



In Vitro Diagnosis of Anaphylaxis: an Update

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Abstract

Purpose of review The management of anaphylaxis, after a rapid clinical-based hypothesis and prompt treatment introduction, includes diagnosis confirmation. We reviewed main laboratory methods available for diagnosing anaphylaxis and its etiology. Then, we searched for new methods that can gain utility in the next few years.

Recent findings Serum tryptase is still the most accurate and available method for diagnosis during the acute phase, although it is necessary to measure baseline levels after a period of time. However, measurement of other mediators, such as histamine and lipid metabolites, is available in many sites with good accuracy. For etiologic diagnosis, serum-specific IgE, component-resolved diagnosis (CRD), and basophil activation test (BAT) are available and sometimes helpful, and the new mast cell activation test (MAT) has been recently proposed to help confirming activation of those cells by a specific trigger.

Summary In vitro tests can help clinicians to confirm anaphylaxis diagnosis and sometimes its etiology, but many triggers cannot be confirmed by laboratory methods. Then, allergist consultation after an anaphylactic reaction is mandatory to order the most appropriate tests and, if indicated, to perform in vivo tests.

Introduction

The definition of anaphylaxis for clinical use includes the concepts of a serious, generalized, or systemic immediate hypersensitivity reaction that can be life-threatening or fatal. Laboratory tests often are not

helpful in diagnosing anaphylaxis at the time of the patient presentation [1[•]]. However, patients should follow up with a specialist for further investigation and to help prevent further episodes.

Some authors classified anaphylaxis into phenotypes, and this classification would include the preferential affected organ of system, age, underlying conditions, and the presence of cofactors and/or risk factors. In addition, biomarkers would be useful for confirming the diagnosis, defining prognosis, predicting risk and severity, and monitoring therapeutic response. Biomarkers should allow the identification of different mechanisms directly related to phenotypes [2[•]].

Regardless of the phenotype, mast cells and/or basophils are the central effector cells involved in

anaphylaxis. During this acute event, detecting mediators related to mast cell/basophil activation would be good biomarkers. The in vitro diagnosis of anaphylaxis includes the measurement of several mediators such as tryptase, as well as other mediators such as histamine, chymase, carboxypeptidase A3, platelet-activating factor (PAF) and other products derived from mast cells or basophils. Histamine, an important mediator of anaphylaxis, induces several signs and symptoms that include bronchoconstriction, cutaneous flushing, headache, and, eventually, systemic hemodynamic manifestations, for example, hypotension and arrhythmia [1[•], 2[•], 3].

In this review paper, we discuss the main strategies available to improve in vitro diagnosis of anaphylaxis. Then, we summarize our main findings in Table 1.

Inflammatory mediators

Histamine

Histamine is a biogenic amine with important biological activities in anaphylactic reactions, such as vasodilation and smooth muscle contraction. It is secreted by mast cells and basophils upon specific and nonspecific activation. Despite being the most important inflammatory mediator in anaphylaxis, the usefulness of measuring histamine in serum or plasma as a marker of mast cell or basophil activation is limited, because its half-life in plasma is short. On the other hand, it is also detectable in other biological fluids, such as urine. Both

Table 1. Role of in vitro tests in anaphylaxis diagnosis

Laboratory test	Anaphylaxis diagnosis during attack	Anaphylaxis severity after attack	Anaphylaxis etiology after attack
Urinary histamine	+	nk	–
Serum tryptase	+	+	–
Serum PG	+	+	–
Serum Cys-LT	+	nk	–
Serum PAF	+	+	–
sIgE	–	–	+
CRD	–	–	+
BAT	–	–	+
MAT	–	–	+

Plus (+) refers that the role in anaphylaxis has been shown, and minus (–) means that it has not been demonstrated. Those that have not been explored are marked as not known (nk). PG, prostaglandin; Cys-LT, cysteinyl leukotrienes; PAF, platelet-activating factor; sIgE, serum-specific IgE; CRD, component-resolved diagnosis; BAT, basophil activation test; MAT, mast cell activation test

histamine and its metabolite methyl-histamine can be measured in plasma and/or 24-h urine collection. Some methods have been used to measure histamine, including enzyme-linked immunoassay, but they are laborious and expensive [4[•], 5^{••}].

To measure levels of histamine metabolites in urine is preferable than in plasma, particularly on urine collected over 24 h. It is a noninvasive technique to monitor global histamine turnover. Its metabolites may be measured by high-performance liquid chromatography (HPLC) and HPLC coupled to mass spectrometry [4[•], 5^{••}].

Histamine levels in plasma increase within 5 to 10 min from the start of anaphylaxis and return to its normal level within 30 to 60 min. Although histamine is rapidly converted to its metabolites, these metabolites are more stable and can be measured in plasma and urine within 30 to 60 min of the anaphylactic event [5^{••}, 6].

Nassiri et al found that histamine measurement seems inadequate under most circumstances because of its short half-life. However, it could be used to detect anaphylaxis if assessed early after anaphylaxis onset [7].

