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Anaphylaxis

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Summary

Anaphylaxis is the most severe systemic hypersensitivity reaction, and it can be life-threatening or even fatal. It involves the activation of multiple immune and non immune pathways beyond IgE, thus exhibiting different phenotypes. New symptoms of hypersensitivity caused by chemotherapy drugs, monoclonal antibodies, and biological agents have been suggested to be recognized as anaphylaxis phenotypes. No biomarker has been described that allows an unequivocal diagnosis of anaphylaxis. Moreover, more biomarkers for specific endotypes are needed to stratify severity, to predict risk, and to optimaze tretament choice in the individual patient.

Food, drugs and stinging insects represent the most commly identified triggers. Idiopathic anaphylaxis is a diagnosis of exclusion and it can hide a clonal mast cell disorder.

Individual risk factors and co-factors may influence the severity of anaphylaxis or its onset, and they should be identified to implement the appropriate measures to prevent recurrence.

Prompt recognition and treatment are critical in anaphylaxis, adrenaline being the first-line saving therapy. Individualized anaphylaxis action plan should include avoidance measures, prescription of an adrenaline autoinjector, education, optimal management of relevant comorbidities, venom specific immunotherapy, food oral immunotherapy, and drug desensitization, when appropriate.

However, the quality of acute and long-term anaphylaxis management is variable influencing the poor outcomes experienced by many patients. Clinical practice guidelines have the potential to improve outcomes, but they often prove challenging to implement in routine clinical care.

Introduction

Anaphylaxis is the most severe systemic hypersensitivity reaction, it involves multiple organ systems, can be caused by a number of triggers and conditions, and be deadly. A number of slightly different definitions for anaphylaxis has been used in different Guidelines (1-6). In these guidelines, the independently developed definitions of anaphylaxis for clinical use all include the concepts of a serious, generalized or systemic, allergic or hypersensitivity reaction that can be life-threatening or fatal. Importantly, none of the definitions include the word "shock" (1-3). The correct term "anaphylaxis" is preferred to "anaphylactic shock" because shock is not necessarily present in patients with anaphylaxis and it is used in preference to terms such as "anaphylactoid reaction", or "pseudo-anaphylaxis" (1-3). All Guidelines report that anaphylaxis is a "life-threatening reaction", even though in general mortality or morbidity do not seem to have increased in recent decades (7-9), despite the vast majority of anaphylaxis reactions are not treated properly with prompt administration of adrenaline. However, as it is not possible to predict the severity of reaction and early adrenaline administration may reduce the risk, the concept of "life-threatening" must be present in the anaphylaxis definition (10).

The anaphylaxis guidelines of the World Allergy Organization (WAO) (2) and of the European Academy of Allergy and Clinical Immunology (EAACI) (1) have established 3 sets of clinical crite-

ria for the diagnosis of anaphylaxis, confirming the proposal of the second symposium on the definition and management of anaphylaxis summary report - Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium (4). In short, anaphylaxis is highly likely in the case of an acute onset of an illness (minutes to several hours) affecting at least 2 different organs (*e.g.* skin-mucosal tissue, airways, gastrointestinal apparatus, cardiovascular system) or in the case of a a reduced blood pressure after exposure to known allergen for that patient (minutes to several hours). Because anaphylaxis mimics common syndromes, such as asthma and urticaria, and it can present without hypotension, its diagnosis is often missed or delayed.

For a number of good clinical reasons, very recently the Anaphylaxis Committee of the WAO proposed to revisit the definition and clinical criteria of anaphylaxis, in order to better capture the reality of anaphylaxis, simplify diagnosis, thus inproving the management (10). This proposal will certainly be the subject of worldwide discussion. It will probably has to take into account that there is a new understanding that atypical symptoms, such as pain, chills, fever can be seen during chemotherapy-induced anaphylaxis characterized by hypotension, desaturation, and cardiovascular collapse, which can lead to a new classification of anaphylaxis pathways (11). Moreover, clonal disorders, such as monoclonal mast cell activation syndrome, are considered part of the wide spectrum of anaphylaxis (11).

A minority of patients exhibit biphasic allergic reactions induced by a variety of causes,, in which signs and symptoms of anaphylaxis recur hours after initial resolution of anaphylaxis without re-exposure to the trigger (12). In addition to the biphasic reactions, patients who have IgE reactive with the oligosaccharide galactose-alpha-1,3-galactose, which is present in mammalian meat and in some therapeutic antibodies, can exhibit anaphylaxis after a delay of several hours during which no signs or symptoms are present (13).

Several classifications were proposed to assess the degree of severity of anaphylaxis; the most used in clinical practice is Ring's (14). New proposed severity scores from Brown and EAACI guidelines suggest simpler criteria, namely dividing reactions in mild, moderate or severe, or in grades according to local (grade 1) or systemic involvement (grade 2, 3), respectively (15, 16). In the latter, however, such proposed grading might be confusing for Hymenoptera venom allergy, given that local reactions are referred to local cutaneous involvement, rather than generalized urticaria.

Epidemiology and triggers

There is some evidence that the incidence of anaphylaxis may be increasing, but this may be due to changing clinical definitions or thresholds for presentation or admission.

A systemic review of the epidemiology of anaphylaxis in Europe (17) found the incidence rates for all-cause anaphylaxis ranging from 1.5 to 7.9 per 100,000 person-years. These data indicated that an estimated 0.3% (95% CI 0.1-0.5) of the population experience anaphylaxis at some point in their lives, with food, drugs, stinging insects, and latex being the most commonly identified triggers. Overall, the case fatality ratio from anaphylaxis was low, estimated at under 0.0001% (17).

A review by a Working Group of the American College of Allergy, Asthma, and Immunology including a number of non-European studies, concluded that the overall incidence of anaphylaxis was between 30-60 cases per 100,000 person-years and 950 cases per 100,000 person/years, with a lifetime prevalence 0.05-2.0% (18). The lower estimates by Panesar *et al.* may reflect differences in diagnostic criteria for anaphylaxis between Europe and North America.

