

Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis

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Background: Platelet-activating factor (PAF) is an important mediator and correlates with anaphylaxis severity. How well PAF correlates with severity relative to histamine or tryptase is not known.

Objective: To analyze the levels of PAF, histamine, and tryptase as a function of severity in patients with acute allergic reactions. **Methods:** PAF, histamine, and tryptase levels were measured in blood samples collected from 23 healthy volunteers and from 41 patients during acute allergic reactions. Reactions were stratified by severity from grade 1 (least severe) to grade 3 (most severe).

Results: Among the 3 reaction grades, there were significant differences by ANOVA for PAF ($P < .0001$). The proportion of elevated PAF values increased across severity groups ($P = .0009$). Increased PAF levels were observed in 20%, 66.7%, and 100% of the patients with grades 1, 2, and 3 allergic reactions, respectively. While the proportion of elevated histamine values increased from 40% to 57% to 70% across grades 1, 2, and 3, respectively, these were not significantly different ($P = .40$). For tryptase, the proportion of elevated values increased monotonically from 0 in grade 1 to 4.8% in grade 2 to 60% in grade 3 ($P = .0002$).

Conclusions: The PAF level was significantly elevated in proportion to the severity of acute allergic reactions. Whereas the PAF level was elevated in all patients with severe anaphylaxis, this was not true for either histamine or tryptase. Neither histamine nor tryptase showed as good correlations with severity scores as did PAF. These data are consistent with a pivotal role for PAF as a mediator of anaphylaxis. (*J Allergy Clin Immunol* 2012;■■■:■■■-■■■.)

Key words: Platelet-activating factor; histamine; tryptase; anaphylaxis; emergency department

Anaphylactic reactions are rapid, potentially fatal, immediate hypersensitivity reactions with multisystem involvement. The major manifestations include bronchoconstriction, pulmonary

Abbreviations used

PAF: Platelet-activating factor

PAF-AH: PAF acetylhydrolase

hypertension, systemic hypotension, and vascular leakage.^{1,2} Biochemical mediators and chemotactic substances are released systemically during the degranulation of mast cells and basophils.³ These include preformed chemicals, such as histamine, tryptase, chymase, and heparin, and newly synthesized lipid mediators, such as prostaglandin D₂, leukotrienes, and platelet-activating factor (PAF).⁴ PAF is a proinflammatory phospholipid synthesized and secreted by mast cells, monocytes, and fixed tissue macrophages.⁵ The binding of PAF to its receptor on target cells—platelets, monocytes, macrophages, and neutrophils—results in many of the manifestations of acute allergic reactions and anaphylaxis.²

Exogenous PAF reproduces many of the manifestations of experimental anaphylaxis in animals.⁶ *In vitro*, PAF is rapidly synthesized and released from antigen-stimulated mast cells and basophils.⁷ *In vivo*, high levels of circulating PAF have been detected after antigen challenge in sensitized animals,⁸ and in human anaphylaxis.^{9,10} PAF receptor antagonists, which inhibit the binding of PAF to the receptor, reduce anaphylaxis-related mortality in animal models.^{5,11} PAF receptor knockout mice exhibited a lower mortality rate to systemic anaphylaxis than did wild-type mice.¹² Moreover, enzymatic inactivation of PAF by PAF acetylhydrolase (PAF-AH) was shown to protect against anaphylaxis in mice pretreated with recombinant human PAF-AH.¹³ Conversely, deficiency of PAF-AH predisposed patients to severe or fatal anaphylaxis.¹⁰

The purpose of this study was to analyze the levels of PAF, histamine, and tryptase as a function of severity in patients treated for acute allergic reactions.

METHODS

Study design

This was a cross-sectional study of 41 patients who presented to the emergency department of a university teaching hospital with acute allergic reactions. Twenty-three healthy volunteers served as controls. The study was approved by the Research Ethics Board at St Michael's Hospital. Informed consent was obtained from all volunteers, patients, or their parents or guardians.

Patients were recruited into the study if they met case-definition criteria for allergic reactions as described by Brown et al.¹⁴ The severity of the allergic reactions was also evaluated according to the criteria described by Brown et al.¹⁴ Grade 1 patients had acute allergic reactions with cutaneous involvement and no other organ system involvement. Those with grade 2 reactions had mild-to-moderate manifestations of anaphylaxis (systolic blood pressure > 90 mm Hg, respiratory rate < 25 breaths per minute). Patients with grade 3 reactions had severe manifestations, with cutaneous, gastrointestinal, and potentially life-threatening respiratory or cardiovascular signs and symptoms.

