



Phenotypes, endotypes and biomarkers in anaphylaxis: current insights

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Purpose of review

The aim of the review is to describe the different clinical pictures of anaphylaxis (phenotypes), in relation to the underlying mechanisms and potential biomarkers, to describe anaphylaxis endotypes. This may aid in achieving a better understanding, management and outcomes of such severe reactions.

Recent findings

Different anaphylaxis phenotypes have been outlined, ranging from the classical type-I-like to those suggestive of cytokine-storm-like or complement-mediated reactions. Underlying mechanisms differ and biomarkers of cells and systems involved are being identified (tryptase, IL-6, bradykinin etc.)

Summary

Identifying specific phenotypes/endotypes will allow the application of precision medicine in patients with anaphylaxis, providing insights to the most appropriate approach in each case.

Keywords

anaphylaxis, biomarkers, endotypes, mast cell, phenotypes

INTRODUCTION

Anaphylaxis is a systemic reaction resulting from the noxious effects of mediators released by the sudden activation of mast cells and/or basophils. Nevertheless, anaphylactic reactions are varied regarding nature and route of exposure to triggers, organ involvement, severity and time course, among other characteristics. Therefore, instead of considering anaphylaxis a single entity, it should be considered a syndrome in which different phenotypes and/or endotypes may be described, linked to the activation of different cells and physiopathological systems, ultimately leading to a wide variety of clinical manifestations. This review aims to describe the potential mechanisms involved in anaphylactic reactions, resulting in a number of distinct phenotypes, and to evaluate the usefulness of biomarkers to endotype patients with anaphylaxis. Such classification may aid in achieving a better understanding, management and outcomes of such severe reactions.

ANAPHYLAXIS PHENOTYPES

Anaphylactic reactions are complex clinical pictures conditioned by a wide variety of intrinsic and extrinsic factors, rendering a number of phenotypes depending on which characteristic is assessed [1], as shown in Fig. 1.

Several authors have identified drugs and stings as elicitors usually associated with more severe reactions [2,3] either due to the administration route, nature of the allergen or intrinsic factors of the patient (age, underlying conditions, medications). Different organ involvement occurs; some patients may develop preferentially cutaneous, respiratory, cardiovascular or gastrointestinal symptoms, depending on age, underlying conditions (i.e. asthma or mast cell disorders) or eliciting allergen [4].

Severity is conditioned by several factors. Causing agent, degree of sensitization, medical conditions of the patient and presence of cofactors have been described as relevant [5^{••}]. The time course of the reaction, despite usually being immediate and rapid, in some occasions may be delayed (i.e. delayed red-meat anaphylaxis), with an ulterior

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KEY POINTS

- Anaphylaxis is a complex syndrome which may exhibit different phenotypes.
- Pathophysiology of anaphylaxis involves multiple pathways beyond IgE, and several biomarkers may help to identify specific anaphylactic endotypes.
- Endotype-driven acute and long-term management of patients with anaphylaxis requires further research.
- There is an urgent need for a point-of-care diagnostic biomarker in anaphylaxis.

progression to a short-lived reaction or to a protracted or biphasic course. Again, this may be trigger, host or treatment dependent [6,7]. Cofactors such as exercise, nonsteroidal anti-inflammatory drugs or alcohol may amplify the allergic reaction increasing severity and/or decreasing the threshold of the reaction [8,9].

Until date, no univocal relationship between the clinical expression and the underlying mechanisms has been established, despite some data indicate the preferential involvement of specific pathways in some reactions. A recent classification of anaphylaxis phenotypes has been proposed: type-I-like reactions, cytokine storm-like reactions,

mixed reactions and complement-mediated reactions [10[■]]. Type-I-like reactions are characterized by classical allergic symptoms, frequently (but not always) IgE-dependent. 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.

Biomarkers in anaphylaxis

Moving into the era of precision medicine, biomarkers will be important to allow endotyping of patients, to personalize management and to develop new treatments for anaphylaxis. 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' [11]. Biomarkers in anaphylaxis are critical to confirm diagnosis, define prognosis, predict risk and severity, and to monitor therapeutic response [11,12[■],13]. The ideal biomarker should be highly specific, sensitive, predictive, rapid and easy to measure, cheap, stable *in vivo* and *in vitro*, noninvasive and relevant to guide clinical decisions (Table 1).