Moreover, it is estimated that 1 in every 3,000 inpatients in US hospitals suffer from an anaphylactic reaction with a risk of death around 1%, accounting for 500 to 1000 deaths annually in this country (19).

Based on these statistics, anaphylaxis would fit well the definition of a rare disease, although it is not currently listed in rare diseases registries (20). In public health terms, anaphylaxis is considered to be an uncommon cause of death (2, 21-23). The case fatality rate is difficult to ascertain with accuracy. Accurate anaphylaxis mortality data are hampered by the limited recognition of this condition among health professionals, the absence of historical details from eyewitnesses, incomplete death scene investigations, paucity of specific pathologic findings at postmortem examination, and the under-notification of anaphylaxis, particularly in the International Classification of Diseases, Injuries and Cause of Death (ICD) (2, 20, 24). Although currently misclassified in the ICD, anaphylaxis is now one of the principal headings in the "Allergic and hypersensitivity conditions" section recently compiled for the forthcoming 11th Revision of ICD (ICD-11). Thanks to this inclusion, it is expected that anaphylaxis should be a public health priority and that it should therefore be formally added into the list of rare diseases in order to support awareness and quality clinical management of patients.

As for the triggers, a pan-European registry for severe allergic reactions collecting 3333 cases of anaphylaxis, showed that allergic reactions were mainly caused by food and insect venom and less often by drugs (25). Most reactions occurred within 30 min of exposure (80.5%); a delay of 4+ hours was mainly seen in drug anaphylaxis (6.7%). Symptom patterns differed by elicitor, with the skin being affected most often (84.1%). Usually previous milder reaction to the same allergen was reported by 34.2% (25).

Data collected in the European Anaphylaxis Registry from 2007 to 2015 (25), allowed to characterize anaphylaxis in children and adolescents (26). Food items were the most frequent trigger (66%), followed by insect venom (19%). Cow's milk and hen's egg were prevalent elicitors in the first 2 years, hazelnut and cashew in preschoolaged children, and peanut at all ages.

There was a continuous shift from food- to insect venom- and drug-induced anaphylaxis up to age 10 years. Vomiting and cough were prevalent symptoms in the first decade of life, and subjective symptoms (nausea, throat tightness, and dizziness) were prevalent later in life. Most incidents occurred in private homes (46%) and outdoors (19%). One third of the patients had experienced anaphylaxis previously (26).

Looking at epidemiological data from from intensive care units pediatric anaphylaxis admissions, 1989 patients were reported from 2010 to 2015 in the United States and Canada, the most common identified trigger being food (mainly peanuts) (27). One percent of patients died because of critical anaphylaxis, and identified triggers for fatal cases were food (peanuts and milk) and blood products (27).

Comparing European patients aged > 65 (elderly: 1, 123) with adults (18-64 years: 5.768) regarding elicitors, symptoms, comorbidities, and treatment measures, insect venoms were the most frequent elicitor in the group ($p < 0.001$), followed by drugs like analgesics and antibiotics (28). Food allergens elicited less frequently anaphylaxis ($p < 0.001$). Skin symptoms occurred less frequently in elderly patients (77%, $p < 0.001$). The clinical symptoms were more severe in the elderly (51% experiencing grade III/IV reactions), in particular when skin symptoms ($p < 0.001$) were absent. Most strikingly, a loss of consciousness (33%, $p < 0.001$) and preexisting cardiovascular comorbidity (59%, $p < 0.001$) were more prevalent in the elderly (28).

Risk factors and co-factors

The risk to develop severe reactions like anaphylaxis, may depend on several factors, including the allergens an individual patient is sensitized to, the degree of sensitization, the quality of binding allergens, probably also the relative proportions of antigen-specific immunoglobulin subtypes, the route of allergen application, and finally, the presence and 'amount' of risk factors and cofactors (29). According to some authors, 'risk factor' is a general term covering any factor, which may lead to more severe allergic reactions, including augmenting factors (also called aggravating factors), concomitant diseases and cofactors (30), while others distinguish between risk factors and co-factors (2, 31).

Very recently, processing the data from the European Anaphylaxis Registry (122 centers in 11 European countries) higher age (not related to concomitant cardiovascular or other diseases) and concomitant mastocytosis have been identified as the most important predictors for an increased risk of severe anaphylaxis (32). Vigorous physical exercise, male sex, and psychological burden were more often associated with severe reactions. Moreover, intake of beta-blockers and ACE inhibitor (ACE-I) in temporal proximity to allergen increased the risk to develop severe anaphylaxis exposition in logistic regression analysis, while ASA and AT-2 did not (32). Indeed, it was recently shown that beta-blockers (BBs) and the ACE inhibitor (ACEI) ramipril can directly promote mast cell activation and are associated with increased odds for severe anaphylaxis (33).

In contrast, a systematic review and metaanalysis of studies that assessed the influence of BBs and ACEIs on anaphylaxis showed low quality of evidence that the use of BBs and ACEI increases the severity of anaphylaxis, due to differences in the control of confounders arising from the concomitant presence of cardiovascular diseases (34).

In a large observational cohort study performed in the United States from 2005 to 2014, age of 65 years or older, medication as a trigger, and presence of comorbid conditions (specifically cardiac and lung disease) were associated with significantly higher odds of severe anaphylaxis (35). On the other hand, evidence showing that respiratory disease increases the severity of anaphylaxis according to a recent a systematic review and metaanalysis is low to moderate, although studies do not usually assess the importance of severity of asthma (36).

A genetic diversity should be also included among the host factors influencing anaphylaxis in some cases of food and drug allergy (37, 38). Polymorphisms affecting metabolism of mediators of anaphylaxis also can influence anaphylaxis severity, since PAF-AH activity levels inversely correlated with severity of anaphylaxis (39, 40), and subjects with variants in angiotensinogen were reported to have increased rates of Hymenoptera venom allergy (41). D816V mutations are found in some patients with mast cell disorders and recurrent anaphylaxis to Hymenoptera stings (42, 43).

