

REVIEW ARTICLE

# Anaphylaxis: opportunities of stratified medicine for diagnosis and risk assessment

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anaphylaxis; augmentation factor; cofactor; component-resolved diagnostics; personalized medicine.

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## Abstract

The risk to develop anaphylaxis depends on the sensitization pattern, the proportion of the involved immunoglobulin classes, the avidity and affinity of immunoglobulins to bind an allergen, characteristics of the allergen, the route of allergen application, and, last but not least, the presence of cofactors of anaphylaxis. To be able to calculate the risk to develop anaphylaxis and to anticipate the severity of the reactions under certain conditions, it is necessary to understand how all these factors interact with each other. Important progress for risk assessment in anaphylaxis is based on component-resolved stratified diagnostics, which allow to (i) determine a patient's sensitization pattern on a molecular basis, (ii) correlate clinical responses to defined sensitization patterns, and (iii) better identify cross-reactive allergens. Together with the increasing knowledge regarding the role and mode of action of cofactors of anaphylaxis, these data pave the way to unscramble the complex interactions determining the clinical relevance of sensitizations, the risk of anaphylaxis, and the severity of reactions. As a consequence, this understanding allows to better determine the individual risk in response to an identified allergen and results in more specific advices and education for our patients to prevent further life-threatening anaphylactic reactions.

Anaphylaxis was first described by Portier and Richet in 1902 (1) and can be defined as a 'severe, life-threatening generalized or systemic' allergic type I reaction involving more than one organ system (2). Depending on the strength of the symptoms, anaphylactic reactions can be clinically categorized according to the classification of Ring and Messmer (3). Corresponding to the definition of immediate-type allergic reactions, anaphylaxis is classically expected to be mediated via allergen-specific IgE antibodies bound to high-affinity IgE receptors (FcεRI) on the surface of tissue-resident mast cells as well as circulating basophils. Cross-linking of FcεRI-IgE by binding of multivalent allergens induces degranulation (4), and the release of a large panel of mainly vasoactive mediators like histamine mediates the clinical symptoms of anaphylaxis. In addition, alternative pathways of anaphylaxis were described. In mice, allergen-specific IgG even in the absence of IgE also induces strong anaphylaxis via basophils and even neutrophils following allergen contact (5–7). An important mediator of basophil-dependent anaphylaxis is the platelet-activating factor (PAF), and a correlation between serum PAF levels and the severity of anaphylaxis

was described (8). Thus, alternative pathways to FcεRI-IgE may be also relevant in human anaphylaxis.

In addition to the increasing knowledge of underlying mechanisms and the role of certain mediators other than histamine, data from a rising number of anaphylaxis registries in Germany, France, Norway, Great Britain, and Canada help to accumulate evidence on the direct elicitors of anaphylaxis and direct more attention to the role of cofactors underlying anaphylaxis development (9). Cofactors are allergen-independent extrinsic or intrinsic factors able to modulate the outcome of type I allergic reactions. In particular, after application of low allergen doses or allergens with low potency, which alone would not be sufficient to trigger clinically significant anaphylaxis, the presence of cofactors is of crucial importance. Epidemiological data show that cofactors of anaphylaxis are relevant in up to 39% of all food-dependent anaphylactic reactions in adults (10). Cofactors are most relevant in the context of food allergy, and the most important cofactor is exercise. The best defined cofactor-related allergic reaction is called 'wheat-dependent exercise-induced anaphylaxis' (WDEIA) (11). It is important to know that in WDEIA

patients, in contrast to other wheat-allergic patients, the presence of exercise as a cofactor acts not only as an intensifier of the allergic reaction but also as an indispensable prerequisite to trigger anaphylaxis. Palosuo et al. could show that WDEIA is elicited by omega-5-gliadin (Tri a 19), a protein that represents only a small fraction of wheat allergen preparations (12). To detect omega-5-gliadin-specific IgE, it is necessary to use either recombinant or purified protein. Therefore, omega-5-gliadin was one of the first recombinantly produced allergens that proved to better stratify diagnostics and risk assessment for anaphylaxis: Detecting IgE binding to omega-5-gliadin became a standard diagnostic tool in the daily allergist's routine and also defined a new entity, WDEIA. Recombinant or isolated native allergens are the basis for component-based diagnostics, which becomes more and more established. The individual sensitization patterns help (i) to dissect primary sensitizations from cross-reactivity and (ii) to determine whether allergic reactions are caused by allergens associated with a risk of severe anaphylaxis or milder reactions. Together with the increasing knowledge regarding the mode of action of cofactors of anaphylaxis and the role of effector cells of anaphylaxis other than mast cells, the use of recombinant allergens could allow to distinguish well-defined anaphylaxis risk patterns or even to define other new entities as it was possible in the case of WDEIA. It is the aim of this review to give a comprehensive overview of the relevant parts known today that together determine the risk of anaphylaxis in an individual patient. A better understanding of the interaction of all those factors is obligatory to better explain the pathogenesis of anaphylaxis, to establish the correct diagnosis, and to take the necessary preventive measures.

