Anaphylaxis: Clinical patterns, mediator release, and severity

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Background: Prospective human studies of anaphylaxis and its mechanisms have been limited, with few severe cases or examining only 1 or 2 mediators.
Objectives: We wanted to define the clinical patterns of anaphylaxis and relationships between mediators and severity.
Methods: Data were collected during treatment and before discharge. Serial blood samples were taken for assays of mast cell tryptase, histamine, anaphylatoxins (C3a, C4a, C5a), cytokines (IL-2, IL-6, IL-10), soluble tumor necrosis factor receptor I, and platelet activating factor acetyl hydrolase. Principal component analysis defined mediator patterns, and logistic regression identified risk factors and mediator patterns associated with reaction severity and delayed reactions.
Results: Of 412 reactions in 402 people, 315 met the definition for anaphylaxis by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network. Of 97 severe reactions 45 (46%) were hypotensive, 23 (24%) were hypoxemic, and 29 (30%) were mixed. One patient died. Severe reactions were associated with older age, pre-existing lung disease, and drug causation. Delayed deteriorations treated with epinephrine occurred in 29 of 315 anaphylaxis cases (9.2%) and were more common after hypotensive reactions and with pre-existing lung disease. Twenty-two of the 29 delayed deteriorations (76%) occurred within 4 hours of initial epinephrine treatment. Of the remaining 7 cases, 2 were severe and occurred after initially severe reactions, within 10 hours. All mediators were associated with severity, and 1 group (mast cell tryptase, histamine, IL-6, IL-10, and tumor necrosis factor receptor I) was also associated with delayed deteriorations. Low platelet activating factor acetyl hydrolase activity was associated with severe reactions.
Conclusion: The results suggest that multiple inflammatory pathways drive reaction severity and support recommendations for safe observation periods after initial treatment. (J Allergy Clin Immunol 2013;132:1141-9.)

Key words: *Anaphylaxis, emergency department, mast cell tryptase, histamine, interleukin, soluble tumor necrosis factor receptor I, platelet activating factor, platelet activating factor acetyl hydrolase, biphasic anaphylaxis*

Anaphylaxis is a serious, life-threatening, generalized hypersensitivity reaction that can occur via immunologic (either IgE-dependent or IgE-independent) or nonimmunologic mechanisms.[1] Typically, it is a multiple organ phenomenon that affects the skin with obvious generalized erythema-urticaria, plus cardiovascular or respiratory compromise or both.[2] However, recognition can be difficult because skin manifestations are subtle or absent in up to 20% of cases,[3] and different reaction patterns may cause clinical uncertainty and can delay treatment. In a study of 164 fatal reactions, epinephrine was given before cardiac arrest in only 14% of cases, and it appeared that diagnostic confusion with asthma delayed treatment with epinephrine in patients with a predominantly respiratory reaction pattern.[4] Another area of uncertainty relates to the optimal period of observation because of the risk of delayed deteriorations (prolonged or "biphasic" anaphylaxis).[2]

Animal and human studies have implicated a broad range of mediators in anaphylaxis, including mediators released from mast cell and basophil storage vesicles, newly synthesized cytokines and chemokines, lipid-derived mediators, complement products, contact system activation (bradykinin), and a variety of products of eosinophil activation.[5] However, the relationships or correlations between different mediators and their associations with reaction severity have not been fully defined. Human studies have been limited, enrolling relatively few severe cases, focusing on one trigger only (eg, insect sting challenge studies) or examining only 1 or 2 mediators.

We therefore studied the clinical and biochemical features of a large cohort of people who presented to emergency departments

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Abbreviations used
ED: Emergency department
IQR: Interquartile range
MCT: Mast cell tryptase
NIAID/FAAN: National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network
PAF: Platelet activating factor
PAF-AH: PAF acetylhydrolase
PCA: Principal component analysis
TNFRI: Tumor necrosis factor receptor I

(EDs) with anaphylaxis, specifically aiming to define the different clinical patterns and the relationships between multiple biochemical mediators and reaction severity.

METHODS

Participants

Eight Australian EDs prospectively recruited patients between June 1, 2006, and February 3, 2009. These hospitals were broadly representative of Australian EDs; 3 that received only adult patients were city tertiary hospitals, and 5 that received adults and children were 2 city tertiary hospitals, 2 outer urban general hospitals, and 1 country regional hospital. Because of the need for resuscitation, sample and data collection were started before obtaining fully informed written consent. Ethics approval was obtained from the human research ethics committees for each site and included approval to commence blood sample collection before formal patient consent being obtained if urgent treatment took precedence. Once the study could be explained to them, patients were able to order all samples and data to be destroyed if they did not wish to be involved. A sample size was not prespecified, and we aimed to recruit as many cases as possible over the 2- to 3-year period of funding.

Inclusion criteria for initial enrollment were any acute-onset (minutes to hours) illness with typical skin features (generalized hives, pruritus or flushing, swollen lips or tongue, or a combination), with or without involvement of other organ systems (respiratory, cardiovascular, gastrointestinal), or any acute onset (minutes to hours) of hypotension or bronchospasm when anaphylaxis was considered possible even if typical skin features were not present. Patients were not enrolled if the treating doctor judged reaction severity to be insufficient for insertion of an intravenous cannula. There were no age restrictions on enrollment.