It is of note that variations in metabolism of mediators, can influence not only the manifestations of anaphylaxis, but theoretically also the ability to recover from these manifestations in patients who have experienced anaphylaxis and survived the episod even though not treated (44).

Risk factors for severe anaphylaxis may be different also according to the trigger, as demonstrated in the case of HV allergy. Mastocytosis and monoclonal mast cell activation syndrome (MMAS) are well known risk factor for severe and even fatal anaphylaxis due to Hymenoptera stings, while this association is less clear for drug hypersensitivity (45).

Also risk factors for fatal anaphylaxis vary according to cause. For fatal drug anaphylaxis, previous cardiovascular morbidity and older age are risk factors, with beta-lactam antibiotics, general anesthetic agents, and radiocontrast injections the commonest triggers (46). For fatal food anaphylaxis, delayed adrenaline administration is a risk factor; common triggers are nuts, seafood, and in children, milk. For fatal venom anaphylaxis, risk factors include middle age, male sex, white race, cardiovascular disease, and possibly mastocytosis; insect triggers vary by region. Upright posture was reported a a feature of fatal anaphylaxis to both food and venom (46).

The so-called co-factors may explain why an allergen can either be tolerated or trigger a mild reaction or, in the same patients, induce a severe anaphylaxis. They may have two different effects: lowering the threshold, so that severe allergic reactions may be observed at much lower doses of allergen; increasing the severity, meaning that more severe reactions are elicited by the same dose of food or anaphylaxis is observed for the first time. Co-factors play a role in 30% of all anaphylactic reactions in adults and in 18% of children. The most frequent co-factors are exercise, non-steroidal anti-inflammatory drugs (NSAID), alchool, but menstruation, infections, medications other than NSAID (*e.g.* antiacids), extreme air temperature, cannabis use, stress and disruption of routine have been also reported (47).
The vast majority (90%) of excercise-induced anaphylaxis are related to food ingestion (FDEIA) (29, 48). Most of the data for FDEIA focusses on wheat triggers, being ω-5 gliadin the culprit proteins in most cases (49), even though in Mediterranea area lipid transfer proteins (LTPs) seem to be the most frequent sensitizer (50). In other studies (31) NSAID were the most frequent co-factor enhanced food allergy, followed by exercise, with LTP allergens being again the allergen most frequently involved.
The underlying mechanisms in FDEIA are still unclear and several hypotheses have been proposed, like exercise increasing gastrointestinal permeability, increasing activity of tissue transglutaminase in the gut mucosa, inducing blood flow redistribution, and finally, exercise increasing histamine release from basophils because of an increase of plasma osmolarity (51).
The frequent implication of cofactors in anaphylaxis highlights the importance of recognizing and including them into diagnostic workup (52).

Phenotypes and endotypes

Anaphylaxis involves the activation of multiple pathways (**table I**). Its endotypes can be divided according to the underlying mechanism and/or the effector cells involved in the reaction. A recent classification of anaphylaxis phenotypes has been proposed: type-I-like reactions, cytokine storm-like reactions, mixed reactions and complement-mediated reactions (1). Endotypes, underlying these phenotypes, are based on biological and molecular mediators supported by biomarkers. Type-I-like reactions are characterized by classical allergic symptoms (*e.g.* urticaria, pruritus, shortness of breath, throat tightness, nausea, vomiting, diarrhea, cardiovascular collapse), frequently (but not always) IgE-dependent, due to foods, drugs, Hymenoptera venoms, and environmental allergens. Cytokine storm-like reactions, as well as mixed reactions, are usually elicited by chemotherapy or biological agents and additionally induce some atypical symptoms such as chills, fever or pain. Finally, reactions mediated by complement can be induced by contrast dyes or dialysis membranes, among others, and provoke hypotension and desaturation.

Type-I-like reactions
These reactions represent the vast majority and include immune-mediated and non-immune-mediated pathogenesis. Antibody dependent anaphylaxis includes IgE-mediated and IgG-mediated reactions. In IgE-mediated reactions, anaphylaxis initiated by an allergen interacting with allergen-specific IgE (sIgE) bound to its high-affinity receptor (Fce RI) expressed on effector cells, mainly mast cell and basophils (44). Mast cell mediators responsable for allergic symptoms are represented by preformed mediators stored in the cytoplasmic granules released by degranulation such as histamine, the proteases tryptase and chymase, carboxypeptidase A and proteoglycans (with heparin as the major component); newly generated proinflammatory lipid mediators (*i.e.* prostaglandins, leukotrienes and PAF); and newly synthesized growth factors, cytokines and chemokines.
Mast cell, IgE and Fce RI depletion in animal models suppresses anaphylaxis (53-55), indicating that this pathway is crucial. In human anaphylaxis, the use of anti-IgE antibody, omalizumab, as an adjuvant treatment in food and venom immunotherapy reduces the risk (56, 57) and it prevents anaphylaxis in patients with systemic mastocytosis (58, 59). However, the presence of antigen-specific IgE antibodies does not indicate that the person necessarily will exhibit any, let alone severe, clinical reactivity to the recognized antigens. On the other hand, severe anaphylaxis can occur despite low levels or undetectable specific-IgE (sIgE) (44), suggesting the existence of IgE-independent mechanisms.
Not definitive evidence of IgG-mediated anaphylaxis in human subjects is present to date. It has been suggested that IgG-mediated anaphylaxis in humans requires considerably more antigen than IgE-mediated anaphylaxis, such as in reactions to infused drugs such as contrast media and antivenoms, due to the lower affinity of IgG binding by Fcg RIII than of IgE binding by Fce RI (60). Indeed, cases of anaphylaxis were reported after treatment with therapeutic monoclonal antibodies (mAbs) without detectable levels of anti-drug IgE (61, 62).
Basophils have been shown to be dispensable for IgE-mediated anaphylaxis but play a crucial role in IgG-mediated anaphylaxis in murine models, through their release of PAF and their ability to bind immune complexes via the low-affinity IgG receptor Fcg RIII (63). Recently, an emergency department study recruiting 31 patients with acute anaphylaxis, predominantly to Hymenoptera venom, showed that human anaphylaxis involves a substantial reduction in numbers of circulating basophils, which inversely correlate with serum CCL2 levels, a major basophil chemotactic factor, thus implying an important and specific role for basophils in the pathophysiology of human anaphylaxis (64).
Several drugs can induce direct nonimmunologic type-1-like activation of mast cells/basophils by basic secretagogues, including vancomycin, NSAIDS, opiates, fluoroquinolones and neuromuscular blocking agents. For example, opiates induce histamine release presumably through a mechanism that involves