### Epidemiological aspects

The available epidemiological data are widely varying due to incongruent definitions of anaphylaxis, investigations of different subpopulations, and a lack in reporting or misdiagnosis. As anaphylactic reactions are acute and therefore often managed in the emergency situation, it is difficult to perform prospective studies on elicitors, and treatments and validation of the diagnosis are not always possible. Data from retrospective studies show a lifetime prevalence of anaphylaxis between 0.05% and 2% with a correlating incidence rate of lowest 1.95 (Turkey, ICD-10 based) (13), and highest 103 (Spain, based on medical records) (14), cases of anaphylaxis per 100 000 person-years. A series of studies confirmed food allergens, drugs, and hymenoptera venoms as the most prevalent elicitors of anaphylaxis (15–18). However, the relative importance of these groups differs with age. In young patients, the most important allergens are food and hymenoptera venoms, but with increasing age, drugs gain more and more importance and represent the second most important allergens already in patients above the age of thirty. A very similar effect can be observed regarding the subgroup of food allergens. In young children, allergens from cow's milk, hen's egg, and wheat dominate. In older children, nuts and peanuts become relevant, while nuts, wheat, celery, and

seafood are the dominant food allergens eliciting anaphylaxis in adults (19). The number of anaphylaxis-related fatalities ranges from 0.33 to 0.87 per 1 000 000 inhabitants per year (20). In Germany between 2004 and 2008, 150 fatalities were registered, correlating with a rate of 0.46 per 1 000 000 inhabitants per year (21). However, a high number of unreported cases can be expected. Although the absolute numbers differ, there is a broad consensus that the incidence of anaphylaxis is rapidly increasing (22, 23). Identifying the right preventive measures for anaphylaxis will be one of the major healthcare issues in Western countries in the next years. To understand which conditions favor the development of such severe anaphylactic reactions is a necessary prerequisite to prevent anaphylaxis and anaphylaxis-related death.

### Factors determining the risk to develop anaphylaxis

Risk factors for anaphylaxis are circumstances that increase the risk to develop anaphylaxis following a contact to an allergen an individual is sensitized to. It is well accepted that the clinical manifestations of systemic type I allergic reactions depend on a complex process involving a cascade of events in different organ systems. As all these components can be involved in regulating the elicitation, the risk to develop anaphylaxis, in theory, depends on a complex formula. This formula would need to include (i) the allergens an individual patient is sensitized to, (ii) the degree of sensitization, (iii) the quality of binding allergens, (iv) probably also the relative proportions of antigen-specific immunoglobulin subtypes, (v) the route of allergen application, and finally, (vi) the presence and 'amount' of cofactors (Fig. 1). If all those factors were known, the risk to develop systemic type I reactions and the grade of the reactions would be rather predictable. Therefore, it is a vision in allergology to determine all those parameters for each individual patient to calculate his/her risk and to better advise them and their doctors. This stratification for diagnosis and risk assessment is the new frontier of allergology.

### Sensitization

Sensitization to any type I allergen leads to the development of IgM and then IgG antibody-producing plasma cells.

