefit from this novel approach [5, 7]. After more than 15 years, now these advances have been consolidated. Indeed, at present, hundreds of recombinant or natural highly purified components are available in single-plexed and in multiplexed diagnostic kits [6•].

The possibility to identify the real (specific, genuine) allergen in a mixture of other allergens belonging to an allergen extract has improved the diagnostic armamentarium of the allergist. So, for example, the peanut allergen (Arachis hypogea) is known to contain 17 components (October 2018), each one with its own characteristics [8]. Unfortunately, not all the known components are commercially available for the diagnosis but those that are present may be enough for a suitable description (at molecular level) of the peanut sensitization. Recent

Table 1. List of allergens and molecules

Type	Consideration	Allergens	Source	OAS	Urticaria	Gastroenteric	Anaphylaxis	Relevant component families	Representative components
Drug	Probably, MAD does not represent the most efficient approach in drug anaphylaxis.	Penicillins		No	Yes	Yes	Yes		Pholcodine, morphine, chlorhexidine, tetanus toxoid, suxamethonium, protamine, penicilloyl V and G, gelatin, chymopapain, cefaclor, ampicilloyl, amoxicilloyl, ACTH, insulin.
		Biological drugs		No	Yes	Yes	Yes		Only for research use
Contact/latex	MAD may represent an efficient approach	Latex		No	Yes	Yes	Yes		Hev b 1, Hev b 3, Hev b 6, Hev b 8, Hev b 11
Food	MAD may represent an efficient approach	PR-10	Fruits	yes	No	No	No		Mal d 1, Ara h 8, Cor a 1, Pru p 1, Act d 8, GLy m 4
		LTP	Fruits, seed	Yes	Yes	Yes	Yes		Cor a 8, Pru p 3, Mal d 3
		Cupins	Nuts, peanuts, hazelnut, walnuts, etc.	Yes	Yes	Yes	Yes	2S albumin	Ara h 2, Cor a 9, Cor a 14
								7S globulin	Ara h 1
								11S globulin	Ara h 3
		Profilins	Grasses, trees, weeds.						Cor a 2, Pru p 4, Act d 9, Tri a 12
		CCD	Various						Mux f 3, HuLTF, Ana c 2
		Parvalbumins	Fishes	Yes	Yes	Yes	Yes		Cyp c 1, Cod a 1
		Tropomyosin	Shrimp, snails, shellfish	Yes	Yes	Yes	Yes		Der p 10, Pen a 1, Ani s N
		Various	Milk	Yes	Yes	Yes	Yes		Bos d 4, Bos d 6, Bos d 8
			Egg	Yes	Yes	Yes	Yes		Gal d 1, Gal d2, Gald 3, Gal d 4, Gal d 5
			Soy	Yes	Yes	Yes	Yes		Gly m 4, Gly m 6
			Wheat	Yes	Yes	Yes	Yes		Tri a 12, Tri a 14, Tri a 19
			Meat	No	Yes	Yes	Yes		Alpha-Gal
Hymenoptera	MAD may represent a fundamental approach	Various		No	Yes	No	Yes		Api m 1, Api m 2, Api m 3, Api m 4, Ves v 1, Ves v 5, Pol d 5
Idiopathic	Extended MAD is the unique approach available	Various		No	Yes	Yes	Yes		All components (allergen arrays should be preferred)