opioid receptors in mast cell (65). Vancomycin is able to directly activate mast cell leading to histamine release in the 'red man syndrome' through a calcium-dependent mechanism that involves activation of phospholipase C and phospholipase A2 (66). An alternative mechanism, based on non IgE-mediated mast cells activation by means of the G-protein-coupled receptor X2 (MRGPRX2) has been identified. The receptor MRGPRX2, which is expressed on mast cells and other cells, has been shown to be activated by quinolone antibiotics, such as ciprofloxacin and levofloxacin; general anesthetics, such as atracuronium and rocuronium; icatibant; and other drugs with Tetrahydroisoquinoline (THIQ) motifs (67), even though its partecipation has not been confirmed in human subjects.

Contact and coagulation system can be activated in anaphylaxis through immunological and nonimmunological mechanisms (**table I**). The latter was related with oversulfated condroitin-contaminated heparin causing severe anaphylaxis through direct activation of factor XII (FXII) of the contact system and release of bradykinin (68). During the acute phase of human anaphylaxis, a strong consumption of contact system factors has been observed, associated with mast cell degranulation and increased plasma heparin levels, being heparin a potent FXII activator (69).

Finally, an activating mutation in c-KIT D816V promotes mast cell proliferation in patients with clonal mast cell disorders, including mastocytosis, in whom type-I-like anaphylaxis may occur with or without known triggers, with or without specific IgE sensitization (70).

Cytokine storm-like reactions

Cytokine storm-like reactions are caused by release of proinflammatory mediators, such as TNF-a, IL-1B, and IL-6, and the target cells include monocytes, macrophages, mast cells, and other immune cells with FcgR (11, 71). Triggers for these reactions include chimeric, humanized, and human mAbs and chemotherapy, including oxaliplatin. Reactions are characterized by chills, fever, generalized malaise followed by hypotension, desaturation, and cardiovascular collapse.

Similar to infusion-related reactions, cytokine-release reactions to mAbs can occur at first infusion, even though they have also been seen after several exposures. The difference between the two reactions is the self-limiting nature of infusion-related reactions on repeat exposure and the response to premedication (72-74). Premedication with anti-inflammatory COX-1 inhibitors and corticosteroids can decrease the intensity of cytokine release reactions but does not protect from severe reactions.

Mixed reactions

Mixed reactions with features of type I – and cytokine storm – like reactions can be seen with chemotherapy and mAbs in which pruritus, hives, and swelling are associated with chills, fever, hypotension, and desaturation (11, 72).

Complement-mediated reactions

Complement-mediated anaphylaxis may also occurr through immunological and nonimmunological mechanisms (**table I**). The anaphylatoxins C3a and C5a are potent inflammatory mediators generated upon activation of the complement cascade. Mast cell, ba-

Table I *- Immunologic and nonimmunologic pathways in anaphylaxis.*

Type of anaphylaxis pathway	Mechanism	Effector cell	Main mediator involved
Immunologic	IgE-dependent	Mast cell/basophil	Histamine, tryptase, chymase, carboxipeptidase,
	IgG-dependent	Basophil/Macrophage/Neutrophil	heparin, PAF PAF
	Complement system	Mast cell/macrophage	Histamine, PAF
	Contact system/Coagulation system (Kallikrein-FXII system)	Endotelial cells	Bradykinin
Nonimmunologic (*physical factors, ethanol, drugs*)	Complement system	Mast cells	
	Mast cell/basophil activation		
	- *Quinolones*	Mast cells	Histamine, tryptase
	- *Neuromuscolar blockers*	Mast cells	Chymase, Heparin, PAF
	- *NSAIDs*	Mast cells	
	- *Opiates*	Mast cells	
	- *Vancomicin*	Mast cells	
	- *Contact system/Coagulation system*	Endothelial cells	Bradykinin

sophils and monocytes/macrophages express receptors for C3a and C5a (75), and release histamine and/or PAF in response to exposure to these complement fragments. In human and mice anaphylaxis, complement activation by peanut (76, 77) or wasp-sting acted synergistically with IgE-dependent mast cell activation (78).
Activation of complement without immune complex formation has been shown to induce anaphylaxis in the absence of specific IgG or IgE. This mechanism has been described in association with hemodialysis, liposomal drug infusion, radiocontrast media, polyethylene-glycol infusion and micellar solvents containing amphiphilic lipids (*e.g.* Cremophor EL, diluent in propofol or paclitaxel) or liposomal doxorubicin (79-81).