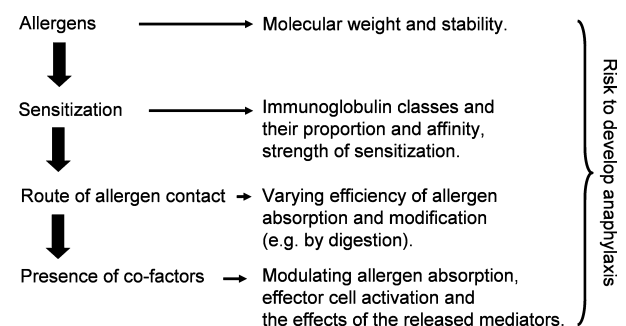


**Figure 1** Factors together determining the risk to develop anaphylaxis.

Under the influence of the Th2 cytokines, IL-4 and IL-13, some B cells switch to the production of IgE antibodies, a necessary prerequisite for the induction of type I allergy. Whether B cells can also directly switch to IgE production, which may favor the preferential production of low-affinity IgE antibodies, is still a matter of debate (24, 25). The quantitative relationship between antigen-specific antibodies of those subclasses is basically always the same with very high IgG, intermediate IgM, and very low IgE levels. However, depending on the antigen and possibly the susceptibility of an individual, some evoke more efficient IgE production than others. Moreover, the anaphylactic potential of IgE antibodies also differs with the affinity to the allergen (25). Therefore, the level of IgE in the serum is not a good predictor of anaphylaxis; however, the proportion of antigen-specific IgE to total serum IgE can be already a much better estimate of a possible risk. Underlying are probably effects of IgE on FcεR density and occupancy and a relevance of the number of epitopes recognized on a certain multivalent allergen (26). In addition to known features of the allergen such as stability in response to heat, acidity, and other characteristics, the clinical relevance of a given sensitization can be assessed much better. There is a broad consensus that allergen-specific IgG is irrelevant for the evaluation of type I allergy, especially for food allergy, because food-specific IgG production is a physiological immune response following contact to food. However, in mice, it is widely accepted that anaphylaxis can be induced via alternative pathways with allergen binding to IgG activating, for example, basophils and neutrophils as effector cells (5, 27–30). Thus, it cannot be ruled out that under certain conditions, alternate pathways are also functional in humans. In this respect, it is likely that we will identify certain allergens or other particular conditions favoring IgE-independent type I reactions. In contrast, in other conditions such as allergen-specific immunotherapy, the induction of IgG4 antibodies is one possible mode of action modulating IgE-dependent type I reactions, but whether this is the protective mechanism is still a matter of debate (31). In conclusion, there is evidence that the risk to develop anaphylaxis and the severity of anaphylaxis are not determined by allergen-specific IgE alone and that the presence of non-IgE immunoglobulins may determine or at least modulate the outcome of anaphylaxis (16). Importantly, sensitization itself seems to have a direct influence on the bioavailability of the allergen as intestinal absorption in mice following oral uptake of the allergen was higher than in nonsensitized control mice (32), which is even multiplied by the addition of exercise. Matsuo et al. also found that ASA and exercise increased the intestinal uptake of the allergen gliadin in humans, with a tendency for higher gliadin uptake in sensitized individuals (33). These results show that in regard to intestinally absorbed allergens, for example, food allergens and some drugs, the extent of sensitization directly modulates also the bioavailability of the allergen and therefore, next to the mere presence of specific IgE antibodies themselves, is an important factor determining the risk of anaphylaxis.

### Sensitization pattern

Component-resolved allergen diagnosis (CRD) using recombinant or purified single allergens to detect IgE in the serum advanced the field of allergy diagnostics tremendously. Based on the characterization of more and more allergens and the classification of allergen protein families (34, 35), component-resolved allergen diagnosis (CRD) is a rapidly growing area leading to several advantages in comparison with diagnostics with allergen extracts. Allergen extracts usually are standardized in regard to (some) major allergens, but not in regard to minor allergens (36). This can be of clinical relevance as in, for example, WDEIA where wheat extracts show low sensitivity for detecting IgE binding to omega-5-gliadin (37). Even in regard to the contents of major allergens and thus specificity of allergen extracts, unexpected problems were identified: Re-evaluation of wasp venom extracts revealed low sensitivity compared with analyses using two major allergens, Ves v1 and Ves v5. Consequently, a method called 'spiking' of the total extract with the single major allergen Ves v5 was used to increase the sensitivity of the extract (38). Thus, detecting IgE using allergen extracts is often less sensitive in regard to single allergens and often less specific based on the mixture of ingredients. In addition, the composition of the extracts is often not defined, and depending on the methods of production of extracts, the composition may vary. As described above, some allergens are underrepresented resulting in low sensitivity. Moreover, cross-reactivity as underlying cause for detected polysensitization could not be identified using extracts to detect specific IgE. Meanwhile, a steadily increasing panel of single allergens is available for diagnostic purposes also as a chip-based method allowing allergists to exactly specify the sensitization pattern of an individual patient. This way, interpretations in regard to the functional consequences of a given sensitization are more precise in view of the molecular structure, epitope–IgE interaction, and stability during heating or digestion of a specific allergen allowing to better estimate a risk also of anaphylactic reactions (39–44). Therefore, in contrast to the low predictive value of extract-based IgE antibody determination, CRD makes it possible to better predict the individual disease manifestation in regard to severity and persistence on the basis of the sensitization pattern of the patient.