Tryptase

The major prestored enzyme of human mast cells secretory granules is tryptase. Tryptase is a serine protease present in large amounts in mast cells and in lower amounts in basophils. It is highly specific of these cells, providing information about mast cell distribution, number, and activation. Within a few minutes since the initial symptoms of anaphylaxis, mature tryptase released from mast cells and basophils can be detected in the serum [2[•], 4[•], 8].

Serum tryptase levels in blood samples taken from 15 to 180 min after symptom onset can support the clinical diagnosis of anaphylaxis in some but not all patients. This increase is transient and typically resolves in 24 to 48 h. Previous data suggested that blood samples should be collected preferably between 30 and 180 min after the initial reaction [1[•], 2[•], 8]. Nevertheless, in a large case series of perioperative anaphylaxis in the UK, it has been shown that serum tryptase collected as soon as the reaction is stabilized increased sensitivity and tryptase peak levels [9]. Serum tryptase concentrations above 11.4 ng/mL suggest mast cell activation, and their levels during anaphylactic reaction can be correlated with severity. Nevertheless, sensitivity of isolated tryptase measurement during an acute reaction is suboptimal, remaining low in up to 40% of anaphylaxis episodes, due to several reasons [1[•], 2[•], 8].

It is not possible to discriminate total and mature tryptase using commercially available immunoassays. Total tryptase levels reflect mast cell and basophil burden, while mature tryptase is released only at the time of activation. Patients with low baseline tryptase levels can have increases during anaphylaxis that do not reach levels higher than 11.4 ng/mL. Thus, levels of 2 ng/mL *plus* 1.2 times baseline are considered significantly increased [1[•], 8].

Although tryptase specificity is high, its sensitivity is low because it is released from different mast cell subsets and basophils, depending on the trigger. It has been shown that tryptase levels are lower in mucosal mast cells

than in cutaneous and perivascular mast cells [8]. Then, it is easy to understand that food-induced anaphylactic reactions are associated with lower tryptase levels than drug-induced anaphylaxis, particularly if a drug is administered intravenously.

Nassiri et al described that tryptase is more stable than histamine and can be used to detect anaphylaxis in a subgroup of patients, but it is crucial to know the tryptase baseline level of the patient [7].

Other mediators of inflammation

Lipid mediators are arachidonic acid-derived proinflammatory and vaso-active mediators, which are de novo synthesized in active mast cells and basophils. Prostaglandin (Pg) D₂, and cysteinyl leukotrienes (cys-LT) LTE₄ are the major lipid mediators, shortly released after mast cell activation. They can be elevated in anaphylaxis. Metabolites of LTC₄ and particular PGD₂ seemed more useful than histamine and tryptase, due to its stability and to their increased levels in serum up to 2 h after onset of anaphylaxis. PGF₂ has a very low baseline level, little variability in the basal concentration among patients, a profound increase after anaphylaxis, and a correlation with severity [1[•], 4[•], 7].

Other mast cell proteases, such as chymase and carboxypeptidase, have been detected during anaphylaxis, but there are no commercial assays available [8].

PAF is a proinflammatory potent phospholipid-derived mediator released by several types of cells, including mast cells, platelets, and others, in response to various stimuli. Serum PAF levels were directly correlated with the severity of anaphylaxis [4[•], 10].

Levels of other serum inflammatory mediators, such as TNF- α , IL-6, and IL-1b, can be increased in patients with anaphylaxis, but their sensitivity or specificity has not been demonstrated [8].

Specific serum IgE (sIgE)

The most well-known mechanism of mast cell and basophil activation is type I hypersensitivity reaction, firstly described by Gell and Coombs. It occurs when specific IgE linked to its high-affinity receptor Fc ϵ RI on the surface of mast cells or basophils recognizes and bounds to the allergen. Initially, there is a first phase named sensitization, when IgE will start to be produced by B cells. At a primary contact, the allergen is taken up by a professional antigen-presenting cells (APCs), such as dendritic cells (DCs). After taking up the allergen, they mature and migrate to regional lymph nodes, so that they can present these antigens to naive CD4⁺ T cells (Th0). Th0 cells will differentiate into Th2 cells, which begin to secrete IL-4, which is essential to induce the isotype switching from IgM to IgE class antibodies in B lymphocytes [11, 12]. Once produced by plasma cells, IgE binds to Fc ϵ RI on the surface of blood basophils and tissue resident mast cells.

Upon a repeated exposure to a bi- or multivalent allergen, cross-linking of Fc ϵ RI-bound IgE induces activation of those effector cells, and the immediate release of preformed mediators such as histamine and various proteases induces

the immediate reaction, with symptoms as urticaria, flushing, angioedema, bronchospasm, and hypotension (early or immediate phase of type I hypersensitivity reaction) [3, 11, 12]. Moreover, effector cells start *de novo* synthesis of many inflammatory mediators such as certain Cys-LTs, PGs, and cytokines, which explain the “late phase” phenomena [3]. This late phase is followed by the release of many cytokines, as IL-1, IL-13, IL4, and IL-5, which induce activation of local cells and recruitment of other inflammatory cells (neutrophils, basophils, eosinophils, monocytes, and lymphocytes) to the site of inflammation [11, 12].