Biomarkers

A biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (82). The ideal biomarker should be highly specific, sensitive, predictive, rapid and easy to measure, cheap, stable *in vivo* and *in vitro*, and noninvasive. Biomarkers in anaphylaxis hold the potential for improving diagnosis, stratification of severity, risk prediction, and therapeutic management, even tough they have no role for the moment in acute management.
Tryptase is considered a largely mast cell-derived product, being present in much lower amounts also in basophils. Mature β-tryptase is stored in mast cell granules and released on activation, such as in anaphylaxis, whereas α- and β-protryptases are secreted constitutively by mast cells, and therefore increased blood levels might indicate increased mast cell burden rather than anaphylaxis (83). Tryptase is much more stable than histamine and blood samples for its measurement are optimally obtained 15 minutes to 2-3 hours after symptom onset (84). Even though commercial methods measure total serum tryptase (immature and mature), this assay is still the best routine biomarker available to assess mast cell activation. Increased serum tryptase levels often support the clinical diagnosis of anaphylaxis from insect stings or injected medications and in patients who are hypotensive; however, levels are often within normal limits in patients with anaphylaxis triggered by food and in those who are normotensive (85). Furthermore, we must bear in mind that in anaphylactic reactions in which the main involved effector cell is not the mast cell, tryptase may not rise. Serial measurement of tryptase levels during an anaphylactic episode, and measurement of a baseline level after recovery are reported to be more useful than measurement at only one point in time (85). The "20% + 2 formula" has been validated in clinical practice and currently considered significant in clinical practice as a criterion of severe systemic mast cell activation and mast cell activation syndrome (MCAS) (86).
Histamine is also a marker of mast cell and basophil activation. Blood samples for measurement of its levels are optimally obtained 15-30 minutes after symptom onset. In fact, plasma histamine peaks within 5-10 min of the onset of symptoms and declines to baseline within 30 min as a result of rapid metabolism by N -methyltransferase and diamine oxidase (2).
Blood tests for other biomarkers, such as chymase (87), carboxypeptidase A3 (88), and CCL-2 (89) remain experimental.
Despite mast cell heparin has been reported to activate the plasma contact system during anaphylaxis, there are no available assays to measure it directly. Anti-Xa is an indirect measure of plasma heparin, but commercial assays are not sensitive enough (69).
PAF is a potent phospholipid-derived mediator implicated in platelet aggregation and it is secreted by mast cells, monocytes and fixed tissue macrophages (90). A limited number of reports have assessed concentrations of PAF or platelet-activating factor acetylhydrolase (PAF-AH), an enzyme responsible for the rapid degradation of PAF, after anaphylaxis in human subjects. In these reports circulating PAF levels were increased, and circulating PAF-AH activity was inversely correlated with the severity of anaphylaxis (39, 40, 91). One of the challenges with measurement of PAF and PAF AH in a routine clinical setting is its very short half-life and special sampling and transport precautions that are required thus making it an unattractive candidate for routine use (84).
CysLTs are potential mediators of anaphylaxis and are synthesized from arachidonic acid by a variety of cells, including mast cells, basophils, and macrophages (44). Several reports show that levels of some of these products, namely LTE4, 2,3-dinor-9a,11b-PGF2, and 9a,11b-PGF2, are increased during the onset of anaphylaxis (92, 93). However, like histamine, they have to be measured by 24-h urine collection, meaning that the sensitivity might be low.
Finally, levels of other serum inflammatory mediators, such as TNF-a, IL-6, and IL-1b, can be increased in patients with cytokine storm-like reactions and anaphylaxis, but their sensitivity or specificity has not been demonstrated. Among them, IL-6 has been pointed out as a potential biomarker for identifying and managing cytokine-release reactions (72).

Diagnosis

If the trigger is highly suspect, an accurate clinical history collection togheter with conventional diagnostic tools are sufficient to confirm the diagnosis. However, in many cases, especially in polysensitised patients, other procedure may help to confirm the cause, for istance component-resolved diagnosis (CRD), and basophil activation test (BAT).

Skin testing

The rationale of skin testing lies in the presence of an IgE-mediated pathogenesis and mast cell involvement. If performed within 2 to 4 weeks after anaphylaxis, skin tests are highly specific for type I reactions to Hymenoptera venoms (HV), foods, drugs (*e.g.*

beta-lactams, general anesthetics, platins). Unlike HV and drug allergies where both prick and intradermal test should be performed, only skin prick test (SPT) are indicated for food allergy diagnosis. Associations between SPT wheal size and severity of reaction on food challenge have been observed in a few studies, but these findings have not been consistent among studies (94).
In HV allergy, the sensitivity of the prick test is lower than the one of the intradermal test; intradermal tests should be performed even in case of positive prick test to identify correctly the cutaneous end-point which will be useful in VIT follow-up; in case of negative tests in subjects with a suggestive history, tests should be repeated after 1-2 months (95).
As for immediate hypersensitivity reactions to beta-lactams (BL), skin tests are more sensitive than in vitro test (96); even tought they become negative with time (93), they should be performed with great caution in case of anaphylaxis (97). It is important to enphasize that skin testing to penicillins requires major and minor determinants (93), the latter being available only in some European countries (98).
Skin testing in perioperative anaphylaxis are useful and all drugs/agents used before the reaction should be tested. An IgE-mediated mechanism has not been demonstrated for all drugs/agents, and a validation of skin testing is lacking. Testing and subsequent interpretation should be performed by experienced personnel using standardised concentrations as several drug groups, especially NMBAs and opioids, can cause irritant skin reactions (99).
For antibiotis other than beta-lactams as well as many other drugs, skin testing lacks well-defined predictive values. Positive skin tests with nonirritant concentrations are suggestive of drug-specific IgE; however, negative skin tests are less helpful due to unclear negative predictive values (100).
Finally, patients with cytokine storm-like reactions and complement activation are likely to have negative skin test results, indicating the lack of IgE participation, but patients with mixed reactions can have positive skin test results (71).

Serum specific IgE

Together with clinical history and SPTs, serum specific IgE (ssIgE) determination is commonly used for diagnosis of food allergy, thus reducing the need for food challenge (101). However, the clinical utility of sIgE for assessing risk of severe reactions has not been yet established (94).
The sensitivity of serological tests using HV whole extracts is generally lower than that of skin tests, and for *Vespula spp.* it is lower than the one for bee venom. In general, *in vitro* tests for the search of specific IgE toward the whole extract of venom can be negative in up to 20% of patients with positive skin tests, wher eas approximately 10% of patients with negative skin test are positive at *in vitro* test, suggesting to perform both tests (102).
SsIgE determination in BL allergic reactions has to be performed togheter to skin testing since cases with immediate hypersensitivity reactions to BL with negative ST and positive ssIgE have been reported (103). As for ST, also the sensitivity of ssIgE decreases with time, thus suggesting to be performed as soon as possible after the reaction (104). In patient with BL anaphylaxis, clearly positive ssIgE can be useful for avoiding both ST and drug provocation test (DPT) (105).
SsIgE quantification can be used for a limited number of drugs in the perioperative setting. The reported sensitivity and specificity are very good for sIgE for latex and chlorhexidine, but show great variation for NMBAs and morfines (99).
In general, ssIgE determination cannot be used for the evaluation of the majority of drugs able to induce IgE-mediated reactions (104).