The potential of CRD is especially valuable to diagnose food, and in particular fruit and vegetable, allergies (45). The prolamin superfamily (alpha-amylase and protease inhibitors, nonspecific lipid transfer proteins (nsLTPs), and storage proteins like 2S albumins) and the protein family of cupins (seed storage proteins such as vicilin and legumin) were identified as plant allergens associated with high anaphylactic potential (40). In contrast, PR-10 (Bet v 1 homologous) proteins, which are important cross-reactive components in whole allergen extracts, usually elicit only localized allergic reactions such as in the oral allergy syndrome (OAS), because like profilins, PR-10 proteins are usually labile and easily degraded by heat or acidity. These PR-10 proteins together with profilins (e.g. Bet v 2) and cross-reactive carbohydrate determinants (CCDs) are present in many plants and interfere

with the specificity of extract-based IgE determination (40–44). PR-10 proteins are especially relevant to north and middle Europe. In contrast, in southern Europe, cross-sensitization to LTPs is more common. Those allergens are stable to heat and digestion and therefore have a much higher potential to induce anaphylactic reactions (46). Most dangerous in this regard are storage protein allergens of different protein families. Sensitizations to CCDs in food or venoms are probably without remarkable clinical relevance, and the primary sensitization may derive from either pollen or venoms, but rarely from food. Similarly, PR-10 proteins or profilins from pollen are usually the primary source of sensitization and not the food proteins. In contrast, LTPs and storage proteins from food are believed to lead to the primary sensitization responsible for severe reactions. An overview of the most relevant components of food allergens is shown in Tables 1 and 2.

One very good example of stratified diagnostics in allergology is the use of omega-5-gliadin, which belongs to the prolamins family and is the main allergen eliciting WDEIA (12). As described above, the omega-5-gliadin fraction in wheat allergen extracts is very small; therefore, conventional sIgE tests for wheat can be negative or very low in WDEIA patients. Measuring IgE specific for omega-5-gliadin in these patients is diagnostic, and detecting IgE specific for omega-5-gliadin was shown to have a high positive predictive value and therefore strongly indicates WDEIA (47). Diagnosing WDEIA instead of conventional wheat allergy is very important for patients' management as it may allow them to be exposed to limited amounts of flour products under controlled conditions. Similarly, in peanut allergy, CRD allows to discriminate between a dangerous primary sensitization to peanut storage proteins Ara h 1 (vicilin), Ara h 2 (2S albumin), Ara h 3 (legumin), Ara h 6 (2S albumin) and, although probably in most cases cross-reactive, potentially dangerous Ara h 9 (nsLTP) and mostly cross-reactive sensitization to Ara h 8 (a PR-10 protein and Bet v 1 homologue) (48–52). For now, the best positive predictive value for peanut allergy was reported for Ara h 2 with a specificity of 100% at a cut-off level of >1.8 kUA/l and 95% at a cutoff level of >1.4 kUA/l (53). As with other food allergens, the Ara h 1/2/3 pattern dominates in the USA and Europe, while the LTP Ara h 9 is the most relevant peanut allergen in southern Europe (54, 55). As in other settings, CCDs may play a role: If no IgE to available recombinant peanut allergens is detectable despite measurable IgE to whole peanut extract, CCDs may be responsible. IgE to CCDs can be measured using horseradish peroxidase or bromelain (nAna c 2 or direct rMUXF3 chain), which are both rich in CCDs. Similarly, CRD-based analyses are established also for other vegetables and fruits (56). In conclusion, in many patients sensitized to plant ingredients, CRD provides exact individual sensitization patterns, which finally allow to better predict the individual risk of anaphylaxis.

In case of animal-derived food allergens, CRD can also help to differentiate relevant from less relevant sensitization patterns. In patients sensitized to egg white, IgE to ovomucoid (Gal d 1), which is heat and digestion stable, is associated with a higher risk of persistent and severe reactions. Gal

**Table 1** Important allergenic components of relevant allergens [adapted from Ebo DG et al., Sastre J et al., García BE et al. and Verma AK et al. (78–81)]

