Anaphylaxis: mediators, biomarkers and microenvironments

Short title: Biomarkers of anaphylaxis

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ABSTRACT

The life-threatening nature of the anaphylactic reactions promotes a growing interest to discover new biomarkers that could support their better diagnosis and prevention. However, both the clinical features and the etiopathology of anaphylaxis are very diverse, hindering the elucidation of valuable molecular indicators of disease.

Most studies of anaphylaxis have focused on the immune system. Precisely, anaphylactic reactions are characterized primarily by IgE-mediated activation of mast cells and basophils and the release of several mediators. Among them, determinations of the serum tryptase levels is the main in vitro test used to confirm the reaction and there are no available biomarkers with predictive capacity for this pathological event. However, recent research has postulated that alternative pathways, cell types and systems are involved. Consequently, different molecular products have been explored and indicated as potential biomarkers, but none of them have been translated into the clinical practice yet. Precisely, vasoactive agents, proteases, proteoglycans, lipids, interleukins, cytokines, products of the complement-contact and coagulation systems, circulating proteins, extracellular vesicles, microRNAs and metabolites have been found to be altered in patients with anaphylaxis. The recognition of biological processes and molecular pathways interacting in the magnitude of the microenvironments switched on in anaphylaxis will notably nourish the clinical practice and the recognition of better molecular markers. Therefore, this article covers a broad review of the different mediators described in anaphylaxis and their proposal as biomarkers of this pathological event, as well as their role in the molecular basis of the reaction.

Keywords: Anaphylaxis. Biomarker. Mast cells. Mediators. Microenvironments.
RESUMEN

La naturaleza potencialmente mortal de las reacciones anafilácticas promueve un creciente interés por descubrir nuevos biomarcadores que puedan ayudar a su diagnóstico y prevención. Sin embargo, tanto las características clínicas como la etiopatología de la anafilaxia son muy diversas, lo que dificulta la identificación de marcadores moleculares precisos.

La mayoría de los estudios sobre la anafilaxia se han centrado en el sistema inmunitario. En concreto, estas reacciones se caracterizan principalmente por la activación de mastocitos y basófilos mediada por IgE y la liberación de diferentes mediadores. Entre ellos, la determinación de los niveles de triptasa sérica es la principal prueba in vitro utilizada para confirmar la reacción y no existen biomarcadores con capacidad predictiva.

Sin embargo, estudios recientes han postulado que existen otros tipos celulares, sistemas y vías de señalización alternativos implicados en la anafilaxia. En consecuencia, se han explorado e identificado diferentes moléculas como potenciales biomarcadores, pero ninguno de ellos se ha trasladado aún a la práctica clínica. Precisamente, los agentes vasoactivos, las proteasas, los proteoglicanos, los derivados lipídicos, las interleuquinas, las citoquinas, los productos de los sistemas de complemento-contacto y de coagulación, las proteínas circulantes, las vesículas extracelulares, los microARN y los metabolitos, se encuentran alterados en pacientes con anafilaxia. El reconocimiento de los procesos biológicos y de las vías moleculares que interactúan en la magnitud de los microambientes implicados en la anafilaxia mejorará notablemente la práctica clínica y el reconocimiento de marcadores moleculares. Por lo tanto, este artículo abarca una amplia revisión de los
distinctos mediadores descritos en la anafilaxia y de su propuesta como biomarcadores de este evento patológico, así como su implicación en las bases moleculares de la reacción.


**INTRODUCTION**

Anaphylaxis is an acute systemic reaction and the most severe manifestation of allergic disorders. Currently, the incidence of this pathological event is considered to be underdiagnosed due to the lack of effective biomarkers. Specifically, the main molecular marker used in clinical practice is serum tryptase, which is not elevated in most cases. In this regard, anaphylactic reactions are classified into different phenotypes and endotypes, pointing to mast cells (MCs) and basophils as the main releasers of this biomarker. However, other molecular and cellular components have also been characterized for human anaphylaxis [1].

The term "biomarker" includes any biological observation that replicates or predicts a clinically relevant endpoint. However, they can also serve to prognosticate pathological conditions, to identify different cells involved in their etiopathogenesis, or to assess an individual's response to various treatments. In clinical practice, a biomarker is usually a mediator involved in the cellular basis of the pathology [2]. Recent key statements in anaphylaxis point to the necessity to identify reliable diagnostic, predictive and prognostic biomarkers [3]. Therefore, the aims of this review are to delve into the different molecules altered during anaphylaxis and to determine their role in the different microenvironments involved in the reaction.

1. **ANAPHYLAXIS**
The World Allergy Organization defines anaphylaxis as “a serious systemic hypersensitivity reaction that is usually rapid in onset and may cause death. Severe anaphylaxis is characterized by potentially life-threatening compromise in airway, breathing and/or the circulation, and may occur without typical skin features or circulatory shock being present” [4]. Among the systems affected, the most common signs and symptoms are observed in the skin and mucous membranes (80-90%), followed by the respiratory system (70%). Nevertheless, less common is the involvement of the gastrointestinal (45%), cardiovascular (45%) and the nervous systems (15%) [5,6].

Worldwide, there has been an elevation in the incidence and prevalence of anaphylaxis over the last two decades [7–10]. This pathological event may be caused by different substances, being food and drugs the most common triggers. Precisely, foods are the most frequent in children, while drugs are more prevalent in adults [11].

However, epidemiological data are underestimated because of the absence of biomarkers and the ambiguity of the diagnosis of anaphylaxis [4,5].

The diagnosis of this pathological event is based on the recognition of its different signs and symptoms [4,12]. Moreover, it could be complemented by the measurement of serum tryptase (sT), the main biomarker used in clinical practice [4]. Over the years, many studies have evidenced the increase of this molecule in patients’ sera [13–17]. This protein peaks appear from 15 minutes after the onset of reaction symptoms and last for several hours [18,19]. However, sT levels do not correlate always with anaphylaxis severity. These one remain low in 36-50% of the events [14,20]. Specifically in mild cases where the reaction is not well diagnosed clinically [20].