Component Resolved Diagnosis (CRD)

CRD, using single molecules or panels of allergens, is a new tool which has revolutionized allergy diagnosis in recent years, helping to improve diagnostic accuracy, and in some cases providing information on risk assessment and consequently on management (106). Nevertheless, we must note that is only able to assess sensitization and not clinical reactivity.
As for food allergy, CRD may be helpful in complicated, polysensitized patients, mixed food intake, as well as in cofactor-enhanced food allergy (ω-5-gliadin, and nonspecific lipid transfer proteins (nsLTP) (31, 50, 107) and in red meat delayed anaphylaxis (α-Gal) (108).
Anaphylaxis has been associated with certain components, such as seed storage proteins (2S albumins, 7S vicilins, and 11S legumines) or nonspecific lipid transfer proteins (nsLTPs) (109), even tough severity risk attributed to specific molecules may vary according to other factors such as geographic variations, degree of allergen exposure, cosensitizations, and cofactors (110).
In the field of HV allergy, CRD may discriminate between primary sensitization and cross-reactivity in patients with double/multiple positivity in diagnostic tests with whole extracts (especially in case of bee and yellow jacket double sensitization), allowing the specialist to choose the most suitable venom for specific immunotherapy (VIT), avoiding unnecessary VIT and reducing the risk of side effects (111). CRD may be useful in patients with negative allergy tests and a proven history of a previous systemic reaction, including those with mast cell disorders, who could benefit from VIT. In honeybee venom allergy, different sensitization profiles have been identified, which could be associated with a greater risk of VIT failure or treatment side effects (112, 113).
Latex allergy may be an important cause of anaphylaxis, even though its incidence has decreased in the last 10 years. In this regard, monosensitization to Hev b 8 (profilin) suggests cross-re-

active and asymptomatic sensitization, whereas markers of genuine allergy like Hev b 1, Hev b 3, Hev b 5, and Hev b 6, may potentially induce severe reactions, thus making it necessary to apply avoidance measures (114).
A diagnosis of idiophatic anaphylaxis (IA) is based on exclusion of known triggers of anaphylaxis, as well as conditions that can masquerade as anaphylaxis (115). The diagnostic utility of an allergen microarray (ImmunoCAP ISAC) in the detection of possible allergenic triggers in patients with unexplained anaphylaxis has been evaluated, showing evidence of sensitization to newly identified allergens (mainly wheat, shrimp and peanut) that had not been detected during routine allergy workup, even tough an allergen challenge procedure to confirm the diagnosis was not performed (116). Finally, CRD has no application in the field of drug allergy.

Basophil Activation Test (BAT)

Among blood-cell based tests, BAT is the most widely used in Europe for diagnostic purposes, in selected situations, and in highly specialized laboratories.
The BAT has shown to be more accurate than IgE sensitization tests and able to distinguish individuals that were clinically allergic from those who were tolerant to some food like peanut, showing a high specificity, thus dispensing from doing food provocation test (117).
The BAT can identify approximately two thirds of HV allergic patients with positive history and negative skin and serological tests (118). It is also recommended in patients with double positive results and inconclusive results of *in vivo* or *in vitro* tests with recombinant allergens (119). The role of BAT as a diagnostic tool in patients with mastcell disorders and negative venom-specific IgE and skin test results is still controversial (95).
Taking into account the diagnostic difficulties specific to drug allergy and risks related to provocation test in the case of anaphylaxis, the BAT should theoretically represent a safer opportunity to be improved. Several studies over the last 15 years have reported the diagnostic accuracy of BAT for allergy to a range of drugs including betalactams, quinolones, platins, and neuromuscular blocking agents (NMBAs) (120).

Provocation test

The food allergen provocation test (FAPT) provides a gold standard diagnostic for food-related adverse reactions leading to appropriate food avoidance (101). However, a severe anaphylactic reaction to a given food with highly positive IgE tests, and a history of several reactions to the same food, will in most cases not need a FAPT (121). The use of CRD in some cases may help to grading the risk of a positive reaction to FAPT (122, 123).
The sting challenge has a low predictive value as a patient with a negative test might still react on a field sting. It differs from other provocation tests in that incremental allergen exposure is not possible and insect biology and several other factors may influence the test result. Even if it may have an indication during VIT to verify its efficacy, it is contraindicated in untreated patients and after stopping the treatment (124).
Drug provocation test (DPT) is a part of drug allergy workup. The majority of the studies were perfomed with BL. The European guidelines and the U.S. practice parameter give different indications to the DPT with BL, and a significant heterogeneity in European current practice has been recently demonstrated (105). However, in the case of anaphylaxis DPT should be avoided regardless of the type of drug involved.