| English name   | Latin name                    | Allergen  | Protein family            |
|----------------|-------------------------------|-----------|---------------------------|
| Peanut         | <i>Arachis hypogaea</i>       | Ara h 1   | 7S globulin (vicilin)     |
|                |                               | Ara h 2   | 2S albumin                |
|                |                               | Ara h 3   | 11S globulin (legumin)    |
|                |                               | Ara h 4   | 11S globulin (legumin)    |
|                |                               | Ara h 5   | Profilin                  |
|                |                               | Ara h 6   | 2S albumin                |
|                |                               | Ara h 7   | 2S albumin                |
|                |                               | Ara h 8   | PR10 (Bet v 1 homologue)  |
|                |                               | Ara h 9   | nsLTP                     |
|                |                               | Ara h 10  | 18 kDa Oleosin            |
| Soy            | <i>Glycine max</i>            | Gly m 1   | nsLTP                     |
|                |                               | Gly m 2   | Defensin                  |
|                |                               | Gly m 3   | Profilin                  |
|                |                               | Gly m 4   | PR10 (Bet v 1 homologue)  |
|                |                               | Gly m 5   | 7S globulin (vicilin)     |
|                |                               | Gly m 6   | 11S globulin (legumin)    |
| Lentil         | <i>Lens culinaris</i>         | Len c 1   | 7S globulin (vicilin)     |
|                |                               | Len c 2   | Seed biotinylated protein |
|                |                               | Len c 3   | nsLTP                     |
| Pea            | <i>Pisum sativum</i>          | Pis s 1   | 7S globulin (vicilin)     |
|                |                               | Pis s 2   | Conviciilin               |
| Chickpea       | <i>Cicer arietinum</i>        | Cic a 2S  | 2S albumin                |
|                |                               | Cic a 11S | 11S globulin (legumin)    |
|                |                               | Globulin  | (legumin)                 |
| Pistachio nut  | <i>Pistacia vera</i>          | Pis v 1   | 2S albumin                |
|                |                               | Pis v 2   | 11S globulin (legumin)    |
|                |                               | Pis v 3   | 7S globulin (vicilin)     |
| Cashew nut     | <i>Anacardium occidentale</i> | Ana o 1   | 7S globulin (vicilin)     |
|                |                               | Ana o 2   | 11S globulin (legumin)    |
|                |                               | Ana o 3   | 2S albumin                |
| Sesame         | <i>Sesamum indicum</i>        | Ses i 1   | 2S albumin                |
|                |                               | Ses i 2   | 2S albumin                |
|                |                               | Ses i 3   | 7S globulin (vicilin)     |
|                |                               | Ses i 4   | Oleosin                   |
|                |                               | Ses i 5   | Oleosin                   |
|                |                               | Ses i 6   | 11S globulin (legumin)    |
|                |                               | Ses i 7   | 11S globulin (legumin)    |
| Sunflower seed | <i>Helianthus annuus</i>      | Hel a 2   | Profilin                  |
|                |                               | Hel a 3   | nsLTP                     |
| Mustard        | <i>Brassica nigra</i>         | Sin a 1   | 2S albumin                |
|                |                               | Sin a 2   | 11S globulin (legumin)    |

**Table 1** (Continued)

| English name | Latin name                | Allergen    | Protein family                      |
|--------------|---------------------------|-------------|-------------------------------------|
| Almond       | <i>Prunus dulcis</i>      | Sin a 3     | nsLTP                               |
|              |                           | Sin a 4     | Profilin                            |
|              |                           | Pru du 2S   | 2S albumin                          |
|              |                           | Pru du 3    | nsLTP                               |
|              |                           | Pru du 4    | Profilin                            |
|              |                           | Pru du 6    | 11S globulin (legumin)              |
| Hazelnut     | <i>Corylus avellana</i>   | Cor a 1     | PR10 (Bet v 1 homologue)            |
|              |                           | Cor a 2     | Profilin                            |
|              |                           | Cor a 8     | nsLTP                               |
|              |                           | Cor a 9     | 11S globulin (legumine)             |
|              |                           | Cor a 11    | 7S globulin (vicilin)               |
|              |                           | Cor a 12    | Oleolin                             |
|              |                           | Cor a 13    | Oleolin                             |
|              |                           | Cor a 14    | 2S albumin                          |
|              |                           | Jug r 1     | 2S albumin                          |
|              |                           | Jug r 2     | 7S globulin (vicilin)               |
| Walnut       | <i>Juglans regia</i>      | Jug r 3     | nsLTP                               |
|              |                           | Jug r 4     | 11S globulin (legumine)             |
| Wheat        | <i>Triticum aestivum</i>  | Tri a aA/TI | $\alpha$ -amylase/trypsin inhibitor |
|              |                           | Tri a 14    | nsLTP                               |
|              |                           | Tri a 19    | $\Omega$ -5-gliadine                |
| Apple        | <i>Malus domestica</i>    | Mal d 1     | PR10 (Bet v 1 homologue)            |
|              |                           | Mal d 3     | nsLTP                               |
| Kiwi         | <i>Actinidia delinosa</i> | Act d 1     | Cysteinoprotease                    |
|              |                           | Act d 2     | Thaumatol-like protein              |
|              |                           | Act d 4     | Phytocystatin                       |
|              |                           | Act d 5     | Kiwellicin                          |
|              |                           | Act d 6     | Pectin methylesterase inhibitor     |
|              |                           | Act d 7     | Pectin methylesterase               |
|              |                           | Act d 8     | PR10 (Bet v 1 homologue)            |
|              |                           | Act d 9     | Profilin                            |
|              |                           | Act d 10    | nsLTP                               |
|              |                           | Act d 11    | Major latex protein                 |
| Peach        | <i>Prunus persica</i>     | Pru p 1     | PR10 (Bet v 1 homologue)            |
|              |                           | Pru p 2     | Thaumatol-like protein              |
|              |                           | Pru p 3     | nsLTP                               |
|              |                           | Pru p 4     | Profilin                            |
| Carrot       | <i>Daucus carota</i>      | Dau c 1     | PR10 (Bet v 1 homologue)            |
|              |                           | Dau c 4     | Profilin                            |