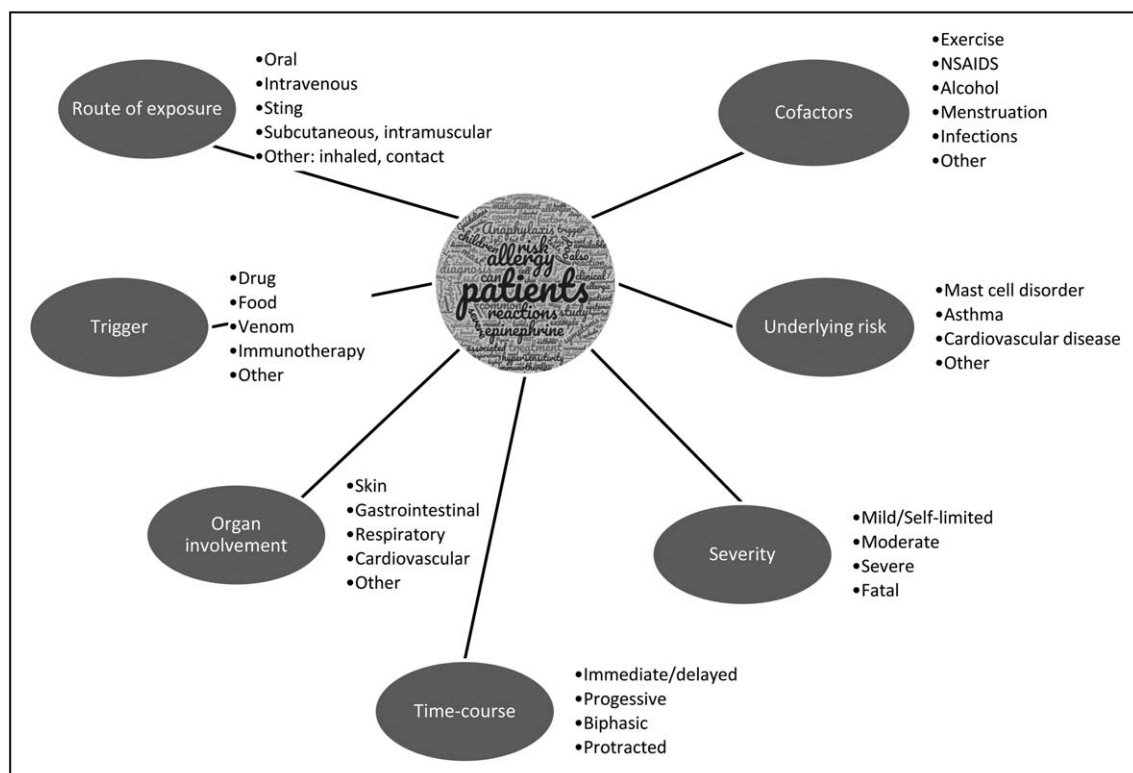


FIGURE 1. Factors influencing anaphylaxis phenotypes.

Table 1. Features of an 'ideal' biomarker in anaphylaxis

Biomarker choice
Related to anaphylaxis mechanisms
Long half-life in blood
Easily accessible sampling
Assay
Sensitive and specific
Rapid and easy
Robust and reproducible method
Noninvasive
Patient management
Improve anaphylaxis diagnosis
Improve stratification of anaphylaxis severity
Improve risk prediction of anaphylaxis
Improve therapeutic management

Adapted from [14].

Biomarkers should allow the identification of different mechanisms directly related to phenotypes and/or endotypes. Despite intramuscular adrenaline is considered the treatment of choice in anaphylaxis, independently of the phenotype, additional measures and long-term management may differ. Thus, biomarker-guided therapy could improve outcomes. In Table 2, the potential function of different biomarkers in anaphylaxis is shown.

Tryptase is a serine protease present in large amounts in human mast cell and in much lower amounts in basophils. It is highly specific of these cells, providing information about mast cell distribution, number and activation. Serum tryptase concentrations above 11.4 ng/ml suggest mast cell/basophil activation [15] and levels during anaphylaxis correlate with severity [16–19]. However, the main limitation of tryptase is that sensitivity is suboptimal, remaining low in up to 40% of anaphylaxis [16–19], due to several reasons. At least four tryptase genes have been described [20], and 27% of the population lack alpha-tryptase genes, limiting peak plasma levels [21]. Therefore, increases above 2 ng/ml and 1.2 times baseline concentration are currently considered significant [22]. In addition, tryptase contents of mast cell vary, being lower in mucosal than in perivascular and cutaneous mast cell. Available commercial methods measure immature and mature tryptase. To increase diagnostic performance in anaphylaxis, only tetrameric mature beta-tryptase, the form released during mast cell-driven anaphylaxis, should be measured. However, total serum tryptase is still the best routine biomarker available to assess mast cell activation, being a robust, easy and reproducible method, although