Despite of the large variability of sT values, the clinical threshold for this protein in anaphylaxis is set to a value equal or over 11.4ng/ml [14,21]. Nevertheless, there are also
cases of MC activation with sT levels below this cut-off point [15,22,23]. Therefore, it has been seen that is recommended to consider sT at least 24 hours after the episode [4,19]. Specifically, the difference between the acute peak of this protein and its baseline value was greater in anaphylaxis than in other diseases related to MC activation [19]. Accordingly, other different sT thresholds have been proposed for the diagnosis of this pathological event such as an increase of 20%+2ng/ml in acute condition compared to baseline [15,19,24]. In addition, a study carried out in Hymenoptera venom allergic patients defined MC activation if acute sT values ≥135% relative to basal [22]. Precisely, a recent study points to an improved diagnostic capacity of tryptase when a personalized medicine is applied considering the patient's baseline status [25].

2. PATHOPHYSIOLOGY OF ANAPHYLAXIS

Anaphylaxis is an acute and systemic reaction where several organs and systems are involved. This is caused by the simultaneous activation of multiple molecular mechanisms and the sudden release of mediators which give rise to the signs and symptoms of this pathological event [26]. From years, studies have been focused on the analysis of the immune component, although the importance of other altered systems in the etiopathogenesis of anaphylaxis has been demonstrated [27,28]. Likely, other cells, still unexplored, contribute to the release of these or other mediators (TABLE 1). Therefore, understanding the plethora of specific microenvironments is the beginning to identify appropriate biomarkers and apply a correct management associated with precision medicine [29].

2.1 Immune System

Classically, anaphylaxis has been considered a type I hypersensitivity reaction taking place through an IgE-mediated immunological mechanism. When it occurs, mediators are
released from activated MCs and basophils giving rise to the sign and symptoms of this pathological event [18]. However, degranulation of these cells can be achieved by different surface receptors and mechanisms attending to the nature of the stimulus (FIGURE 1) [30,31].

IgE-mediated reactions are divided in two phases: one of sensitization and other of re-exposure/activation. In primary sensitization, allergens induce the release of IgE into circulation, which binds to the high-affinity receptor for IgE (FcεRI) on the surface of MCs and basophils [32,33]. However, the onset of symptoms takes place after a new exposure to the allergen, where the activation of MCs and basophils and the sudden release of mediators occurs [34,35].

However, some patients experience anaphylaxis with undetectable allergen-specific IgE levels by skin or in vitro testing. Furthermore, elevated levels of allergen-specific IgE have been found in subjects who have not experienced a reaction [34,36–38]. Therefore, these events evidenced the presence of other process and cells involved in the reaction.

The existence of IgG-mediated anaphylaxis was firstly characterized in mouse models [39,40]. Currently, this pathway has been described in patients and could be also responsible for numerous episodes of IgE-independent reactions, although the number of studies in humans still remains limited [41–43]. IgG antibodies act through the binding to Fc gamma receptors (FcγRs) that are expressed in different cell types such as macrophages, dendritic cells, neutrophils, platelets, basophils and MCs [43–46]. Specifically, neutrophils have been described as the main cells involved in IgG-mediated human anaphylaxis [41,42,44]. Their activation is produced by the formation of IgG-allergen immune complexes and the subsequent binding to FcγRs receptors on their surface, leading to the release of many protein and lipid mediators [35,36,43]. Among
them, platelet activating factor (PAF) is one of the main molecules stored in neutrophils and its role in the pathogenesis of anaphylaxis has been importantly evidenced both in mice and humans [43,47].

2.1.1 Anaphylactic mediators released by immune cells

In anaphylaxis, the release of molecules previously stored in granules or newly synthesized has been classically understood as derived from MCs and basophils. However, the discovery of other cell types and the subsequent release of other mediators endow anaphylactic reactions of a wide nature. Among the broad number of molecules released, tryptase is the main one used as biomarker in the clinical practice, although many others have been proposed.

**Histamine**

Histamine is a diamine prestored in granules of MC and basophils and is considered, together with tryptase, one of the main mediators of anaphylactic reactions [48,49]. The mechanism of action of this vasoactive peptide is through four receptors (H1, H2, H3 and H4) that are expressed in numerous cell types [49–51]. Their activation give rise to different intracellular signalling pathways inducing a large part of the symptomatology of anaphylaxis such as the increase in vascular dilatation and permeability, as well as constriction of the airways [34,52,53].

In anaphylaxis, plasma levels of histamine increase becoming a promising biomarker [13,54,55]. A study of 97 patients presenting to the emergency department shows that almost half of them present elevated serum histamine levels. Likewise, another study in 76 patients found that there is a significant elevation of the molecule levels in severe reactions compared to moderate reactions [54,55]. Moreover, its detection in body fluids
is simple and some studies have even proposed it as a more sensitive diagnostic molecule than tryptase. However, its use as a potential diagnostic marker has several limitations [55,56]. Among them, its short half-life of approximately 30 minutes, peaking 5-10 minutes after the initiation of the reaction, makes it difficult to collect samples for optimal measurement [56,57].

**Enzymes: proteases and peroxidases**

Among the proteases released by the degranulation of effector cells, sT is the most relevant and easily detectable molecule in blood samples of patients with anaphylaxis. However, other proteases have been pointed as candidate biomarkers [36].

**Carboxypeptidase A** is a zinc-dependent metalloprotein and can also be found as a preformed mediator in basophils [56,58,59]. The levels of this protein have been found elevated in serum of patients with anaphylaxis and in those who died by drug-induced reactions [60]. However, their levels do not necessarily correlate with those of sT and may even appear to be increased in cases where sT levels remain low [36].