Table 2. List of components available for the molecular allergy diagnosis

Common components available on the market (in alphabetic order, Oct 2018)
Act d 1, Act d 2, Act d 5, Aln g 1, Alt a 1, Amb a 1, Ana c 2, Ana o 3, Ani s 1, Ani s 3, Api g 1, Api m 1, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9, Art v 1, Art v 3, Asp f 3, Asp f 4, Asp f 6, Ber e 1, Bet v 1, Bet v 6, Bla g 1, Bla g 2, Bos d 4, Bos d 5, Bos d 6, Bos d 8, Can f 1, Can f 2, Can f 3, Che a 1, Cla h 8, Cor a 1.01, Cor a 1.04, Cor a 8, Cor a 9, Cor a 14, Cup a 1, Cyp c 1, Der f 1, Der f 2, Der p 1, Der p 10, Der p 2, Equ c 1, Fag e 2, Fel d 1, Fel d 2, Fel d 4, Gad c 1, Gal d 1, Gal d 2, Gal d 3, Gal d 4, Gal d 5, Gly m 4, Gly m 5, Gly m 6, Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 8, Hev b 11, Jug r 1, Jug r 2, Mal d 1, Ole e 1, Par j 2, Pen m 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p 12, Pla a 1, Pla l 1, Pol d 5, Pru p 3, Ses i 1, Ves v 5
Components only available in Thermofisher ImmunoCAP system
Act d 8, Alkalase, Alt a 6, Ana o 2, Api m 4, Asp f 1, Asp f 2, Asp o 21, avinase, Bet v 2, Bet v 4, Bla g 5, Bla g 7, Blo t 5, Bos d Lact, Can f 5, Cry j 1, Cyn d 1, Equ c 3, Gliadin, Jug r 3, alpha-Gal, Lep d 2, Mer a 1, Maxatase, Mus m 1, MUXF3, Ole e 7, Ole e 9, Pen m 2, Pen m 4, Phl p 4, Phl p 11, Pla a 2, Pla a 3, Pru p 1, Pru p 4, Sal k 1, Sus s, Sus s, Tri a 14, Tri a 19.01, Tri a aA_TI, Ves v 1
Components only available in the MADx ALEX
Act d 10, Alng4, Amb a 4, Api m 10, Apig2, Apig6, Apim2, Betv2, Blag4, Blag5, Bos d 2, Cor a 11, Dau c 1, Der p 10, Der p 11, Der p 23, Derp7, Fra e 1, Glyd2, Glym8, Hom s LF, Lolp1, Mac i 2S Albumin, Mala s 1, Mala s 11, Mala s 5, Mala s 6, Mala s 9, Mald2, Mald3, Musm1, Ole e 2, Pap s 2S Albumin, Per a 7, Phod2, Sin a 1, Sola l 6, Tri a Gliadin, Vitv1

precision medicine approaches [9] as well as the recent reconsideration of the different strategies of allergy diagnostics [10••] considered that not only the top-down diagnostic strategy can be used for the identification of the allergen causing anaphylaxis [11••] but also a bottom-up strategy, starting from the use of extended analysis of the IgE profile supported by the use of molecular allergens, can be used. In both contexts, an accurate description of the patients' IgE profile is needed.

From a practical point of view, as shown in Table 1, the list of allergens and molecules that can be associated with signs and/or symptoms of anaphylaxis can be used to decide whether a single (or a small group) of components can be tested in vitro. In this situation, a single-plexed approach (namely, the specific request of IgE assays to a well-defined list of allergens) seems to be the most reliable approach. For example, in a clinically clear picture of milk allergy, milk components such as nBos d 4—α-lactalbumin, nBos d 5—β-lactoglobulin, and Bos d 8—casein are assayed. Similarly, for egg allergy, Gal d 1—ovomucoid, Gal d 2—ovalbumin, Gal d 3—ovotransferrin, Gal d 4—lysozyme C, and Gal d 5—serum albumin can be assayed in a single-plexed manner.

Two main producers have the widest choice of components: Thermofisher with ImmunoCAP and/or ImmunoCAP ISAC (for a total of 131 different components) and MacroArray Diagnostics with ALEX (126 components). In Table 2, the components available in both systems are listed together with the component specific for each producer. It is evident that, in the presence of a more complex clinical picture (in particular, when an allergen is only suspected or an anaphylaxis occurred in the context of a pollen-food syndrome), it may be useful an approach based on the multiplexed technology.