Idiopathic anaphylaxis and mastcell diseases

Idiopathic anaphylaxis is a diagnosis of exclusion and mandates careful consideration of all recognizable and rare causes of anaphylaxis (115, 125, 126). The clinical manifestations and management of acute episodes are the same as for other forms of anaphylaxis. A good clinical history is paramount to direct further investigations. Idiopathic anaphylaxis represents an opportunity for identification of previously unrecognized novel triggers and also for identification of mastocytosis or clonal mast cell disorders. Particular attention should be paid to "hidden allergens," cofactors (*i.e.* wheat-dependent exercise-induced anaphylaxis), galactose alpha-1,3 galactose (a carbohydrate contained in red meat) allergy, pigeon tick bite (Argax reflexus), and Anisakis simplex allergy (115). Out of the 30 cases of IA with no signs of cutaneous mastocytosis, 47% were found to have an aberrant MC population and were subsequently diagnosed with clonal mastcell (MC) disorder (127). Similarly, the presence of a clonal mast cell population with a diagnosis of IA was reported, in whom there were no features of urticaria pigmentosa or histological evidence for systemic mastocytosis on bone marrow (BM) biopsy (128). These findings demonstrate a need for robust criteria for BM examination in cases of suspected clonal MC disorders in the context of IA. The only available validated tool is the Spanish Network on Mastocytosis (Red Española de Mastocitosis - REMA) scoring system, based on a combined clinical (*i.e.* gender and clinical symptoms) and laboratory (baseline tryptase value with "cut-off" of 15-25 ng/mL) parameters, to predict underlying MC clonality in patients presenting with systemic MC activation symptoms, including anaphylaxis (129).
Other differential diagnoses include "allergy-mimics" such as asthma masquerading as anaphylaxis, undifferentiated somatoform disorder, panic attacks, globus hystericus, vocal cord dysfunction, scombroid poisoning, vasoactive amine intolerance, carcinoid syndrome and phaeochromocytoma (115). Diagnosis must be revisited in cases with recurrent episodes, where there is paucity of clinical signs and/or in the context paucity of refractoriness to corticosteroid therapy.

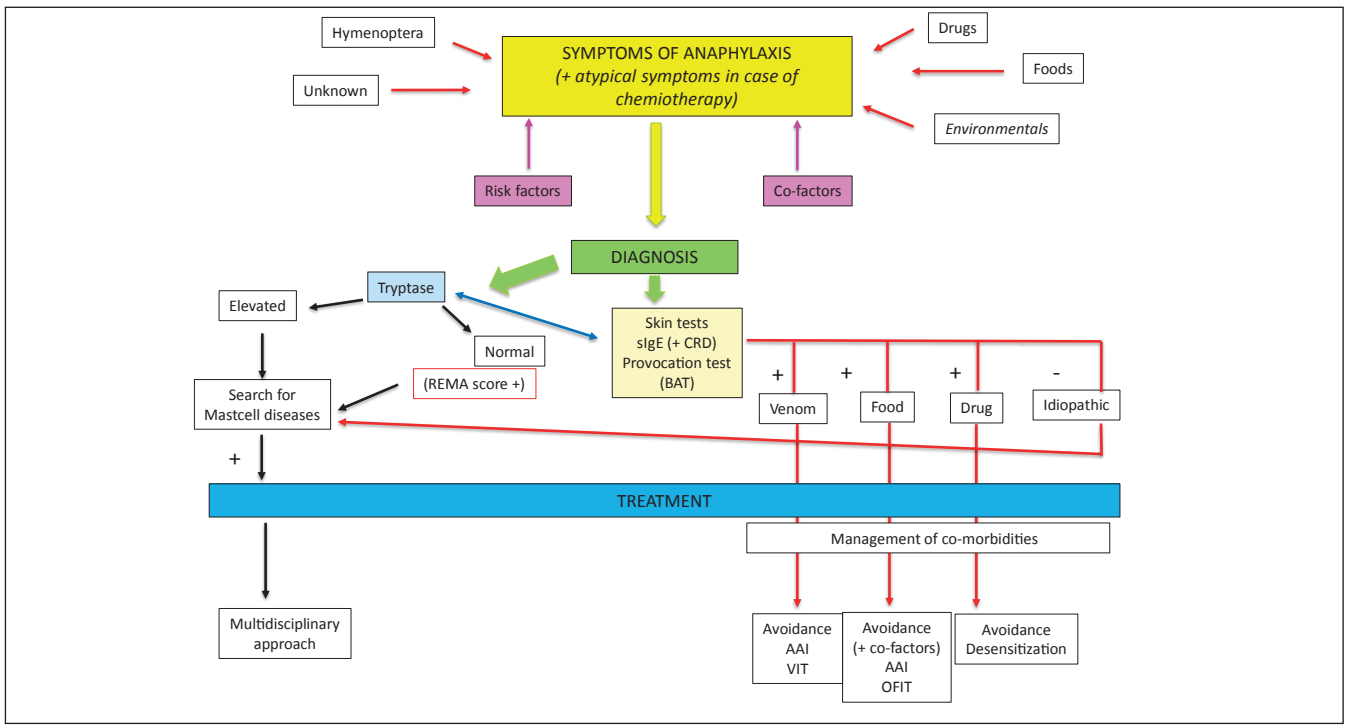
Treatment and management

Patients with a history of anaphylaxis have an increased risk of severe reactions in the future, thus indicating that secondary prevention measures are of paramount important, as suggested by different international guidelines (1, 2). The most important preventive measures include the identification, and consequent avoidance of triggers and co-factors, the recognition by the patient of the first symptoms indicative of anaphylaxis, the availability of an (AAI) and management training, optimal management of relevant co-morbidities, venom specific immunotherapy, food oral immunotherapy, and drug desensitization, when indicated (**figure 1**).