**Chymase** is a serine proteinase whose biological effects include the activation of the renin angiotensin system (RAS). Specifically, chymase activity is heparin-dependent, and its levels has been observed elevated in serum of anaphylactic patients up to 24 hours after the onset of the reaction [61,62]. In addition, an available study in patients who have died of anaphylaxis exhibit not only increased chymase levels, but also their value correlated with those of sT [63]. Moreover, the RAS is the main blood pressure homeostatic regulator contributing also to other processes, such as proliferation, fibrosis and inflammation [64]. Precisely, a study of 50 patients with anaphylaxis due to hymenoptera venom showed that **renin, angiotensinogen, angiotensin I** and
angiotensin II levels decreased significantly and in accordance with the severity of the reaction [65].

Elastase is a serine protease mainly released by neutrophils, although it can also be present in the granules of basophils and MCs [66]. This protein can cleave the kinin light chain activating the contact system [61,67]. On the other hand, myeloperoxidase (MPO) is a potent oxidizing enzyme contained in neutrophil azurophilic granules. precisely, neutrophil extracellular traps, detected as DNA-MPO complexes together with elastase, has been described as increased during human anaphylaxis correlating with the severity of the episodes [41,43,47].

Lipidic molecules

Bioactive lipids are involved in immune function and vascular biology. Consequently, their relevance in allergic diseases and anaphylaxis has also been recognized both molecularly and likely as a complementary biomarker of sT [68,69].

PAF is a biologically active phospholipid with a primary capacity of inducing platelet aggregation and which plays key roles in cardiovascular pathophysiology [70]. This mediator is released by abundant cell types and can be involved in processes such as inflammation, proliferation or cell adhesion, among others [71,72]. In anaphylaxis, PAF induces the release of several cytokines, as well as participates in bronchoconstriction, hypotension, and endothelial permeability [73]. In addition, its elevation is related with hemodynamic changes that occur in severe cases, including alterations in right ventricular pressure and total pulmonary resistance [44]. Consequently, this molecule has been proposed as a potential therapeutic target for this pathological event [53]. PAF is one of the most widely evaluated mediators as biomarkers in anaphylaxis, although it is not extrapolated to clinical practice. Studies carried out by Vadas et al. revealed that this
molecule increased in the plasma of adults and children according to the severity of the reaction [16,74]. Furthermore, when comparing with other biomarkers, as histamine and tryptase, it was observed that PAF correctly diagnosed a higher percentage of patients [16]. However, this mediator presents short half-life because it is rapidly inactivated. This fact represents a problem for its application in clinical practice since patients with anaphylaxis often attend the emergency department within minutes or even hours after the onset of symptoms [74]. The main enzyme involved in PAF degradation is PAF acetylhydrolase (PAF-AH) [75,76]. In turn, the activity of this enzyme has also been proposed as a possible molecular marker for anaphylaxis and different studies suggest that its levels drop during the reaction [17,74,77].

Sphingosine-1-phosphate (S1P) is a bioactive lipid involved in a broad spectrum of cellular process and can be supplied to plasma from MCs, erythrocytes, platelets, endothelial and smooth muscle cells [78]. This molecule plays a key role in the signaling of vascular homeostasis and allergic disorders [68]. In addition, the balance between intra- and extracellular S1P and their functional receptors attributes relevant features to several human inflammatory and allergic responses [79,80]. Intracellular levels of this molecule can be regulated by sphingosine kinases and are essential to maintain endothelial barrier function [81]. Precisely, in vivo systemic anaphylactic response has been associated with rapid depletion of circulating S1P concentrations [78,82].

Derived from arachidonic acid (AA) are synthesized in anaphylaxis and grouped into families including prostaglandins (PGE2, PGD2, PGF2α), prostacyclin (PGI2), cysteinylation-leukotrienes (LTC4, LTD4, and LTE4) and thromboxanes (TXA2) [83,84]. These molecules are derived from membrane bilayers through cyclooxygenase (COX) action or the lipoxygenase pathway. Precisely, early studies in anaphylaxis observed the
presence of slow reacting substances with biologic activity after antigenic stimulation on sensitized tissues [85]. Years later, those are considered a blend of prostaglandins and cysteinyl-leukotrienes released mainly through MC activation [35,86]. In addition, some of these molecules have been considered as valuable biomarkers in anaphylaxis [84,87,88]. Interestingly, non-steroidal anti-inflammatory drugs has been related with the increase of PGE2 and the production and overexpression of adenosine receptor 3 (A3) [89,90].

**Cytokines and chemokines**

Cytokines are released by a wide variety of cells, although the main contributors are those of the immune system. These proteins present diversity of functions and play a key role in immune responses, being mainly involved in inflammatory processes [91,92]. Specifically, in anaphylaxis, increased levels of several cytokines have been observed in the most severe cases [17].

**Tumor necrosis factor-α (TNFα)** is one of the most studied cytokines and during this pathological event it is mainly released by the activation and degranulation of MCs [93]. Indeed, increased levels of TNFα have been described in experimental models, and in serum samples of patients with anaphylaxis [54,94,95]. In turn, an elevation in the values of its receptor, TNF receptor I (TNFRI), has also been characterized in most severe human cases [17,54]. Accordingly, circulating levels of TNF-like weak inducer of apoptosis (TWEAK) as well as those of its receptor (Fn14) have been shown to increase in patients with anaphylaxis. These proteins have been shown to be crucial in the vascular permeability alterations underlying this pathological event [94].

**Tumor growth factor-β (TGF-β)** is mainly produced by activated MCs and carry out an inhibitory effect on the anaphylactic reaction by blocking the release of
TNFα [93,96]. This protein has been shown increased in serum levels of patients with anaphylaxis [97].

**Interferon-γ (IFNγ)** is a proinflammatory cytokine which plays a key role in the Th1 response [98]. In anaphylaxis, peripheral blood-derived mononuclear cells (PBMCs) obtained from patients have been shown to release higher levels of this protein [99]. In addition, elevated values have been observed in serum of patients with anaphylaxis [54].

**Granulocyte and monocyte growth stimulating factor (GM-CSF)** is a hematopoietic growth factor increased in patients with anaphylaxis. This protein modulates the maturation of several immune and inflammatory cells, including the main effector cells of the reaction [54]. In addition, it also plays a role in inflammation by mediating communication between tissues and immune cells [100].