**Table 1** (Continued)

| English name         | Latin name                  | Allergen | Protein family                     |
|----------------------|-----------------------------|----------|------------------------------------|
| Celery               | <i>Apium graveolens</i>     | Api g 1  | PR10 (Bet v 1 homologue)           |
|                      |                             | Api g 2  | nsLTP                              |
|                      |                             | Api g 3  | Chlorophyll a-b-binding protein    |
|                      |                             | Api g 4  | Profilin                           |
|                      |                             | Api g 5  | FAD containing oxidase             |
| Tomato               | <i>Solanum lycopersicum</i> | Lyc e 1  | Profilin                           |
|                      |                             | Lyc e 2  | Beta-Fructofuranosidase            |
|                      |                             | Lyc e 3  | nsLTP                              |
|                      |                             | Lyc e 4  | Intracellular PR protein TSI-1     |
| Latex                | <i>Hevea brasiliensis</i>   | Hev b 1  | Rubber elongation factor           |
|                      |                             | Hev b 3  | Small rubber particle protein      |
|                      |                             | Hev b 5  | Acid protein                       |
|                      |                             | Hev b 6  | Hevein                             |
|                      |                             | Hev b 8  | Profilin                           |
|                      |                             | Hev b 11 | Chitinase                          |
| Milk (cow)           | <i>Bos domesticus</i>       | Bos d 4  | $\alpha$ -lactalbumin              |
|                      |                             | Bos d 5  | $\beta$ -lactoglobulin             |
|                      |                             | Bos d 6  | Serum albumin                      |
|                      |                             | Bos d 8  | Casein                             |
| Crustaceans (shrimp) | <i>Penaeus aztecus</i>      | Pen a 1  | Tropomyosin                        |
|                      |                             |          |                                    |
| Fish (cod)           | <i>Gadus morhua</i>         | Gad m 1  | Parvalbumin                        |
|                      |                             | Gad m 2  | Beta enolase                       |
|                      |                             | Gad m 3  | Aldolase A                         |
| Egg white (henn)     | <i>Gallus domesticus</i>    | Gal d 1  | Ovomucoid                          |
|                      |                             | Gal d 2  | Ovalbumin                          |
|                      |                             | Gal d 3  | Ovotransferrin                     |
|                      |                             | Gal d 4  | Lysozyme                           |
|                      |                             | Gal d 5  | Serum albumin ( $\alpha$ -livetin) |

d 1 IgE levels of >11 kUA/l are associated with a specificity of 95% with a high risk to develop clinical reactions to heated as well as a raw egg (57). In contrast, sensitization to ovalbumin and presumably ovotransferrin and lysozyme is considered less serious, because it is heat labile and patients may tolerate heated eggs (58). Another important indication for CRD is the diagnosis of meat allergy. In addition to the pork-cat syndrome that is based on cross-reactive IgE antibodies to serum albumin of different species, recently, the oligosaccharide galactose- $\alpha$ -1,3-galactose ( $\alpha$ -gal) was identified as the causative allergen in delayed-type immediate allergy to red meat. As the anaphylactic reaction often is delayed in affected patients, this type of allergy is not only dangerous but may also be difficult to diagnose. The detection of IgE to  $\alpha$ -gal therefore is a helpful tool to prevent misdiagnosis in such patients (59–61). In patients

**Table 2** Most important allergen families

| Protein family                                         | Anaphylactic potential                         |
|--------------------------------------------------------|------------------------------------------------|
| Pathogen related protein<br>PR-10 (Bet v 1-homologues) | Low                                            |
| Profilin                                               | Low (expected for most allergens)              |
| Non specific lipid transfer<br>proteins (nsLTP)        | High                                           |
| 11S globulin                                           | High                                           |
| 7S globulin                                            | High                                           |
| 2S albumin                                             | High                                           |
| Thaumatococin-like proteins (TLP)                      | High (expected for some<br>allergens) (82)     |
| Oleosins                                               | High (expected for some<br>allergens) (83, 84) |
| Tropomyosin                                            | High                                           |
| Parvalbumin                                            | High                                           |

sensitized to cow's milk, shrimp, fish, or other allergological relevant foods, the number of available single allergens and the knowledge regarding their relevance are also rapidly increasing, for example, determining whether all fish types need to be avoided (pan-allergen parvalbumin) or only some types are relevant (e.g. sensitization to enolase) (62–64). Besides food allergy, CRD also plays an important role for diagnosing and interpreting sensitizations in anaphylaxis of other forms such as in hymenoptera venom allergy. Double sensitizations to wasp and bee venom in conventional serum IgE tests using extracts may be discriminated using CRD including CCD-rich proteins (36, 65), and in some patients, even the hymenoptera species responsible for anaphylaxis can be identified (66). Moreover, in case of suspected latex allergy, detection of IgE binding only CCD or profilin can also discriminate irrelevant from relevant latex sensitizations.