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Blood sample collection

Venous blood samples for the measurement of PAF level were collected in EDTA and immediately added to an equal volume of 20% acetic acid (to inactivate endogenous PAF-AH). For the measurement of histamine and tryptase levels, blood samples without anticoagulant were centrifuged for 10 minutes at 4°C to obtain cell-free serum. In all cases, blood was collected before the initiation of therapy.

Materials

PAF ^3H scintillation proximity assay system was supplied by Amersham Biosciences (Oakville, Ontario, Canada). All other chemicals were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada).

Measurement of PAF level

Blood samples were assayed for PAF as described previously¹⁰ by using the PAF-scintillation proximity assay system (Amersham Biosciences). PAF level was measured in the range of 0.2 to 12.8 ng/mL in the sample. Assay specificity and sensitivity were determined by the addition of known amounts of PAF and subjecting each concentration to the same extraction and purification procedures followed by scintillation proximity assay.

Histamine level was measured by ELISA according to manufacturer instructions (EIA Kit; Cayman Chemical, Ann Arbor, Mich). Samples (in triplicate) were centrifuged at 1600g for 20 minutes. The enzyme immune assay was based on the competition between unlabeled derivatized histamine and acetylcholinesterase linked to histamine (tracer) for limited specific mouse antihistamine antibody sites. Derivatization was needed to increase the affinity of histamine to the antibody and thereby increase the sensitivity of the assay.

The concentration of total tryptase was measured in triplicate serum samples by using UniCap-Tryptasefluoro-immunoassay (Pharmacia & Upjohn, Uppsala, Sweden).

Statistical analysis

The large SDs relative to the mean for the chemical mediators suggested that the distributions were skewed and log transformation should be considered. For subjects with 0 values for PAF (observed in controls only), 1 was added prior to log transformation. Values of the chemical mediators between groups were compared with the *t* test (for 2 groups) and ANOVA (for 3 or more groups) when considering the mediators on a continuous scale and the chi-square statistic for categorical variables (ie, considering the mediators as normal vs elevated), as appropriate. Analyses were conducted by using SAS, version 8.2 (SAS, Cary, NC).

RESULTS

Demographics

Forty-one patients and 23 healthy volunteers were enrolled in the study and included in the statistical analyses. The mean age of patients was 35.2 ± 13.9 years (range, 15-74 years), and 26 patients (63%) were female. The mean age of healthy volunteers was 30.8 ± 9.8 years (range, 20-51 years), and 7 volunteers (30%) were female. Prior to presentation in the emergency department, 36 of the 41 patients had known histories of allergy confirmed by appropriate diagnostic testing and were exposed to their respective allergen at the time of the acute reaction. The prevalence of asthma as a function of severity of allergic reactions was as follows: grade 1, 1 of 10 (10%); grade 2, 6 of 21 (28.5%); grade 3, 3 of 10 (30%). Allergic reactions were triggered by foods ($n = 22$), drugs ($n = 12$), and insect stings ($n = 2$), and the remainder were idiopathic ($n = 5$). Ten patients had grade 1 reactions, 21 patients had grade 2 reactions, and 10 patients had grade 3 reactions.

PAF, histamine, and tryptase levels in blood

Platelet activating factor. Analysis on a continuous scale. As a group, mean serum PAF levels among patients with acute allergic reactions (mean $\pm$ SD, 805 ± 595 pg/mL) were significantly greater than those among controls (127 ± 104 pg/mL) whether untransformed ($P < .0001$) or log-transformed ($P < .0001$). PAF levels were observed to be statistically significantly different among the patients with grades 1, 2, and 3 allergic reactions (ANOVA; $P < .0001$ untransformed or log-transformed). Both explained similar amounts of variation (R^2 65% untransformed; 57% transformed).