Regarding to anaphylaxis etiology, serum-specific IgE (sIgE) levels can be measured for food, Hymenoptera venom, latex, chlorhexidine, and drug agents, including antibiotics and chemotherapeutic agents. Depending on the antigen suspected (e.g., foods), accuracy of sIgE levels measurement might be superior to skin tests [8, 13].

Component-resolved diagnosis (CRD)

Because allergen sources can be complex, resolving the different components (allergens) has led to advances in precision medicine by identifying patient-specific IgE antibodies directed against different components [8]. Advances in precision medicine have made it possible to identify and characterize some molecules present in the allergenic extracts, and this has resulted in component-resolved diagnosis (CRD). CRD is an advanced tool capable to help in determining the causal agent or add some information regarding risk of reactions or natural history of the allergic disease. CRD has been used to determine the relative risk of the severity of reactions in specific cases, such as food allergens. Therefore, CRD is a useful tool to help define the course of anaphylaxis in many occasions and provide guidance for management measures [5^{••}, 14].

CRD, which is based on the determination of sIgE concentration against individual allergenic molecules, allows the detection of sensitization against individual components of the allergic source, even against those lacking in the allergen extract. Furthermore, the development of DNA technology has enabled the introduction of individual allergenic molecules into laboratory diagnostics of allergies. Since the CRD has been applied in recent years, future investigation will examine the diagnostic power of this currently expensive method [15].

Basophil activation test (BAT)

Basophils and mast cells originate from a common progenitor cell in bone marrow and share similar phenotypic/biochemical characteristics. It is quite easy to assess basophils because they are circulating granulocytes that respond to allergic stimuli by migrating and accumulating at sites of allergic inflammation. On the other hand, mast cells leave the bone marrow immature and complete their maturation in peripheral tissues, where they ultimately reside. Therefore, evaluation of human mast cells is limited to biopsy specimens from specific tissues. Because both mast cells and basophils are sensitized with the same repertoire of IgE, the basophil activation test (BAT) is considered a true reflection of sensitized mast cells [5^{••}].

BAT is a blood test used to diagnose IgE-mediated reactions to allergens. The flow cytometry BAT is based on the detection of allergen-induced CD63

expression as well as the expression of CD203c on basophils useful for the in vitro diagnosis of patients with IgE-mediated allergy, as observed in several anaphylaxis [5^{••}, 16].

Activating basophils in vitro with allergens, such as food, Hymenoptera venom, and some drugs and measuring surface activation markers or the release of mediators, such as histamine and leukotrienes, could reflect the sensitization and activation of tissue mast cells. BAT should provide reliable information regarding sensitization or severity, but the clinical utility of this method will require further studies [8].

The use of the BAT in peanut-sensitized children revealed peanut concentration-dependent upregulation of CD63 and CD203c in those who were clinically reactive to peanut, contrasting with no significant responses in those who could tolerate peanut ingestion. The BAT reduced the number of food challenges required [17].

BAT has been shown to be useful in evaluating immediate reactions to β -lactam antibiotics, muscle relaxants, platins, and other drugs, as well as in the diagnosis and monitoring of Hymenoptera venom allergies [18, 19]. BAT is not recommended for patients with a history of multiple reactions, NSAID hypersensitivity, or those with food allergies because of their increased histamine release at baseline [5^{••}].

Mast cell activation test (MAT)

A new mast cell activation test (MAT) demonstrated utility in experiments involving primary human blood-derived mast cells generated from CD117 peripheral blood precursors. Diagnostic cutoffs with high levels of discrimination were validated and found to be more reproducible compared with the BATs and CRDs [20^{••}].

Bahri et al developed this robust and reproducible mast cell-based assay to improve the diagnosis of IgE-mediated allergy using human mast cells derived from human progenitor cells. Mast cells sensitized with sera from patients allergic to some allergens (food, pollen, wasp) demonstrated allergen-specific and dose-dependent degranulation by using both expression of surface activation markers (CD63 and CD107a) and functional assays (PGD2 and beta-hexosaminidase release). They compared this new test (MAT) with other commonly used diagnostic tests and observed that MAT appeared to confer superior diagnostic accuracy in distinguishing between patients with true peanut allergy and those who are sensitized but tolerant to peanut [20^{••}].

The role of these laboratory tests is described in Table 1.

Conclusions

Anaphylaxis diagnosis is still clinically based, but sometimes a clear picture definition is hardly supported only by signs and symptoms. In vitro tests as serum tryptase or the measurement of other mediators can help in confirming the clinical suspicion. After being clinically stabilized, all patients should undergo allergy consultation to confirm etiology and avoid future recurrences. Thus in vitro tests as sIgE and BAT can help to define culprit agents and to reduce the need of future skin tests or challenges.

Compliance with ethical standards

Conflict of interest

Rosana Câmara Agondi, Jorge Kalil, Pedro Giavina-Bianchi, and Marcelo Vivolo Aun declare no conflicts of interest.

Human and animal rights and informed consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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