Acute treatment: adrenaline

The acute management and treatment of anaphylaxis depend on early recognition and prompt use of adrenaline, as it is the first-line treatment of anaphylaxis and it is life-saving (1-6). Treatment of anaphylactic reactions in the hospital setting should adhere as closely as possible to guidelines. Indeed, only 27.1% of European patients with anaphylaxis treated by a health professional received adrenaline and, in total, 10.5% received a second dose (130). Interestingly, successful administration was less frequent in German-speaking countries (minimum 19.6%) than in Greece, France, and Spain (maximum 66.7%). Nevertheless, over the last decade adrenaline administration from a health professional almost doubled to reach 30.6% in 2015-2017, probably reflecting improved guideline distribution and awareness (130).
All patients with a history of anaphylactic reaction should be provided with adrenaline autoinjectors to be injected into the vastus lateralis muscle (1-6). There are six absolute indications for a prescription of an adrenaline auto-injector (1): previous anaphylaxis with food, latex, aeroallergens such as animals or other unavoidable triggers; previous exercise-induced anaphylaxis; previous IA; co-existent unstable or moderate to severe, persistent asthma with food allergy; venom allergy in adults with previous systemic reactions (unless receiving maintenance VIT) and children with more than systemic cutaneous reactions; and underlying mast cell disorder and any previous systemic reaction. European Guidelines (1) suggest also to consider prescribing at least one adrenaline auto-injector with any of the following additional factors (especially if more than one is present): previous mild-to-moderate allergic reaction to peanut and/or tree nut; teenager or young adult with a food allergy; emote from medical help and previous mild-to-moderate allergic reaction to a food, venom, latex, or aeroallergens; previous mild-to-moderate allergic reaction to traces of food. Indications for prescription of a second adrenaline auto-injector have been also suggested (1). Of note, the European Medicines Agency (EMA),

Figure 1 *- Algorithm for the management of anaphylaxis in clinical practice.*



VIT (venom immunotherapy); AAI (adrenaline autoinjector); OFIT (oral food immunotherapy); BAT (basophil activation test); CRD (component resolved diagnosis).

after evaluation of all available data, recommended healthcare professionals to prescribe two autoinjectors (European Medicine Agency, 26 June 2015. EMA/411622/2015).
According to the data from the Anaphylaxis Registry (131), an AAI prescription was offered to only 37% of the patients ouside specialized centers compared to 84% of the patients in specialized allergy centers, highlighting the need of better education for primary healthcare and emergency physicians to follow guidelines. In the multivariate analysis, the elicitor of the reaction (less prescriptions in patients with food allergy than in those with venom allergy), age of the patient (less prescriptions in babies and elderly patients), mastocytosis as comorbidity, severity of the reaction, and reimbursement/ availability of the autoinjector influence physician's decision to prescribe one (131).
An integral part of the anaphylaxis action plan is represented by advising patients to carry the device with them at all times, as well as instructing the patient on how to use the autoinjector through educational material and practical training (1, 2). Nevertheless, in Europe few lay- or self-treated cases receive an autoinjector (14.7%), even tough clinical severity considerably influence the likelihood of receiving adrenaline (130). Moreover, it is alarming that no change in successful administration by lay emergency respondents was found over the last 10 years (130), underlining the persistence of several gaps in the management of severe allergic reactions (132).
Although many patients are afraid to use their AAI (133), no significant adverse effects have been reported, with the exception of the known onset of tachycardia, tremors, and peripheral vasoconstriction (134).
Finally, corticosteroids and antihistamines are not lifesaving, they have not been demonstrated to prevent biphasic anaphylaxis and their use should never delay adrenaline administration (1, 2).

Specific immunotherapy and desensitization

Specific subcutaneous immunotherapy for hymenoptera venom is the only treatment able to protect patients from systemic reactions after subsequent stings (protection against reported in 91-96% of cases, 77-84% for bee allergy) (135). Nonetheless, VIT offerslong lasting protection upon re-sting even after discontinuation of treatment, and increases dramatically the quality of life of HVA patients (95, 135). It is effective and safe even in patients with mastcell diseases (136). As rush and ultra-rush protocols offer rapid protection from re-sting as early as the maintenance dose is achieved, they should be offered to patients with severe reactions.
Even though oral food immunotherapy may increase the amount of a tolerated dose over time (137), and enhances sustained unresponsiveness that persists after cessation of therapy (138), there are currently no established oral immunotherapy treatment protocols for food-induced anaphylaxis. Since significant systemic side-effects can occur, currently this treatment is not recommended in clinical practice (101).

Drug desensitization to drug is a highly effective readministration strategy for those patients who develop hypersensitivity reactions to their needed medications, like chemotherapeutic agents, mAbs (72, 139, 140), antibiotics (141) and many other drugs (142). Of note, it has been documented that carboplatin-desensitized patients had a non-statistically significant lifespan advantage over nonallergic controls, indicating that the efficacy of carboplatin was not reduced in allergic patients and that desensitization protocols are as effective as regular infusions (140). Even patients presenting with type I and cytokine-release reactions to mAbs are thought to be candidates for desensitization (11).
The anti-IgE mAb Omalizumab has been shown to be a successful treatment for reducing the number and severity of anaphylactic reactions in association with VIT (58) or with food oral immunotherapy (143).

Conclusions

Anaphylaxis is the most severe allergic reaction, it involves multiple organ systems, can be caused by a number of triggers and conditions, and be deadly. Although rapid advances in allergy and immunology concerning the identification of new allergens, biomarkers and cofactors, as well as the availability of new diagnostic tools, there are still many gaps in evidence and knowledge. There is still much to be done to identify genetic and epigenetic markers and cofactors for determining risk of anaphylaxis to specific allergens, performing an individual risk assessment, and preventing future episodes by developing personalized risk reduction strategies. Gaps in knowledge and anaphylaxis management have been observed at different levels, at the level of patients, community as well as physicians (131). Diagnosis of anaphylaxis, evaluation of the severity of the allergic reaction, and the use of adrenaline is insufficient for many physicians and a gap between best practice and Emergency Department (ED) care has been also reported. These findings highlight the need for an easier definition of anaphylaxis especially for non-allergists to improve the diagnosis and consequently the appropriate treatment with adrenaline.
Further identified gaps in the management of anaphylaxis include infrequent or delayed use of AAI by the patients for acute allergic reactions, as well as inadequate AAI training, and prescription rates for patients at risk (144). A recent review of a number of English language anaphylaxis management plans underlines a wide variety of content, with no plans having 100% of the recommended material (145). Therefore, more appropriate training for patients, families and caregivers of patients are necessary.
Finally, very few studies are being designed to determine how to increase adherence to existing anaphylaxis guidelines and best practice through integrated knowledge translation strategies.

Conflict of interests

The authors declare that they have no conflict of interests.

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