Allergen arrays were first described in early 2000 [12] and, after more than 15 years of clinical use, their role is well established [6]. Advantages of the allergen arrays are mainly represented by the large number of whole extracts from allergen sources or allergen components that can be assayed in a single run and by the small amount of blood required. Disadvantages are represented by the cost, the absence of certain components in the array, the list of allergens that is not decided by the allergist, the complexity of the interpretation in some poly-sensitized patients, and the possibility of detecting sensitization to unexpected components [13]. The Thermofisher ImmunoCAP ISAC [14] is a 112 component microarray and the MADx ALEX is a *282* macro-array containing 156 extractive allergens and 126 recombinant or highly purified components [15]. Thus, for its content of molecular components alone, ISAC can be easily interpreted if data from skin prick test and/or specific IgE analysis are available. On the contrary, ALEX, containing both the extractive allergens and the relevant molecular components, could not require previous first or second level assays for its interpretation.

The aim of this work was to revise the literature on the use of MAD in the diagnosis of anaphylaxis.

Table 3. The most frequent Allergens described in the last 10 years in Medline

Allergens	2007–2018	2015–2018	Allergens	2007–2018	2015–2018
Peanut	305	98	Cereal	19	3
Drug	227	86	Pork	19	9
Nut	176	67	Anise	18	7
Milk	170	63	Apple	18	2
Wheat	158	46	Livetin	18	7
Egg	154	60	Alpha-gal	17	9
Ovalbumin	111	25	Aspirin	17	5
Cow	98	40	Casein	17	7
Fish	90	26	Hop	17	8
Gliadin	67	18	Yellow hornet	16	7
Seed	50	14	Banana	15	5
Shellfish	49	14	Celery	15	2
Honey	48	13	Corn	15	9
Meat	48	24	Beta-lactam	12	4
Peach	43	20	Kiwi	12	2
Cod	42	14	Lentil	12	0
Hazelnut	38	9	Parvalbumin	12	8
Rise	36	13	Pea	12	1
Shrimp	34	17	Spice	12	7
Legume	29	6	Bird	10	2
Crustacea	24	9	Grape	10	3
Pig	24	4	Jelly	10	5
Buckwheat	23	7	Orange	10	4
Cashew	23	7	Rice	10	2
Sesame	23	5	Rocuronium	10	3
Walnut	22	12	Tomato	10	1
Ovomucoid	21	11	Vespula	10	3

Methods

Medline databases were searched for the two keywords “allergy” and “anaphylaxis” in the range of years from 2007 to 2018. More than 7000 works were found. Of these, only papers published in English and discussing human (and not mouse) diseases were maintained. Thus, 2010 articles remained (498—corresponding to the 25%—were published in the years 2015 to 2018) and the study was focused on this selection. Then, by the use

of a data-mining procedure, a list of 681 relevant terms (Table 3) was identified in the abstracts and a Filemaker pro™-dedicated database was used to manage articles with the relevant contents.

Despite the very large number of publications on Molecular Allergy Diagnosis (MAD) in food, cutaneous, and respiratory allergy, the use of MAD in patients with anaphylaxis, at least on the basis of the articles published in Medline, it seems to be mainly relegated to few components and few centers. It is evident, for example, that not all anaphylactic reactions related to the administration of drug can be managed with MAD; on the contrary, anaphylaxis related to Hymenoptera or food can be. However, also in this field, MAD does not seem to be a standard procedure. In practice, only articles where MAD diagnosis was used are reported, while a larger number of articles on the diagnosis of anaphylaxis without MAD were not considered.