**Platelet factor 4 (PF4)** is released by platelets following activation of the coagulation system [101]. Specifically, its levels have been found to be increased in rabbit anaphylactic models [102].

**Chemokines** play a role in attracting and guiding different families of leukocytes [103]. Among these, CCL-2, also known as monocyte chemoattractant protein (MCP-1), has been found increased in mice models and human anaphylactic samples [104–106]. In addition, a study conducted in serum from pediatric patients with anaphylaxis also demonstrated the increase of this chemokine, as well as CCL-11 [107].

**Interleukins**

The term interleukin (IL) was coined to describe cytokines produced by leukocytes, which act on these cells in a specific manner [108]. However, these mediators can be produced by many other cell types (including resident cells) and exert pleiotropic effects [109,110].
Serum levels of **IL-2, IL-4, IL-5, IL-6, IL-10 and IL-13** have been detected increased in patients with severe anaphylaxis [54]. Among these, **IL-2, IL-6 and IL-10** correlates with severity of the episodes, histamine concentration and the occurrence of hypotension [54,106,111]. Specifically, mouse anaphylactic models show that IL-6 is released within the first few hours after allergen exposure [112]. In humans, high levels of IL6 have been observed in hypersensitibiy reactions and anaphylaxis induced by monoclonal antibodies as well as in perioperative patients [106,113]. On the other hand, **IL-33** has been seen to promotes oral anaphylaxis after epicutaneous sensitization in experimental murine models [114].

### 2.1.2 Complement and contact/coagulation systems

Complement, contact and coagulation systems are involved in the pathophysiology of anaphylaxis [61,115]. These molecular cascades are in a constant crosstalk and in continuous amplification and inhibition loops keeping their effect balanced during an inflammatory situation. For example, components of the coagulation system amplify complement activation which, in turn, magnify coagulation and inhibits fibrinolysis [116]. Factors and molecules derived from their proteolytic processes have been observed to be altered in anaphylaxis. Therefore, it does not only indicate a potential function of these systems in hypersensitivity reactions, but also open the possibility of evaluating them as candidate diagnostic biomarkers.

**Complement system**

The complement system is composed of a complex cascade of proteases, participating in both the innate and adaptive immune responses through the activation of three distinguishable pathways: the classical, the alternative, and the lectin ones [117].
In anaphylaxis, complement seems to be mostly activated by the classical pathway through the formation of IgG immunocomplexes. This process derives in the release of C3a, C4a and C5a from the proteolysis of the C3, C4 and C5 components [117,118]. These soluble products, also known as anaphylatoxins, bind to specific G protein coupled receptors (GPCRs) in MCs and basophils stimulating their degranulation [119–121]. Indeed, levels of C3a, C4a and C5a have been found to be elevated in sera of patients with anaphylaxis and seem to be correlated with the severity of the reaction [17]. In addition, there is also clinical evidence that demonstrate the induction of this type of hypersensitivity reaction by agents that directly activate the complement system, which would confirm that anaphylatoxins alone would be capable of triggering the reaction [122].

**Contact/coagulation systems**

The contact system consists of a series of proteases and coagulation factors that are activated in response to inflammatory macromolecular complexes or negatively charged surfaces. In turn, it enables bradykinin (BK) formation and the activation of the intrinsic coagulation cascade whose ultimate goal is clot formation [61]. A relevant glycosaminoglycan released in anaphylaxis is Heparin that exhibits a key function in the activation of the contact system after MC and basophil degranulation. In addition, non-IgE immunologic anaphylactic reactions produced by the injection of oversulfated chondroitin sulfate contaminated heparin have been described [123]. Due to its negative charge, heparin is able to provoke the binding of high-molecular-weight kininogen (HK) to Factor XII (FXII) of the contact system inducing the autoactivation of FXII and the initiation of this cascade. Activated FXII induces the transformation of prekallikrein...
(pKK) into kallikrein (KK), a serine protease that will hydrolyze HK into BK, one of the most potent vasoactive mediators involved during anaphylaxis [61,124–126].

The contact, the coagulation and fibrinolytic systems are intimately related because FXII activation in turn induces, via Factor XI, the conversion of prothrombin to thrombin. This molecule, is capable of transforming fibrinogen into insoluble fibrin which, with the help of Factor XIII, will cause crosslinking of the fibrin polymers creating clots [61].

Overall, a study in serum samples from patients with anaphylaxis mediated by insect stings show that levels of Factor V, Factor VIII and fibrinogen were found to be decreased during the reaction [127]. On the other hand, regarding the contact system, other studies have shown decreased levels of FXII, pKK and HK, as well as elevated BK values in sera from anaphylactic patients [128,129].

2.2 Cardiovascular system

The cardiovascular system is closely related to the pathophysiology of anaphylaxis [130,131]. A recent detailed review exhibits the vascular disorders accounting in this event where an acute increase in vascular permeability, relaxation of vascular smooth muscle and constriction of vessels in the thoracic cavity happen [27].

Cardiovascular disease increases the risk of severe anaphylaxis and activated heart MCs release mediators in the thoracic cavity [130]. In relation, Kounis syndrome, a coronary hypersensitivity disorder caused by massive activation of cardiac MCs and characterized by acute myocardial damage, associates with anaphylaxis [132,133]. Specifically, an increase in troponins has been described in patients with this syndrome and anaphylaxis [134–136]. These proteins are part of the contractile apparatus of both skeletal and cardiac muscle cells and biomarkers of myocardial damage and myocardial infarction [137].
2.2.1 Cells and processes

Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) interact with each other and govern the vascular pathophysiological processes of anaphylactic reactions [27,115].

ECs form a monolayer called endothelium that functions as the physical barrier between blood and tissues regulating vascular permeability and blood pressure [115,138–140]. In anaphylaxis, mediators released during the reaction cause the opening of adherent junctions giving rise to an increase in vascular permeability and stimulating fluid extravasation [58,115].