If cases met the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID/FAAN) consensus clinical definitions (Table I),[2] they were designated anaphylaxis. Severe anaphylaxis was defined by the presence of hypoxemia (oxygen saturations by pulse oximetry $\leq 92\%$), hypotension (systolic blood pressure < 90 mm Hg in an adult), collapse, altered consciousness, or incontinence. The remaining cases of anaphylaxis without these severity features were designated as "moderate."[3] Reactions with typical generalized skin features (generalized itch, erythema, urticaria, angioedema) but not meeting NIAID/FAAN definitions were designated as skin-only reactions. Cases were excluded if they did not meet NIAID/FAAN definitions, or if an alternate (nonallergic) diagnosis was made by discharge from the hospital or at allergy clinic follow-up.

Clinical procedures

Clinical management was not protocol driven; however, guidelines were provided to assist the treating doctors.[6] A structured data sheet was completed at arrival, 1 hour later, and before discharge from the ED. Treatments, physiological parameters, and the presence or absence of individual reaction features were recorded. A predischarge summary sheet recorded demographics, physician opinion as to likely causation, times of likely exposure and reaction onset, prehospital reaction features and treatments, comorbidities, concurrent medications within the past 24 hours (β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, other vasodilators), whether any delayed deterioration (defined simply as any worsening of reaction features) occurred while under observation. Patients were referred for allergy clinic follow-up, and the clinic summaries were obtained to determine final attributed cause. Research nurses checked all data sheets for consistency with prehospital and hospital records and reviewed clinical information systems for representations with delayed deteriorations.

Mediator assays

Collection procedures, assays, normal ranges, and assay characteristics for the laboratory, materials and suppliers are described (see this article's Methods section in the Online Repository at www.jacionline.org). Serum and plasma were collected at ED arrival, 1 hour later, and at discharge. Mast cell tryptase (MCT) was measured at all time points, histamine was measured in the enrollment sample only, and the cytokines (IL-2, IL-6, IL-10 and soluble tumor necrosis factor receptor I [TNFRI]) and anaphylatoxins (C3a, C4a and C5a) were measured at enrollment and 1 hour later. Because of requirements for immediate processing of samples, concentrations of histamine, cytokines, and anaphylatoxins could not be measured in samples collected out of hospital laboratory hours, which varied between hospitals.

Cytokine selection was based on an initial study of a large panel of cytokines and chemokines in 36 severe patients, which found that histamine concentrations peak at enrollment then rapidly decline, and the others peak at either enrollment or 1 hour later and remain high for several hours.[7] Therefore, precise timing of the second sample was not critical. Because of reagent supply shortages, the number of anaphylatoxin assays was limited. Therefore, we analyzed all severe cases and as many of the moderate and skin-only reactions as possible, selected in sequence from the earliest to be enrolled. Platelet-activating factor (PAF) could not be measured because a requirement for collection into customized tubes that contained strong acid prevented sampling across multiple study sites. Therefore, PAF-acetylhydrolase (PAF-AH) was measured, low levels of which have been associated with elevated PAF activity.[8] Sample volumes were often limited so that after assaying plasma histamine, PAF-AH could only be measured in a subset of cases.

Statistical analysis

To explore potential effects of age, comorbidities, concurrent medications, and suspected reaction trigger on severity and the type of severe reaction (hypotensive vs hypoxemic) and on the occurrence of delayed deteriorations, logistic regression analysis was performed. A robust method was used for estimation of standard errors that accounted for clustering (repeat presentations).[9]

NIAID/FAAN definitions and severity were assessed against a positive MCT, defined as above the manufacturer's diagnostic cutoff (11.4 ng/mL) or a Δ-MCT (difference between highest and lowest values) of ≥2.0 ng/mL.[10,11] Positive and negative results were compared between groups with Pearson χ^2. Because of the skewed (non-normal) distribution of most laboratory results, results were presented with median and interquartile range (IQR), and nonparametric tests were used. Correlations between peak mediator concentrations and the 3-tiered reaction grade were tested with Spearman correlation. Differences in peak mediator concentrations between 2 groups were tested with the Wilcoxon rank sum test.

Because high levels of correlation were found between mediators, a principal component analysis (PCA) was performed on log-transformed peak mediator concentrations. PCA and related methods are effective tools for dealing with panels of cytokine and microarray data.[12-14] PCA reduces the dimensionality of a data set that consist of a large number of interrelated variables, transforming the data into a new set of variables or principal components, which are uncorrelated. The derived principal components are ordered so the first few retain most of the variation present in *all* the original variables.

Multivariate logistic regression, again with the use of a robust method to account for repeat presentations, was then used to identify the principal components associated with severe reactions and delayed deteriorations. Sampling weights, corresponding to the inverse of the probability of selection, were applied to regression analyses that involved mediators to account for the