Anaphylaxis to drugs

Drug anaphylaxis articles represent 11% of anaphylaxis articles. It is evident that, despite the very large number of anaphylactic reactions due to drugs, the number of molecules to be assayed by using a specific IgE test is extremely small. Indeed, in October 2018, a list of 17 different drugs (mainly antibiotics and peptide hormones) is available in the Thermofisher catalog. But many other anaphylactic reactions due (or suspected to be related) to drugs have been identified. These included at least 48 different drugs in our search, containing antibiotics, NSAIDs, anesthetics, etc. Unfortunately, these drugs do not usually elicit an IgE response and, for this reason, they cannot be tested in vitro by means of specific IgE assays. These drugs can be assayed by using the basophil activation test that, in this situation, it seems much more powerful than specific IgE tests to identify the possible cause of the anaphylactic reaction [16].

Anaphylaxis to Hymenoptera

Venom anaphylaxis articles represent 11% of recently published articles on anaphylaxis. Indeed, hymenoptera are considered one of the main causes of anaphylaxis. For this reason, the number of articles on this topic is high (228 articles, of which 74 in the last 4 years). Molecular diagnostics is mentioned in several articles: indeed, this seems one of the fields of diagnosis of the cause of anaphylaxis where MAD is more frequently used. For example, for Vespula (Ves), 17 articles describe MAD, of which seven in the recent period, and Ves v 1 (Phospholipase A2) is mentioned in three [5, 17, 18] while Ves v 5 in four articles [5, 17, 18, 19•]. Polystes d. is described in nine articles (five recent) but Pol d 5 is discussed only in one recent article [5]. *Apis mellifera* allergy is the subject of 35 articles of which 12 are recent. In these, Api m 1 is studied in nine [5, 17–23], even if in certain cases, MAD was used to better define characteristics of immunotherapy [21] or to deepen into the natural history of the evolution of venoms [20]. The use of Api m2 is described in four articles [17, 20, 21, 23], that of Api m 4 in three [17, 18, 24] and that of Api m 10 in four [18, 19, 21, 23]. Along this

line, two articles stated that MAD may add value to the classic diagnostic strategies [18, 19•].
Some new components, that should be relevant in the identification of the IgE profile at least in certain countries, have been described: for example, the Pac c 3 from the ant *Pachycondyla chinensis* [25], the Sol g 4.1 from Solenopsis geminate [26], the characterization of venoms from other Asia-Pacific ants [27], and the proteomic analysis of allergens of *Myrmecia pilosula* [28].

Food anaphylaxis

At present, virtually, all foods can cause anaphylaxis in predisposed patients. The most common are related to seed, milk, and seafood [29]. Other allergens (meat, grains) have also been identified as sources of allergens causing anaphylaxis and they will be discussed if some kind of molecular allergy study was associated with them. Many allergens (such as Bet v 1/Mal d 1) are PR-10, proteins that are highly susceptible to heat, low-pH, and gut peptidases. So, these components—with certain exceptions—would never cause a systemic reaction due to their absorption in the gut. However, other heat-, low-pH, and peptidase-resistant components (such as cupins and nsLTPs) have the capability of causing a systemic reaction in sensitized patients.
Fruits were involved in severe reactions for their repertoire of molecular allergens with specific characteristics. So, the following fruits were associated with anaphylaxis in studies published during the last 10 years of Medline: apple, apricot, avocado, banana, berries, blackberry, blueberry, cherry, citrus, coconut, cucumber, kiwi, lemon, mandarin, mango, melon, nectarine, orange, peach, pear, pineapple, pomegranate, strawberry, watermelon. However, molecular components are not available for all (even if a great homology is evident) and for this reason, MAD results are currently restricted to few. So, in the last 4 years, MAD was used in few cases of anaphylaxis. In more detail, *Kiwifruit* was discussed in 12 (but only 2 recent) and MAD was never considered in case of anaphylaxis.
Apple allergens are considered mild antigens, even if Mal d 3 is a nsLTP. However, out of 19 articles in the last 10 years, only two were published more recently and molecular components were not considered.
Peach: 22 articles were published in the last 4 years. Only Pru p 3 (a nsLTP) was considered in 12 [5, 30–37]. Of note, Pru p 3 was studied in different situations, such as the identification of new allergens [34], its effects in the presence of certain drugs [35] and effects of age in developing a sensitization to nsLTP [31, 32]. Other components were not considered.
Grapes: Three recent papers on grapes were published but Vit v 1, the grape nsLTP, was never considered.