On the other hand, VSMCs are responsible for the regulation of vascular tone through their contractile properties. This process is mainly modulated by changes in intracellular calcium levels and membrane depolarizations [27,141,142]. In anaphylaxis, mediators act on these cells causing relaxing or contractile processes in both bronquial and vascular smooth muscle cells [115].

2.2.2 Mediators derived from vessels

In anaphylaxis, ECs function as both receptors and effector cells releasing relevant agents as Nitric oxide (NO) [58,143–145]. This vasodilator and anti-inflammatory molecule is crucial in vascular homeostasis [146]. Precisely, mediators such as histamine and PAF induce its synthesis by activating endothelial NO synthase (eNOS) [52,58,143]. NO plays a key role in the pathophysiology of anaphylaxis by positively feeding back the vasodilatation underlying the reaction by triggering intracellular calcium depletion in VSMCs. In addition, this molecule has a hypotensive effect and decreases the levels of catecholamines [27,147]. Likewise, fractional exhaled NO has been detected increased in patients with anaphylaxis that exhibit respiratory symptoms [148].
**Endothelin-1** is a molecule with vasoconstrictor capacity since upon binding to its receptors on VSMCs induces an increase in the amount of intracellular calcium [149]. Murine models have evidenced that this mediator can induce MC degranulation [150]. In addition, levels of endothelin-1 have been detected to be elevated in tracheae from guinea pigs with anaphylaxis [151].

The **vascular endothelial growth factor (VEGF)** is a molecule released by ECs, VSMCs and MCs, among others, which induce vascular permeability [58,152]. Specifically, levels of this molecule have been found to be elevated in the serum of a patient with anaphylactic shock [153].

### 2.3 Nervous system

The nervous system is composed of the central nervous system (CNS), which includes the brain and spinal cord, and the peripheral nervous system (PNS), consisting of afferent sensory and efferent motor nerves [154]. This system monitors and responds to changes in the internal and external environment [155]. Therefore, it is essential for maintaining body homeostasis and reacting to acute stressors [156,157].

Numerous studies have described the importance of the nervous system in the integration and coordination of the immune response [156,157]. In allergic processes, this system participates in the regulation of antigen presentation, IgE production and MC degranulation, among others [156]. These functions are due to close associations between autonomic nerves and immune cells. Precisely, these cells can be activated through numerous neurotransmitters, and molecules released by their activation can also influence the neuronal activity [156].

In anaphylaxis, the histamine liberated acts through C- and A-γ fibres stimulating the liberation of **substance P (SP)** and **calcitonin gene-related peptide (CGRP)** from the
peripheral nerve endings [158]. These neuropeptides induce further activation of MCs through their specific surface receptors and intensify inflammatory response affecting arteriolar vasodilation and increasing blood flow [156]. In addition, tryptase can activate protease-activated receptor-2 (PAR2) on sensory neurons stimulating the liberation of SP and CGRP [158,159].

On the other hand, IgE and antigen-mediated MC activation induces the release of serotonin [160]. This molecule contributes to clinical signs of anaphylaxis and is associated with the severity of the episode [161]. Specifically, in mice models, serotonin is released in response to ovalbumin and act on the parasympathetic neuronal fibres. This event provokes acetylcholine liberation which has a direct effect inducing bronchoconstriction [162]. Moreover, activated human or mouse platelets contained high concentrations of serotonin and were able to induce significant hypothermia, probably acting through the 5-hydroxytryptamine receptor 7 (5-HT7) [160,163].

Another significant molecule reported to be released by the nervous system during anaphylaxis is adenosine [164]. This one produces vasodilatation, increases vascular permeability, and potentiates mediator release from stimulated MCs [164]. Additionally, adenosine may act on the nervous system causing the release of tachykinins from sensory nerve endings in a similar manner to BK [165].

Finally, clinical studies show the important role of the nervous system in anaphylaxis recovery [166,167]. Epinephrine, norepinephrine, and angiotensin II are molecules elevated minutes after the onset of an anaphylactic episode [167]. These mediators present vasopressor activity, so they compensate the vasodilation and extravasation of liquids produced during the reaction [34].

2.4 Other circulating blood mediators
Blood-derived circulant biomarkers have several advantages as they reflect the individual’s status, are easy to measure, and are obtained by minimally invasive methods [168]. Consequently, together with complement- contact- and coagulation-derived proteins, various anaphylaxis-related circulating molecules have been characterized and proposed as new molecular markers for this pathological event.

2.4.1 Circulating proteins

The blood contains many circulating proteins. Among them, human serum albumin (HSA) stands out since it alone accounts for 55% of the total concentration [169]. A recent study in 112 patients with anaphylaxis showed that both serum protein concentration and HSA levels decreased according to the severity of the reaction. Moreover, these measurements allowed for the first time to indirectly quantify the extravasation underlying this pathological event [25].

In addition, other circulating proteins have also been characterized in anaphylaxis. Apolipoprotein B (ApoB) inversely correlates with severity in a pediatric population of food-induced anaphylaxis [170]. Moreover, the decrease of serum apolipoprotein AI (ApoAI) has been suggested as a good promising biomarker for this pathological event [88]. In addition, a drop in this protein and apolipoprotein E (ApoE) levels has also been reported in the serum of anaphylactic mice models and human patients. However, the reduction of ApoE has also been observed in other inflammatory reactions, so it could not discriminate this pathological event correctly [171]. Similarly, haemoglobin β subunit (HBB) was found to be increased in 150 patients with mild hypersensitivity reactions and anaphylaxis [25].

2.4.2 MicroRNAs
MicroRNAs (miRNAs) are small non-coding RNA molecules (19-25 nucleotides) that govern the translation of messenger RNAs (mRNAs). Indeed, a single miRNA can modulate several mRNAs and the same mRNA can be regulated by different miRNAs [172]. Thus, they participate in a wide range of physiological processes such as proliferation, differentiation or survival, among others [173].

Alterations in circulating miRNAs levels have been associated with a wide variety of diseases [174]. Furthermore, these molecules can be related to the status of the individual, making them non-invasive and promising biomarkers, although they have not yet been incorporated into clinical practice [173,175].