Comparisons of untransformed data demonstrated that PAF serum levels of patients with grade 3 allergic reaction were significantly different from those of patients with grades 1 and 2 allergic reactions (grade 3 vs 1, $P = .0006$, and grade 3 vs 2, $P = .0128$); however, PAF serum levels of patients with grades 1 and 2 allergic reactions were not significantly different (1 vs 2, $P = .0684$, NS). Comparisons between grades of log-transformed data showed that for the log-transformed values, the controls were significantly different than grade 1 ($P = .0002$), grade 2 ($P < .0001$), and grade 3 anaphylaxis ($P < .0001$), while for untransformed data, the controls were not different from grade 1 but were significantly different from grades 2 and 3 anaphylactic reactions.

Analysis as categorical data (normal vs elevated). Using the cutoff of 400 pg/mL as the upper limit of normal, PAF levels were elevated in 26 (63%) of allergic patients combined but in no controls ($P = .0006$ by χ^2 test with 1 degree of freedom). Across the 4 groups, the proportion that was elevated increased monotonically from 0 in controls to 2 (20%) of grade 1, 14 (66.7%) of grade 2, and all 10 (100%) of grade 3 reactions ($P < .0001$ with 3 degrees of freedom).

Histamine. Analysis on a continuous scale. Analysis of the untransformed data of serum histamine on a continuous scale demonstrated that serum histamine levels achieved only borderline significance when log-transformed (ANOVA; $P = .06$) among patients with grades 1, 2, and 3 anaphylaxis. Comparisons between grades showed significant differences between grade 1 versus grade 3 and grade 2 versus grade 3 (untransformed).

Tryptase. Analysis on a continuous scale. Analysis of tryptase serum levels showed these to be significantly different among patients in the 3 allergic reaction groups in both untransformed and log-transformed data (ANOVA; whether untransformed or log-transformed $P < .0001$). Comparisons between grades showed serum tryptase levels to be significantly different in patients with grade 3 allergic reaction than in patients with both grades 1 and 2 allergic reactions whether the data were untransformed or log-transformed (grade 1 vs 3, $P = .0006$; grade 2 vs 3, $P = .0086$). Serum tryptase levels were borderline significant between patients with grade 1 and grade 2 allergic reactions ($P = .0484$).

Comparison of 3 mediators among allergic patients

Analysis on a continuous scale. PAF. Among the 3 grades of allergic subjects themselves, there were significant differences by ANOVA for PAF ($P < .0001$ whether untransformed or log-transformed). The log-transformed data explained slightly more of the variation between groups. Comparisons between grades showed that for the untransformed data, grade 3 was significantly different from grades

1 and 2 (but grades 1 and 2 were not significantly different), and when log-transformed, all 3 grades were significantly different from each other.

Histamine. The 3 grades were significantly different by ANOVA when untransformed but only of borderline significance ($P = .06$) when log-transformed. Comparisons between grades showed significant differences between grade 1 versus grade 3 and grade 2 versus grade 3 (untransformed); there were no significant pairwise differences when log-transformed.

Tryptase. The 3 grades were significantly different by ANOVA whether untransformed or log-transformed ($P < .0001$ for both). Comparisons between grades showed that grade 3 was significantly different from grades 1 and 2 whether untransformed or log-transformed, while grades 1 and 2 were not different.

Analysis as categorical data (normal vs elevated). The proportion of elevated PAF serum levels increased across the 3 grades of allergic reactions ($P = .0009$). The proportion of elevated histamine serum levels¹⁵ (>10 nmol/L) increased from 40% (4 of 10) in patients with grade 1 to 57% (12 of 21) in patients with grade 2 to 70% (7 of 10) in patients with grade 3 anaphylaxis; however, there was no statistically significant difference among these proportions ($P = .40$). The proportion of elevated tryptase serum levels (>11.4 $\mu\text{g/L}$) increased monotonically from 0 (0 of 10) in patients with grade 1 to 4.8% (1 of 21) in patients with grade 2 to 60% (6 of 10) in patients with grade 3 allergic reactions ($P = .0002$). Fig 1 shows the number of samples with elevated serum levels of PAF, histamine, and tryptase according to the severity of the allergic reaction.

The relationship between the start of the allergic reaction and the timing of the blood draw is shown in Fig 2 for each of PAF, histamine, and tryptase levels. Although a trend is noted for decreasing PAF and tryptase levels with increasing time from the start of the reaction to the time of blood draw, the differences for all groups were not statistically significant ($P > .05$).

The levels of PAF, histamine, and tryptase are shown as a function of the etiology of anaphylaxis in Fig 3. Differences between groups (food, medication, sting, other) are not statistically significant for PAF, histamine, or tryptase ($P > .05$).