Alpha-gal syndrome

Allergy to red meat has been associated with a tick bite that, by injecting alpha-gal, sensitizes the patient. For its cross-reactivity with sugars of the red meat, patients may experience several different systemic allergic reactions

up to anaphylaxis after eating red meat. At present, sensitization to alpha-gal has been observed in many different countries of the world: indeed also the number of ticks involved seems to be increasing. But interestingly, as suggested [38•], alpha-gal immune response of different antibody isotypes could be relevant not only in certain idiopathic allergic reactions but also in situations like adverse reactions to heparin and bioprosthetic heart valves, atherosclerosis, inflammatory bowel disease, and seronegative Lyme disease.

Seeds are probably the most frequent cause of anaphylaxis in children and in adults. The number of seeds that caused anaphylaxis is wide (virtually all) and, in more detail, in the last 10 years, the following allergens were associated with an anaphylactic reaction: almond, peanuts, Brasilian nuts, cashew, chestnut, date palm, hazelnut, lupine, macadamia, peanut, pecan, pistachio, ricinus, sesame, sunflower, and walnut. The number of components available for identification of the IgE profile in patients with suspected seed anaphylaxis is large. In the last 4 years, the following molecular reagents were used.

Hazelnut is considered in 40 studies of anaphylaxis (11 more recent) and molecular diagnosis seems to be used more frequently than in other situations: indeed, Cor a 1 is described in five articles [32, 39–42, Cor a 8 in two [30, 40], Cor a 9 in five [22, 32, 40, 42], Cor a 14 in four [22, 32, 40, 42] but Cor a 11 is not used.

Peanut, another common source of anaphylaxis, is considered in 307 articles (of which 100 are recent). MAD tools are relevant: indeed, Ara h 1 is considered in ten articles [32, 43–48, 49•], Ara h 2 in 14 [22, 31, 32, 42, 45–47, 49–52], Ara h 3 in three [45, 47, 49•], Ara h 6 in four [45, 46, 49•, 53], Ara h 8 in six [32, 46, 47, 49•, 51], and Ara h 9 in six [30, 31, 46, 47, 49•]. For peanut, despite the large number of articles, conflicting results were published. For example, the role of MAD was not superior to classic diagnostic methods in a cohort of 72 children with suspected peanut allergy [49•]. On the contrary, Martinet et al. proposed an algorithm based virtually on the same Ara h components, suitable to improve the diagnosis and to reduce the number of oral food challenges [51].

Walnuts are less frequently related to anaphylaxis. Twenty-two articles on this topic, of which, 12 are recent. MAD is rarely used for walnut allergy: Jur r 1 and Jug r 2 in a single article [32], Jug r 3 in two articles [30, 32].

Sesame is described as a cause of anaphylaxis in 23 articles, of which five are recent but MAD with Ses i 1 is not cited in anaphylaxis cases.

Poppy seeds (with Pap s 2), *Brasilian nut* (with Ber e 1), and *Macadamia nut* (with Mac i 2S), despite being considered potentially dangerous and for which components that could be potentially harmful have been characterized, have not been published.

Cashew is mentioned in 23 articles (of which seven are recent) However, Ana o 3, the component available, is described in only one article [54].

Milk was frequently associated with anaphylaxis at the beginning of MAD (180 articles) but in recent years, only 65 articles were published on this topic. However, not only milk but also milk derivatives were associated with the risk of anaphylaxis: butter, cheese, ricotta. However, despite the good number of molecular components available, MAD, at least in recent years, was not widely studied or published. Thus, MAD was included in ten

articles: in particular, Bos d 8 was used in eight [55–60], Bos d 5 in seven, Bos d 4 in three, Bos d6 in two, and Bos d 2 never. Of note, a couple of articles came from countries, such as Iran and Georgia [58, 60] where MAD entered in the routine recently.