Table 2. Potential role of biomarkers in anaphylaxis

Biomarker	Diagnosis	Predictive/ severity	Risk of anaphylaxis
Tryptase	✓	✓	✓
PGD2	x	✓	nk
Histamine	✓	✓	x
Leukotrienes	x	✓	nk
PAF	x	✓	nk
PAF-AH	x	✓	✓
Bradykinin	x	✓	nk
Carboxypeptidase A3	✓	x	nk
Chymase	✓	x	nk
IL-6	✓	✓	nk
TNF- α	x	✓	nk
IL-1- β	x	✓	nk
IL-10	x	✓	nk
IL-2	x	✓	nk
TNF- γ	x	x	nk
sTNFRI	x	✓	nk
IL-5	x	x	nk
IL-4	x	x	nk
IL-13	x	x	nk

Tick refers that the role in anaphylaxis has been shown and cross means that it has not been demonstrated. Those that have not been explored are marked as not-known (nk). IL, interleukin; PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetyl-hydrolase; PGD2, prostaglandin D2; sTNFRI, soluble tumor necrosis factor receptor 1; TNF, tumor necrosis factor.

the long duration of commercial assays precludes its use as a point-of-care diagnostic tool.

Histamine is also a marker of mast cell and basophil activation and correlates with anaphylaxis severity [19]. 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 [23]. Therefore, blood samples need to be obtained at the onset of symptoms, limiting its applicability. Its metabolite, methyl-histamine, can be measured in 24-h urine. Although specific for anaphylaxis, the sensitivity is low because of the difficulties in 24-h sampling.

Chymase and carboxypeptidase A3 are secreted only by mast cell and basophils, and potentially good biomarkers of mast cell activation, but no validated commercial assays are currently available [10²²].

Despite mast cell heparin has been reported to activate the plasma contact system during anaphylaxis [24], 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 [24].

Leukotriene C₄, prostaglandin D₂ and its metabolite (11- β -PGF₂- α) are secreted by activated mast cell and basophils. However, platelets, eosinophils, endothelial and vascular smooth muscle cells also produce them. Like histamine, they have to be measured by 24-h urine collection, so the sensitivity might be low. A study showed high levels during anaphylaxis correlating with severity [25].

Platelet-activating factor (PAF) is a proinflammatory phospholipid synthesized and secreted by mast cell, monocytes, neutrophils and macrophages [26,27]. When PAF binds to its receptor on platelets, monocytes, macrophages and neutrophils, it may induce typical manifestations of anaphylaxis [28]. Because of its rapid inactivation by PAF-acetylhydrolase, it has a short half-life, ranging from 3 to 13 min, limiting its potential use as diagnostic biomarker in anaphylaxis [28]. However, there is a good correlation between PAF levels and the severity of anaphylaxis, whereas low levels of PAF-AH are associated with fatal peanut anaphylaxis, and could therefore be a severity predictor [29].

Different studies have evaluated the role of cytokines [IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN- γ and TNF- α and TNF receptor I (TNFRI)]. Their concentrations are elevated during anaphylactic reactions; however, only IL-2, IL-6, IL-10 and TNFRI correlate with severity [19]. IL-2, IL-6, IL-10 and TNFRI peaked around 100 min and remained sustained for as long as 10 h. Brown *et al.* [30] found that tryptase, histamine, IL-6, IL-10 and TNFRI levels were associated with severity and with delayed deteriorations. Nevertheless, the cell-source of these cytokines may be extremely varied, hampering their role in

endotyping specific mechanisms or cells involved. However, IL-6 levels have been pointed out as a potential biomarker for identifying cytokine-release reactions [31^{***}]. Their measurement provides insight into the phenotype of reactions and can help guide recommendations for desensitization and premedication.