Since PAF is a vasoactive mediator, we examined the end-organ effects at the time of blood draw to determine whether elevated PAF levels correlated with symptoms (Fig 4). Specifically, serum PAF levels were compared in patients with angioedema (group A) to patients with hypotension (group B) (Fig 4). PAF levels in patients with angioedema versus those with hypotension were significant ($P < .001$).

DISCUSSION

Anaphylaxis is considered one of the most serious clinical presentations of allergy and constitutes a medical emergency in both pediatric and adult patients.¹⁶ Systemic anaphylaxis is a relatively uncommon event, with prevalence estimated at 0.05% to 2%,¹⁷ and the rate of fatal anaphylaxis ranges from 2% to 20% of the cases.¹⁸ Most anaphylactic reactions are due to the acute and massive release of mediators from tissue mast cells and blood basophils, which occurs within minutes of exposure to allergen.¹⁹

PAF is an extremely potent biological mediator that is active at concentrations as low as 10^{-12} mol/L.⁸ The role of PAF has been well established in experimental models of anaphylaxis. In murine models of IgE-dependent anaphylaxis, PAF was produced

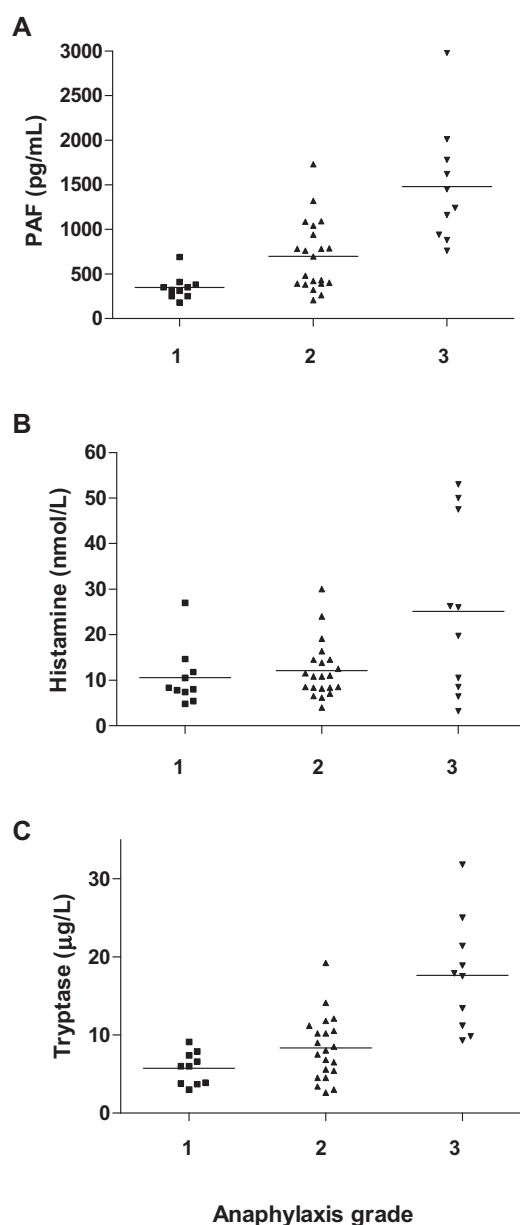


FIG 1. Serum levels of PAF (A), histamine (B), and tryptase (C) as a function of the severity of the allergic reaction.

by cross-linking of allergen-specific IgE bound to FcεR1 on mast cells.⁵ PAF is also a mediator of IgE-independent anaphylaxis in mice, by cross-linking of FcγR_{III} on stimulated macrophages.²⁰ Activated mast cells and stimulated macrophages in both mice and humans secrete histamine and PAF, and both histamine and PAF increase vascular permeability in both species.^{21,22} Administration of exogenous PAF to experimental animals has reproduced the severe manifestations of anaphylaxis,^{6,7} and *in vivo*, endogenous PAF levels have been shown to correlate with the severe manifestations of experimental anaphylaxis.^{6,10} Moreover, PAF levels have been found to correlate with the severity of anaphylaxis and may be an important determinant of the outcome.¹⁰ In conscious mice, both hyperacute PAF shock and active systemic anaphylaxis depended on the vasodilator nitric oxide produced entirely by the constitutive PAF-dependent PI3K-eNOS pathway.²³