Fishes, in particular, their content of parvalbumin, have been consistently associated with anaphylactic reactions. The following species were described in the last 10 years: anchovy, carp, cod, fish, mackerel, salmon, and tuna. The component armamentarium for IgE study in fish is limited to few components, and along this line, also references are few. Indeed, in the last 4 years, 28 articles on anaphylaxis considered fishes, in particular, codfish, salmon, tuna, mackerel, anchovy, etc. Molecular diagnostics (by Gad m 1 and Cyp c 1) was virtually absent, while parvalbumins were cited in three different articles: in two, there was not a direct relationship with fishes, while in the third [10], parvalbumins were described as a family.

Crustaceans and mollusks can be considered a functional family of allergens that is well known to be involved in systemic reactions, being their allergens substantially resistant to cooking, low-pH, and gut peptidases. So, the number of species described to be involved in anaphylaxis is large, including abalone, crustacea, jelly, jellyfish, litopenaeus, lobster, metapenaeus, mollusk, mussel, oyster, prawn, shellfish, shrimp, squid, and cephalopods. However, due to the small (and variable in the commercial source) number of components available, MAD is not frequently used, at least in recent years. Shrimp seems to be the main allergen source of this family. Indeed, 17 articles were published in recent years but in none the available components (namely Pen m 1, Pen m 2, and Pen m 3) were reported.

Vegetables

Also vegetables, mainly for their content in nsLTP, may be cause of systemic reactions in sensitized patients. The following vegetables have been reported in the last 10 years as associated with anaphylaxis: artichoke, carrot, cauliflower, celery, fennel, lettuce, mushroom, potato, pumpkin, spinach, tomato, tuber, zucchini. But, unfortunately, even in this situation, the number of components available is small and, proportionally, the number of articles on this topic, at least in the last 4 years, is small too. Even if many different vegetables (potato, pumpkin seed, spinach, tomato, zucchini, lettuce, mushrooms, artichoke, cauliflowers, celery, cucumber, etc.) are considered responsible of anaphylactic reactions, the use of MAD is restricted to the small number of vegetables where molecular components are available, such as tomato, carrot, and celery). So, anaphylaxis due to tomatoes was described in a single article without MAD, carrot (and the relevant Dau c 1) was never cited, and celery in two articles (without MAD indications).

Hen eggs are frequently described as responsible of anaphylaxis. Indeed, many articles (59 in total and 11 in the last 4 years) are available. A useful review was published in 2015 [61]. MAD in egg allergy was extensively considered: indeed, six articles cite Gal d 1, 25 Gal d 2—Ovalbumin, two Gal d 3, four Gal d 5, and none Gal d 4. Notably, one article discussed the use of egg extracts compared with components concluding that "Egg white

specific IgE showed a similar ability as Gal d 1 and Gal d 2 in differentiating children at risk for egg anaphylaxis, although Gal d 1 and Gal d 2 showed a better specificity" [62]. Other articles focused on regional patterns of sensitization [58, 63, 64].

Wheat and legumes (rice including Basmati rice, buckwheat, cereal, corn, gliadin, hop, millet, soy, tapioca). Soy is another well-known food involved in food allergy and anaphylaxis. For soy, a good number of components are available. However, in recent years, only one article (that also considered the use of components) was published [65]. Buckwheat has 23 articles and of these, 7 are recent. Fag e 2, the relevant component, is considered only in one [66]. In this article, the added value of MAD (based on Fag e 2 and Fag e 5) in predicting buckwheat allergy are shown. Wheat is frequently cited (17 recent articles), but only gliadin is considered, while other components are not taken into consideration. These allergens are also associated with the wheat-dependent exercise-induced anaphylaxis (WDEIA): see in the specific paragraph.