Anaphylaxis endotypes

Due to the life-threatening nature of anaphylaxis, the lack of 'ideal' biomarkers and the difficulty of collecting samples during acute reactions, only limited data on the pathophysiology of human anaphylaxis are available. Although IgE-mediated anaphylaxis is thought to be the main pathway, increasing evidence from animal models supports that alternative pathways may play an important role [32,33]. Anaphylaxis endotypes can be divided according to the underlying mechanism or the effector cells involved in the reaction (Table 3).

Immune-mediated anaphylaxis

Antibody-dependent anaphylaxis includes IgE-mediated and IgG-mediated reactions. In IgE-mediated anaphylaxis, the key effectors cells are mast cell and basophils [34^{*}]. Whereas allergen-specific IgE can be detected in tolerant subjects to allergen exposure, severe anaphylaxis can occur despite low levels or undetectable specific-IgE (sIgE) [35], suggesting the existence of IgE-independent mechanisms. Mast cell, IgE and Fc ϵ RI depletion in animal models [36–39] suppresses anaphylaxis, indicating

Table 3. Endotypes and effector cells in anaphylaxis

Type of anaphylaxis	Mechanism	Effector cell/receptor
Immunologic anaphylaxis	IgE-dependent	Mast cell/basophil: Fc ϵ RI
	IgG-dependent	Macrophage/neutrophil: Fc γ RIII, Fc γ RI
	Complement system	Mast cell/macrophage: C3aR and C5aR
	Contact system	Endothelial cells
Nonimmunologic anaphylaxis	Complement system	Mast cell: C3aR and C5aR
	Mast cell/basophil activation	
	Vancomycin	Mast cell: phospholipase C and A2R
	NSAIDs	Mast cell: CysLTR
	Opiates	Mast cell: opiate receptor
	Quinolones	Mast cell: MRGPRX2
	Neuromuscular blockers	Mast cell: MRGPRX2
	Contact system	Endothelial cell

Fc ϵ RI, receptor for the Fc region of IgE; Fc γ RIII, receptor III for the Fc region of IgG; Fc γ RI, receptor I for the Fc region of IgG; C3aR, receptor for C3a; C5aR, receptor for C5a; phospholipase A2R, phospholipase A2 receptor; CysLTR, receptor for cysteinyl leukotrienes receptor; MRGPRX2, mas-related G-protein-coupled receptor member X2.

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 [40,41], and it prevents anaphylaxis in patients with systemic mastocytosis [42,43]. However, other studies have shown that anaphylaxis can occur after animal allergen immunization followed by parenteral challenge in the absence of the IgE/FCεRI and mast cell axis [44²²]. Thus, compelling evidence observed both in humans and animal models suggest that sIgE levels alone do not fully explain the susceptibility to anaphylaxis.

IgG-mediated anaphylaxis has been mainly explored in animal models. Antigen cross-linking with specific IgG bound to IgG receptors on macrophages, neutrophils and basophils induces PAF release [26]. Manifestations in IgG-dependent anaphylaxis in mice resemble human IgE-dependent anaphylaxis [26,45]. Macrophages are the main effector cells in this pathway; they express FcγRI and release PAF, which is thought to be the main mediator. Nevertheless, macrophages play a role in both IgE and IgG-dependent pathways by responding to mast cell mediators released after IgE/Ag cross-linking and by acting as effector cells in IgG-mediated anaphylaxis. Recently, the role of other cells such as neutrophils has been established. A mouse model of anaphylaxis showed that allergen-specific IgG2 binding to FcγRIV on neutrophils led to activation and release of PAF [46]. On the other hand, several markers of neutrophil activation and trafficking in patients with IgE-dependent anaphylaxis have been described [47]. In mouse models, the depletion of neutrophils completely inhibited anaphylaxis, whereas replenishment with either mouse or human neutrophils restored the anaphylactic potential [46]. In human anaphylaxis, neutrophil activation markers, such as myeloperoxidase and soluble CD62L, are increased, and this observation suggests a role of neutrophils in amplifying the mast cell response to the antigen [48²³].

Also, the amount of antibody and antigen seems to be crucial in determining IgG or IgE-dependent anaphylaxis. Thus, to induce a similar response in IgG-dependent anaphylaxis requires higher doses of antigen than the IgE pathway and higher levels of serum IgG [49]. In this case, IgG antibodies block antigen binding to FcεRI-bound IgE, but IgG/antigen complexes can bind FcγRs [44²²,49,50]. By contrast, when antibody levels are low, only IgE reactions can occur.