### Effector phase: modulators of allergic reactions

The clinical consequences of contact to an allergen in a sensitized individual also differ depending on the route of application/ingestion. The application route is very important, because it determines whether an allergen can act systemically or only locally and, in case of systemic effects, how rapid the allergen is absorbed and distributed. The higher the peak allergen concentrations, the more likely are (severe) anaphylactic reactions. Thus, direct intravenous application of allergens most rapidly elicits (severe) allergic reactions, while mucosal or cutaneous application is less likely to elicit severe systemic reactions.

However, allergen uptake can be modulated. This was proved by studies investigating the effect of augmentation factors like alcohol, intake of acetylsalicylic acid (ASA), or exercise on the intestinal allergen absorption. All these factors are able to increase the intestinal allergen absorption by disturbing the barrier function of the intestinal epithelium. For example, the intake of ASA leads to the downregulation of the expression of claudin-7, a protein which is important

for the functionality of tight junctions. In an *in vitro* model, decreased claudin-7 expression indeed correlates with increased permeability of the model allergen dextran (67). Other studies confirm an increase in the permeability of the small intestine also due to exercise (68). In oral provocation tests with wheat plus exercise, WDEIA patients often show a dose-dependent reactivity (69). Finally, in WDEIA patients, exercise as well as ASA intake leads to an increase in gliadin serum levels correlating with the allergic symptoms (33).

Cofactors are allergen-independent factors modulating the clinical development of the allergic reaction. The large group of cofactors can be classified into three subgroups. The first group consists of patient intrinsic factors that can increase the symptoms of type I allergy without modulating the immunological reaction itself. This group comprises a number of diseases that in most cases act in an organ-specific manner. The second group consists of factors that also have nonimmunological modulating effects on type I allergy, but are patient extrinsic, and those are mostly drugs. Finally, the third group consists of circumstances directly modulating the immune reaction of type I allergy. To distinguish those from the other cofactors, they are often alternatively called 'augmentation factors'. In recent years, the establishment of anaphylaxis registries helped to identify how important cofactors are (Fig. 2). Epidemiological studies observed atopic (31.8%) and cardiovascular (18.5%) diseases to be the most prevalent diseases in patients with anaphylaxis. Other allergies (2.6%) are also quite abundant, while concomitant skin diseases were rarely found (0.7%) (15). Atopic and cardiovascular diseases may favor the development of anaphylaxis and therefore are the most important group I cofactors. In particular, bronchial asthma is known to be an eminent risk factor provoking bronchospasm and often is associated with anaphylaxis-related fatalities (70, 71). Other nonimmunological intrinsic cofactors are mastocytosis and most probably also age (Fig. 3) (72, 73). Worm et al. reported a strong correlation between higher age and an 'increasing risk to develop circulatory symptoms, while in younger age groups,

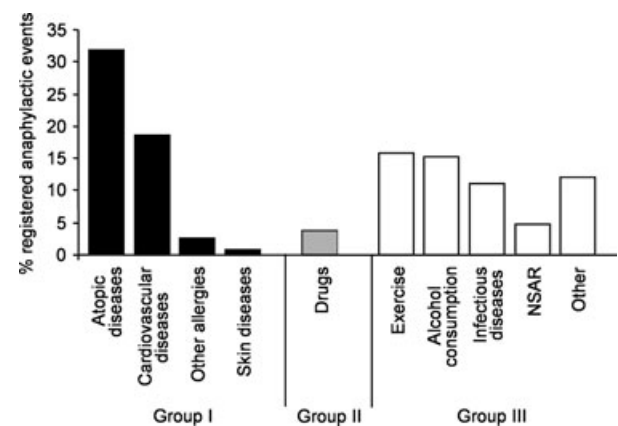


**Figure 2** Highest reported prevalence rates of cofactors in patients who developed anaphylaxis (collocated from different studies as cited in 9, 15).