Diverse studies carried out in mouse models suggested the involvement of these molecules (miR-155, miR-154-5p, miR-26a, miR-26b, miR-122a-5p, miR-135-5p and miR-182-5p) in the allergic inflammatory processes behind anaphylaxis [176–180]. In human samples, the analysis of miRNA levels demonstrated variations in these molecules during this pathological event. Specifically, a study in a pediatric population with food-induced anaphylaxis has shown increased circulating serum levels of miR-21-3p and miR-487b-3p [181]. Similarly, other evidences propose miR-451a as the most relevant biomarker in adult blood samples [88].

2.4.3 Extracellular vesicles

Extracellular vesicles (EVs) are lipidic structures mainly classified according to their size and biogenesis into three subtypes: exosomes, microvesicles and apoptotic bodies [182]. However, although they present different properties such as size, morphology or protein composition, their distinction is still difficult [183].

EVs can be released by many cells in physiological conditions controlling the homeostasis of the organism [184]. They participate in cell-cell communication.
regulating the interaction between different systems [183]. However, EVs are also released under pathological conditions [183,185,186]. Therefore, they have been proposed as a promising source of biomarkers as they reflect the physiological or pathological state of cells. Moreover, they are very stable structures and are easy and inexpensive to obtain as EVs can be isolated from many body fluids, such as blood or saliva [182,187].

The role of these particles in anaphylaxis is practically unknown. Currently, the only study carried out demonstrated the existence of a differential proteomic profile in patients' EVs obtained during the anaphylactic reaction. Among these proteins, an increase in Ficolin-2, CDC42 and alarmin S100A9 levels was demonstrated [188].

2.4.4 Metabolites

Metabolomics as a tool for biomarker discovery has been empowered in the last few decades in different fields, such as allergy [189,190]. In anaphylaxis, a study carried out in guinea pigs demonstrated metabolic changes in challenged animals compared to control [191]. Furthermore, different metabolites are generated from relevant known mediators participating in anaphylaxis, such as histamine. The rapid breakdown of this molecule gives rise to two metabolites (N-methylhistamine and N-methylimidazole octane) detectable in urine up to 24 hours after the onset of the reaction. However, although this sampling is less invasive than blood collection, its sensitivity and specificity is much lower [53,56,57]. In addition, due to its stability both in urine and sera from anaphylactic patients, 9alpha,11beta-PGF2 appears to be a promising lipidic-derived biomarker of this pathological event [84,87,88]. Moreover, an exploratory study published recently point to the relevance of other altered metabolites in anaphylaxis.
Glucose, lipids, cortisol, betaine and oleamide are some of those differential molecular observed accordingly to triggers or severity of the reaction [192].

3. MICROENVIRONMENTS

The relative contribution of mediators, cells and systems to the pathophysiology of anaphylaxis is unknown. Furthermore, the location of effector cells and the half-life and abundance of the molecules released (local or systemic distribution) is likely to mask the recognition of reliable biomarkers. If future studies would decipher the accurate mechanisms activated in these reactions, it would improve an adequate diagnosis of patients focused in precision medicine. Therefore, clarifying the different microenvironments established in anaphylaxis (skin, lung, intestinal, cardiac, vascular, nervous…) will notably nourish the clinical management and the recognition of better molecular markers.

3.1 Mast cells heterogeneity

Undoubtedly IgE-mediated anaphylactic reactions are the best characterized. However, MCs heterogeneity is detrimental to finding a “universal” biomarker of anaphylaxis. Specifically, differences between human and mice, and even between mice strains, indicate marked variations in IgE abundance, MC activity and features of the released mediators [193,194]. Precisely, the degranulation profile of MCs, far from being simple, is not a unique and uniform. Different types of stimuli or triggers induce diverse patterns of exocytosis releasing diverse types of mediators to the surroundings [31]. In detail, MCs induce distinct lipid, cytokines, chemokines secretion profiles, as well as diverse degranulation dynamics depending on FceRI cross-linking or other GPCRs activation [30,195]. For instance, this is the case of gastrointestinal symptoms, where the main MC mediators involved are PAF and serotonin, rather than histamine [196,197].
Moreover, the wide variety of MCs receptors activating specific cellular signalling has reached an enormous relevance. This fact could even have an impact in the diversity of organs affected in anaphylaxis [198,199]. For instance, A3 is found three-fold increased in lung MCs compared to skin MCs [90,200]. Therefore, different anaphylactic microenvironments would be created depending on the nature of each reaction. Consequently, the release of specific mediator’s derived from determined activated cells would explain the clinical differences of this pathological event.

3.2 The vascular wall and homeostatic factors

The endothelium is crucial to understand the development of the reaction due to its direct contact with the blood. At the onset of anaphylaxis many mediators released by the effector cells induce a direct effect on the ECs. Among them, molecules such as histamine, cathepsin G or PAF are able to produce vascular dilation, as well as an increase in endothelial permeability [34,52,53,58,73,201].

On the other hand, products derived from the activation of the different blood systems are also capable of exerting an effect on the vascular wall. This can occur indirectly, such as complement-derived anaphylatoxins, by inducing degranulation of effector cells [202,203], or in a direct manner, such as BK, a potent vasoactive agent capable of inducing vasodilation and increased vascular permeability [44,124]. In addition, a multitude of mediators released by effector cells, including the most relevant molecules (sT, chymase, histamine and PAF), appear to be involved in the regulation of the complement, coagulation and contact systems. Specifically, proteases play an important role amplifying the inflammatory aspects and activating the molecular cascades of complement and coagulation. For instance, chymase has been related to the coagulation pathway [48,56,61]. Similarly, sT and heparin are capable of activating...
complement through an independent loop of the system's own convertases inducing the release of C3a and C5a [53,204]. In turn, complement system can amplify coagulation and inhibit fibrinolysis. Reciprocally, the coagulation system has the capacity to activate the complement system [116].