Spices (including anise, mustard, poppy seed, paprika, and few other exotic spices). Despite a number of spices are available for the diagnostics as extracts (anise, paprika), only mustard has a molecular component, Sin n 1. But in the four articles published in the last 4 years, none considered the use of this component.

Other foods were associated with anaphylaxis but for the absence of available components, do not belong to the aim of this review.

Exercise-induced anaphylaxis

This topic has been recently revised in an extensive manner by Giannetti [67•]. In this review, the role of specific IgE is considered relevant but only omega-5 gliadin (Tri a 19) is considered as a useful component to be used. Of this topic, a large number of works have been published in the past and, at present, little new evidence, related to the use of MAD, can be found. Between these, interestingly, data from countries where MAD use is recent are interesting, in particular to detect differences and similarities with other countries [68]. The role of LTP seems to be confirmed and the role of certain components absorbed by the gut during the exercise is relevant [53]. In addition, the interaction between food and NSAIDs may have an effect in at least 30% of cases supporting the concept that cofactors are relevant in the triggering of an exercise-induced anaphylaxis (EIA).

Idiopathic anaphylaxis

Idiopathic anaphylaxis is an entity diagnosed once a thorough clinical history and allergy sensitization tests have not determined a plausible cause of the anaphylactic reaction. Some currently identified anaphylactic syndromes, such as delayed red meat anaphylaxis, were previously often labeled as idiopathic. Performing a multiplexed component assay, plus single-plexed assays to molecular allergens not present in the microarray (i.e., alpha-gal) seems a reasonable additional diagnostic strategy which may aid in the diagnosis. One single article has evaluated the added value of MAD in this scenario and

yielded the identification of a potential relevant sensitization in one-third of cases of their cohort [69]. In the case of a completely negative result after MAD, the clinician should consider investigating alternative entities, such as mast cell activation syndromes [70].

Conclusions

The problem of anaphylaxis is significant in the recent literature, in particular for diagnosis, prophylaxis, and treatment. Fine tuning the diagnosis of the causative allergen at a molecular level is of utmost importance to enable a correct long-term management of many patients with anaphylaxis. As an example, in the case of hymenoptera venom allergy, some molecules are poorly represented or even absent in therapeutic extracts, rendering an unacceptable risk for patients inadequately treated. Also, in food allergy, a recommendation of avoidance strategies will greatly differ according to the sensitizing allergen. However, at least in recent years, even if the diagnosis of the causative allergen is considered important, it seems that only few centers have the methods (that at present are available on the market) for the identification of the responsible component(s). This should be commented: indeed, it is true that molecular allergy has been introduced in diagnostic procedures only few years ago, but it is also true that MAD is based on a complex array of rules that are known by experts. In other words, until now, MAD does not belong to the standard armamentarium of the allergist. Second, probably, anaphylaxis is a so complex (and serious) problem that scientists are much more focused on the clinic and the treatment and less on the fine molecular identification of the responsible molecules. However, for certain families of allergens, such as hymenoptera venom, the molecular diagnosis is already fundamental and cannot be ruled out. In other situations, such as in food anaphylaxis, the great homology of allergens from different sources increases the complexity of the use of MAD in the identification of a diet suitable to prevent the trigger of anaphylaxis. However, research and formation on MAD will be able to overcome these problems in a near future.

Compliance with Ethical Standards

Conflict of Interest

Enrico Heffler declares that he has no conflict of interest. Victoria Cardona declares that she has no conflict of interest. Olga Luengo declares that she has no conflict of interest. Giovanni Paoletti declares that he has no conflict of interest. Francesca Racca declares that she has no conflict of interest. Francesca Puggioni declares that she has no conflict of interest. Giovanni Melioli declares that he has no conflict of interest. Giorgio Walter Canonica declares that he has no conflict of interest.

Human and Animal rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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