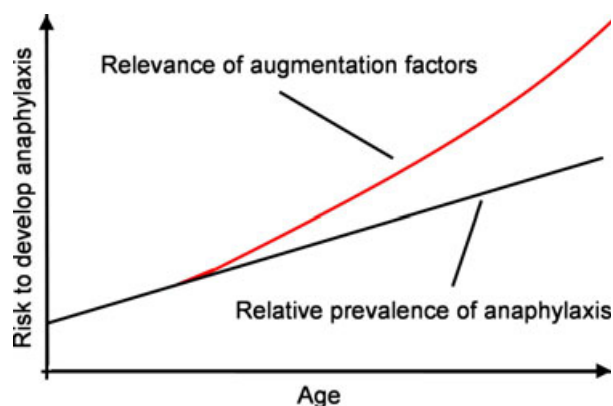


**Figure 3** The risk to develop anaphylaxis and the role of augmentation factors with increasing age (schematically).

the occurrence of respiratory symptoms dominates'. This is in accordance with earlier studies; however, regarding age as a risk factor, conflicting data were also published (15). Data published by Rueff et al. (74) point to male sex as a further risk factor for anaphylaxis especially in the context of hymenoptera sting reactions. Interestingly, young patients with peanut-related anaphylaxis had a high prevalence of atopic diseases (>30% vs <10% in wheat-allergic patients), while older patients with wheat-induced anaphylaxis showed a high prevalence of cardiovascular diseases (>10% vs <5% in peanut-allergic patients) (75).

Drugs that can be attributed to group II cofactors are supposed to play a role in 3.7% of anaphylactic reactions in adults and 2.6–6% of anaphylactic reactions in children. Best known for such effects are ACE inhibitors and beta-adrenergic blockers, which favor anaphylaxis by either increasing the bradykinin levels or inhibiting the counter-regulative tachycardia, which otherwise could stabilize the blood pressure. Other drugs such as codein, fluoroquinolones, muscle relaxants, and certain contrast media may also increase the anaphylactic risk by inducing anaphylactoid or pseudoallergic reactions mainly due to direct histamine release (reviewed in Refs. 9, 76).

Probably most important are group III cofactors, including the augmentation factors exercise, alcohol consumption, infectious diseases, NSAIDs, psychological stress, and other, in this context, rarely described circumstances, such as menstruation. The single most important augmentation factor is exercise playing a role in up to 15.9% of anaphylactic reactions in adults and in up to 10% in children. Some studies, unfortunately without differentiating between adults and children, reported exercise to be relevant in 20.4% of all anaphylactic reactions. The second most important augmentation factor in adults is alcohol consumption (1–15.2%), followed by infectious diseases (1.3–11%) and nonsteroidal antirheumatic (NSAR) drugs with 4.7%. In children, infectious diseases are an important trigger in 2.5–3% of all cases of anaphylaxis, while NSAR like in adults account as augmen-

tation factor for 3% of all anaphylactic reactions. Other augmentation factors, such as psychological stress or menstruation play a role in 8–12.1% of adults and in 10.3–12% of children.

Infectious diseases also act as cofactors of anaphylaxis and are expected to utilize immune pathways to directly modulate mast cell and basophil reactivity. However, underlying mechanisms are still not clarified in detail.

In general, cofactors seem to be less relevant in children: In adults and children, up to 39% and 14–18.3% of all anaphylactic reactions were associated with cofactors, respectively (reviewed in Ref. 9).

## Conclusion

Based on the complex sensitization patterns, the mode of action of cofactors of anaphylaxis, the degree of sensitization to an allergen, and the interaction and the proportion of different allergen-specific immunoglobulin subclasses, an increasingly good estimate of the individual risk to develop (severe) anaphylactic reactions can be given. However, this is just a beginning. Identifying all risk factors and understanding how they interact will ask for more studies and better characterizations both clinically and in regard to diagnostics. Some sensitizations are more often found in the context of cofactor-related anaphylaxis than others (77), suggesting that in this process, the 'character' of the allergen, the route of application, or the unique combination of cofactor and allergen may play a decisive role for the manifestation of anaphylaxis. In some patients, more than one cofactor needs to interact to trigger anaphylaxis, and the same cofactor may differ in respect to consequences depending on the allergen (75). Even consequences of more than one allergen on anaphylaxis development and severity are likely. Therefore, systematic subgroup analyses of larger patient cohorts regarding a possible correlation of certain cofactors or other risk factors in general with certain sensitization patterns are needed, and the emerging possibilities of CRD will help to clarify these questions. In the future, with increasing application of CRD and better awareness of the role of cofactors, anaphylaxis registries may be able to collect a more complete characterization of the patients suffering from anaphylaxis. Increasing understanding will allow to give even better advice and estimate to our patients to prevent anaphylaxis- and anaphylaxis-related fatalities.

## Author contributions

The article was written by Florian Wölbing and Tilo Biedermann.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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