Moreover, an in vitro study of ECs stimulated with anaphylactic patient sera exhibit the relevance of platelets and coagulation processes in anaphylaxis and show alterations in proteins related not only to this system but also to the complement and fibrinolitic ones (FIGURE 2). Most of the increased proteins identified are related to the coagulation process, but it is noteworthy that, among them, the FIX and FX proteins had previously been observed to be decreased sera from patients. This could indicate that the endothelium is actively participating in the consumption of factors derived from these systems during the course of the reaction, which could explain their decrease in serum at the cellular level [115].

3.3 Amplification molecular loops of interleukins

The inflammatory feature of anaphylaxis is partially due to the contribution of ILs to the reactions. These molecules mediate main immune processes but also lead activation to the resident cells setting up positive and negative feedback loops (FIGURE 3).

IL-4 is highly relevant in the development of Th2 lymphocytes and the induction of IgE class-switching in B cells [205]. In turn, IL-13 activates IL-4 signalling pathways, induces IgE production and activates MCs and eosinophils recruitment promoting their survival [205]. Both, IL-4 and IL-13 exacerbate anaphylaxis through activation of a STAT 6-dependent pathway in murine models, increasing sensitivity of target cells to vasoactive mediators [206]. In addition, both molecules could activate a phosphatidyl inositol-3 kinase (PI3K)-dependent pathway to induce eNOS expression and
overproduction of NO [197]. Most of these processes lead to vascular permeability, vasodilation and hypotension influencing the severity of the episode. In addition, the fact that IL-4 and IL-13 shared the alpha chain of their IL-4 receptor (IL-4Rα) has launched the use of anti-IL-4Rα which has been pointed as a relevant therapeutically tool in anaphylaxis [207]. On the other hand, it has been shown that IL-4 and IL-13 can also enhance the response to other anaphylactic mediators in human MCs and macrophages by increasing the expression of their receptors, such as cysteiny LT receptors [208].

IL-33 acts directly on IgE class-switching in B lymphocytes by inducing IL-4 and promoting MC degranulation [209]. Importantly, ILs could amplify other signalling pathways, as IL-33 that induces to release of VEGF from MCs, increasing vascular permeability and contributing to inflammation [210]. Likewise, the effect of IL-6 can be mediated by its binding to IL-6Rs present on the surface of different cells or in its soluble form (sIL-6R) [205]. This is also able to induce ECs to synthesize different factors and proteins of the complement system [211,212]. Finally, in vitro approaches shows the effect of IL-3 in basophils through increasing the expression of the activation markers CD69 and CD203c and by enhancing mediator release in response to FcεR cross-linking [213,214].

In animal models, the role of IL-18 in the initiation of Th2 responses inducing IL-4 and IL-13 synthesis in MCs and basophils, leading to an increase in IgE production have been described [215,216]. Conversely, IL-18/IL-12 coadministration induces the production of IFNγ, stimulate Th1-mediated immune responses and inhibit IgE synthesis [216,217]. Therefore, the administration of these molecules combined, could be considered a treatment for severe allergic disorders [217].
On the other hand, IL-10 modulates MHCII and costimulatory molecules expression on monocytes/macrophages and dendritic cells, limiting the production of pro-inflammatory cytokines and chemokines [218–220]. Therefore, this mediator participates in the resolution of the systemic immune response. Precisely, peripheral T-cell tolerance in immunotherapy is mediated by IL-10 upregulation by allergen-specific T regulatory (Treg) cells [221]. In mouse models, introduction of bioengineered microorganisms to deliver this protein to the intestine decreases food-induced anaphylaxis and prevents IgE-type sensitization to common food allergens [222]. Similarly, IL-12 exhibits a similar function than IL-10. Its oral administration prevents and reverses peanut hypersensitivity. Furthermore, it reduces the release of histamine, specific IgE and IgG1 in mouse model of peanut anaphylaxis [223].

3.4 Communication between microenvironments: miRNAs and EVs

All these data demonstrate the importance of the different microenvironments in anaphylaxis. However, they are not isolated niches since there is communication across them. Therefore, several molecules have been postulated as participants in the interaction between these microenvironments. Among them, miRNAs and EVs have been described as key players in cell-cell communication during the anaphylactic reaction [181,188] (FIGURE 4).

Specifically, miR-155 regulates MC degranulation by modulating their calcium concentration through PI3K levels [179]. In addition, miR-21-3p could participate in this process by its action on Rac signaling, a protein involved in MC degranulation [181,224,225]. In turn, analysis of EVs obtained during anaphylaxis revealed that the main function of their differential proteins was neutrophil degranulation [188].
Specifically, elevation of circulating levels of miR-451a has been associated with increased degranulation of the effector cells of the reaction [88].

Nevertheless, EVs obtained during anaphylaxis have also been reported to induce an increase in \textit{in vitro} endothelial permeability [188]. In addition, they can act as transporters of different miRNAs, which exert their action on the target cell [226]. Precisely, two of them have been characterized in anaphylaxis, miR-135-5p and miR-154-5p, which can regulate allergic inflammation in a p62 protein-dependent manner [179, 227].

In addition, other miRNAs have been described in the regulation of inflammation during anaphylaxis. For instance, miR-26a and miR-26b regulate COX 2 levels, a key enzyme in this process and in the release of the cytokines involved [177]. Conversely, an anti-inflammatory effect of miR-182-5p has been reported as it reduces the levels of IL-27, a cytokine involved in allergic inflammation [180]. Similarly, miR-122 was also found to participate in the inhibition of this process through the regulation of suppressor of cytokine signaling 1 (SOCS1) and Janus kinase 2 (JAK2), both of which control the production of the anti-inflammatory mediator TGF-β [178]. In turn, miR-487b-3p modulates the inflammation underlying the reaction as it participates in JAK2 and IL-9 signaling, a proinflammatory cytokine released by MCs [181]. Likewise, miR-21-3p regulates this process through its role in TNFR signaling, on which a wide variety of cytokines act [181].