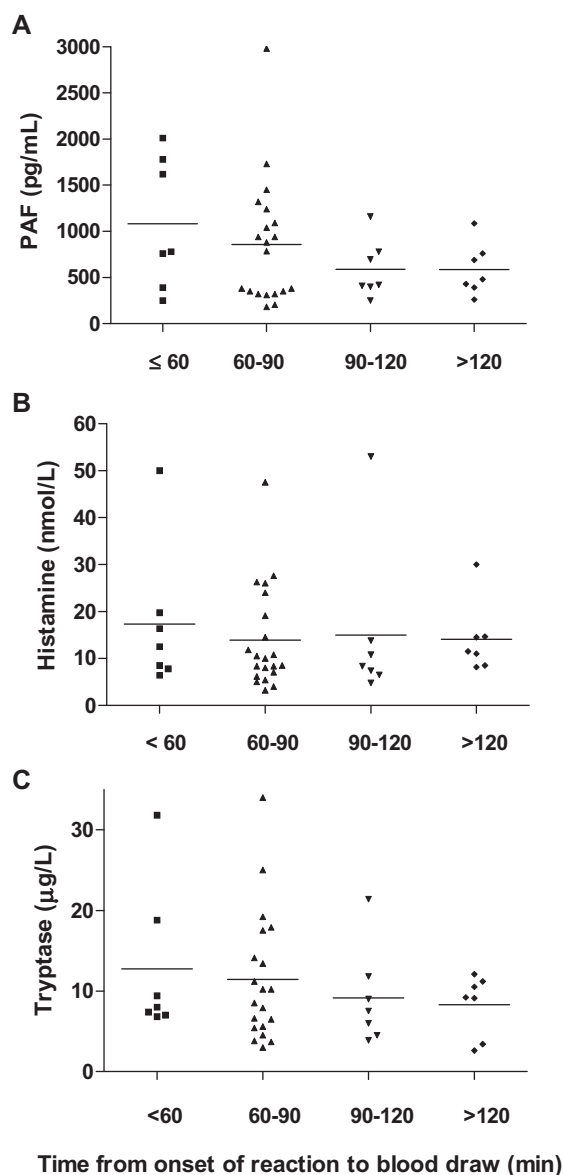


FIG 2. The relationship between the start of the allergic reaction and the timing of the blood draw for each of PAF (A), histamine (B), and tryptase (C).

The half-life of PAF is short, ranging from 3 to 13 minutes, because of its rapid inactivation by the enzyme PAF-AH.¹⁰ This short half-life likely leads to a conservative underestimate of the peak PAF concentrations generated during acute allergic and anaphylactic reactions, because patients with anaphylaxis often present to the emergency department after a considerable period of time has elapsed since their allergic reaction first began and because it takes additional time to obtain consent, enroll in a study, and perform venipuncture for the purpose of clinical investigation. Therefore, the time between the onset of the anaphylactic reaction and the acquisition of a blood sample for analysis may represent the equivalent of several half-lives of PAF.¹⁰ In spite of the time elapsed between the onset of anaphylaxis and the analysis of blood samples, the levels of PAF in patients with grades 1, 2, and 3 anaphylaxis were 2.5, 5, and 10 times, respectively, greater than PAF levels of healthy volunteers.¹⁰