Studies have shown that human IgG receptors are capable of activating macrophages and neutrophils to secrete PAF [51], and then PAF can activate mast cell *in vitro* [52], reinforcing the role of PAF in

human anaphylaxis. However, it is important to keep in mind that PAF can also be produced by mast cell and basophils in response to IgE cross-linking [53].

In patients receiving mAbs, such as infliximab or adalimumab, anaphylaxis can occur in the absence of detectable sIgE but in the presence of specific IgG [54]. The evidence of human IgG-mediated anaphylaxis has been demonstrated in studies showing the presence of increased frequency of a gain-of-function allele of the stimulatory FcγRIIA in patients with common variable immunodeficiency who have IgG-anti-IgA who developed anaphylaxis after intravenous immunoglobulin infusion [55]. Thus, in subjects receiving large amounts of antigen parenterally, with high levels of IgG and undetectable specific IgE, the existence of an IgG-mediated anaphylaxis appears to be plausible [44²²].

Complement-mediated anaphylaxis has also been described. C3a, C4a and C5a, known as anaphylatoxins, are potent inflammatory mediators generated upon activation of the complement cascade. Mast cell, basophils and monocytes/macrophages express receptors for C3a and C5a [56,57] and release histamine and/or PAF in response to exposure to these complement fragments. Injection of low doses of C3a induces wheal-and-flare reactions [58]. The lack of C3a and C5a receptors in mice mast cell is related with reduced IgE-mediated anaphylaxis [59]. In human and mice anaphylaxis, complement activation by peanut [60,61] or wasp-sting acted synergistically with IgE-dependent mast cell activation [62]. Moreover, levels of C3a and C5a produced during human peanut-induced anaphylaxis correlate with severity [60]. In addition, C3a exerts an additive effect on mast cell activation by aggregated IgG through FcγRI [63].

Nonimmunological anaphylaxis

A number of nonimmunological mechanisms have been proposed through mechanisms by which mast cell and basophils could be activated without involvement of IgE, IgG or immune complexes.

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 [44²²,64,65]. These agents activate complement through both the classical and alternative pathways, releasing C3a and C5a.

Several drugs can induce direct nonimmunologic activation of mast cells/basophils by basic secretagogues, including vancomycin, NSAIDs, opiates, fluoroquinolones and neuromuscular blocking agents. 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]. NSAIDs block cyclooxygenase, resulting in decreased levels of PGE2, which can suppress anaphylaxis, and increased levels of cysteinyl leukotrienes, increasing vascular permeability [67]. Opiates induce histamine release presumably through a mechanism that involves opioid receptors in mast cell [68]. Fluoroquinolones and some neuromuscular blocking agents can also directly activate mast cell by binding to Mas-related G-protein-coupled receptor member X2 through a chemical motif present in these drugs [69,70]. Nevertheless, most of these drugs can also induce anaphylaxis via an IgE-mediated mechanism, so a correct allergological diagnosis has to be performed.

Contact and coagulation system can be activated in anaphylaxis through immunological and nonimmunological mechanisms. The latter was related with oversulfated chondroitin-contaminated heparin causing severe anaphylaxis through direct activation of factor XII (FXII) of the contact system and release of bradykinin [71,72]. In human [24,73] and mouse models of IgE-mediated anaphylaxis [74], a strong consumption of contact system factors has been observed, associated with mast cell degranulation and increased plasma heparin levels. Heparin, mainly produced by activated mast cell, is a potent FXII activator. Moreover, the severity of anaphylaxis is correlated bradykinin formation and the intensity of contact system activation. In addition, FXII or bradykinin receptor 2 deficient mice, resistant to bradykinin signaling, were protected from systemic hypotension during anaphylactic reactions [24], indicating contact system activation and contribution to systemic anaphylaxis.

Mast cell mediators may also be involved in the activation of the coagulation system and may play a role in anaphylaxis. Mast cell tryptase has been shown to participate in fibrinolysis through plasmin activation and by facilitating the degradation of fibrinogen.

CONCLUSION

Better understanding of the underlying mechanisms, pathways, cells and mediators involved in anaphylaxis will allow a better management of this severe condition. Different clinical phenotypes may be identified, and biomarkers for specific endotypes

are needed to stratify severity, predict risk and to optimize treatment choice in each case. Recognition of the acute reaction remains a crucial issue, and a point-of-care diagnostic biomarker would be a major achievement.

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Conflicts of interest

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- of special interest
- of outstanding interest

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