4. CONCLUSIONS

In conclusion, different molecules have been identified and proposed as biomarkers in anaphylaxis, but none of them has yet been extrapolated to clinical practice. Probably, the reason is the lack of studies connecting the clinical manifestations of the patient with the
molecular mechanisms underlying the reaction. Accordingly, the recent increase in the understanding of the signaling pathways involved in anaphylaxis has led to new candidate molecular markers. Therefore, a more robust knowledge about the plethora of anaphylactic phenotypes and endotypes would lead to the identification and characterization of better diagnostic and predictive biomarkers that would improve the clinical management of patients and their consequent quality of life.

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**Conflict of interest**

In relation to this manuscript, the authors declare that they have no relevant conflicts of interest.
REFERENCES


Table 1. Table shows mediators released from activated cells and systems in anaphylaxis.

| MC | B | N | MON | MAC | TL | EOS | PLAT | EC | VSMC | E | P | LTB | C/D/E4 | PGD2 | PGE2 | PGF2 | TXA | TXB2 | PAF | S1P | C/C/C | RAS | SP | TNF-alfa | TGF-beta | IFN-gamma | G-CSF | M-CSF | GM-CSF | CCL-2 | PF4 | IL | VEGF | MPO |
|----|---|---|-----|-----|----|-----|------|----|-----|----|---|----|------|-------|-------|-------|-------|-----|-----|----|------|-------|----------|-------|-------|-------|-------|-------|-----|------|-----|

MC: mast cell; B: basophil; N: neutrophil; MON: monocyte; MAC: macrophage; TL: T lymphocyte; EOS: eosinophil; PLAT: platelet; EC: endothelial cell; VSMC: vascular smooth muscle cell; E: erythrocyte; P: plasma; LTB; C, D, E4: leukotrienes B, C, D, E4; PGD2: prostaglandin D2; PGE2: prostaglandin E2; PGF2: prostaglandin F2; TXA: thromboxane A2; TXB2: thromboxane B2; PAF: platelet-activating factor; S1P: sphingosine-1-phosphate; C/C/C: contact, coagulation and complement systems; RAS: renin-angiotensin system; SP: substance P; TNF-alfa: tumor necrosis factor (*TNFα members family including TNFα, TNFRI and TWEAK); TGF-beta: transforming growth factor beta; IFN-gamma: interferon gamma; G-CSF: granulocyte colony-stimulating factor; M-CSF: macrophage colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; CCL-2: C-C motif chemokine ligand 2; PF4: platelet factor 4; IL: interleukin; VEGF: vascular endothelial growth factor; MPO: myeloperoxidase.
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Figure 1. The activation of MCs is addressed through multiple molecular mechanisms in anaphylaxis. Classically, MCs can be activated through the cross-linking of FcεRI (upper left panel). Secondarily, they can also respond to IgG immune-complexes (down left panel). Other ligands and/or signals activate MCs through receptors such as MRGPRX2, complement receptors and many others. APC: antigen presenting cell; Th cell: T helper lymphocytes; B cell: B lymphocytes; FcεRI: Fc epsilon receptor I; FcγRI: Fc gamma receptor I; MRGPRX2: Mas-related G-protein coupled receptor member X2; C3a: complement component 3a; C5a: complement component 5a; C3aR: complement component 3a receptor; C5aR: complement component 5a receptor; PAR2: protease activated receptor 2; CysLTRs: cysteinyl leukotriene receptors.
Figure 2. Relevant proteins identified in *in vitro* EC stimulated with serum from anaphylactic patients adapted from [115]. The complement, coagulation and fibrinolytic systems stand out. Increased proteins are marked in red while decreased proteins are shown in blue. Tissue plasminogen activator: tPA; urokinase uPA; CXCL7: Chemokine (C-X-C motif) ligand 7; HRG: Histidine-rich glycoprotein; CPB2: Carboxypeptidase B2; TSP1: Thrombospondin-1; MMRN1: Multimerin 1; THRBL: Thrombin; LG3BP: Galectin-3-binding protein; FINC: Fibronectin; APOH: Apolipoprotein H; iPROC: inactive Protein C; aPROC: active Protein C; PROS1: Protein S; FV, VII, VIII, IX, X, XI, aXI: Factor V, VII, VIII, IX, X, XI, active XI; C1qC1sC1r: complement components 1q, 1s, 1r complex; C2, 2a, 2b, 3, 3a, 3b, 4, 4a, 4b, 5, 5a, 5b: complement components 2, 2a, 2b, 3, 3a, 3b, 4, 4a, 4b, 5, 5a, 5b; C3bC2b: complement components 3b, 2b complex; C4bp: complement components 4b binding protein; C6cC7C8C9: complement components 6c, 7, 8, 9 complex; VTNC: vitronectin; CFI: complement factor I; CFH: complement factor H; DAF: Complement decay-accelerating factor.
Figure 3. Relevant ILs exhibit positive and negative feedbacks loops in anaphylaxis.

B cell: B lymphocytes; Th2 cell: T helper type 2 lymphocytes; MC: mast cell; MAC: macrophage; EOS: eosinophil; MON: monocyte; MAC: macrophage; DC: dendritic cell; EC: endothelial cell; VSMC: vascular smooth muscle cell; IL: interleukin; VEGF: vascular endothelial growth factor; CysLTR: Cysteinil leukotriene receptor; STAT 6: Signal transducer and activator of transcription 6; PI3K: Phosphatidylinositol 3 kinases; eNOS: endothelial nitric oxide (NO) synthetase.
Figure 4. miRNAs and extracellular vesicles (EVs) described in anaphylaxis. These molecules are involved in degranulation and vascular permeability (left) and in pro/anti-inflammatory effects (right). (MC: mast cell; MON: monocyte; N: neutrophil; PI3K: Phosphatidylinositol 3-kinases; COX-2: cyclooxygenase 2; SOCS1: suppressor of cytokine signaling 1; JAK2: Janus kinase 2; TNFR: Tumor necrosis factor receptor; TGF-β; Transforming growth factor β).