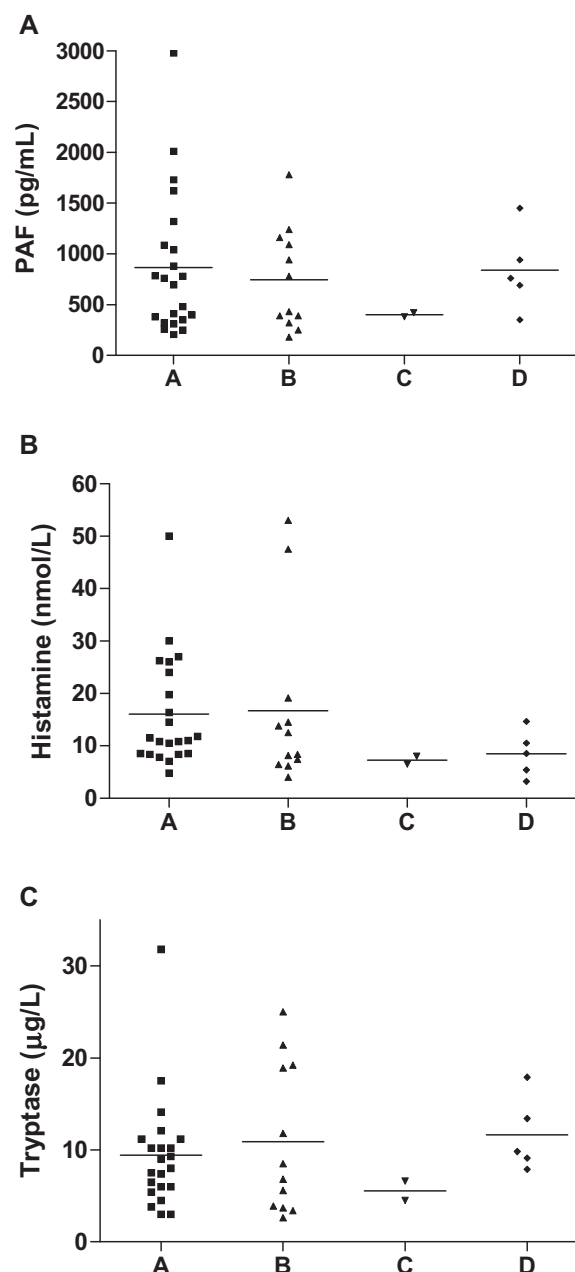


FIG 3. The levels of PAF (A), histamine (B), and tryptase (C) are shown as a function of the etiology of anaphylaxis. A, Reactions induced by foods. B, Reactions induced by medications. C, Reactions induced by stings. D, Idiopathic reactions.

The biological half-life of PAF is determined primarily by the rate of inactivation of PAF to lyso-PAF by PAF-AH,⁸ and so the biological activity of PAF is limited by the rate of its inactivation. Therefore, the slower the inactivation of PAF, the more severe the manifestations of anaphylaxis may be and indeed, a highly significant inverse correlation between PAF-AH activity and PAF levels has been reported.¹⁰ These data suggest that rapid inactivation of PAF may provide protection against severe allergic reactions while impaired PAF-AH activity may be associated with severe anaphylaxis. This protective effect has been seen in mice pretreated with recombinant human PAF-AH.¹³

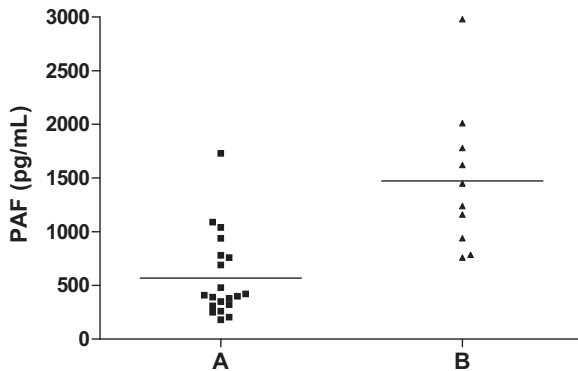


FIG 4. Serum PAF levels in patients with angioedema (A) as compared with patients with hypotension (B).

This current study has confirmed that PAF levels were significantly elevated in patients with acute allergic reactions compared with healthy volunteers. In this study, none of the healthy volunteers had elevated PAF levels. These results are in agreement with previously reported levels of PAF in healthy individuals measured by using the same methodology as in this current study.²⁴ Serum levels of PAF were directly correlated with the severity of the clinical manifestations, with increased PAF levels in 20%, 66.7%, and 100% of patients with grades 1, 2, and 3 allergic reactions, respectively. It is noteworthy that all patients with grade 3 anaphylaxis had consistently elevated levels of PAF.

Neither histamine nor tryptase showed as good a correlation with severity scores in this study as did PAF. In 76 patients presenting to emergency departments with anaphylaxis, histamine was elevated in 35% of moderate reactions and 61% of severe reactions. Tryptase was elevated in 55% of moderate reactions and in 75% of severe reactions.²⁵ In a study of 97 adults with acute allergic reactions presenting to emergency departments, raised histamine and tryptase levels were seen in fewer than 50% of the patients. Histamine levels correlated better with clinical signs than did tryptase levels.²⁶ In a study that measured mediator levels in 19 patients with possible anaphylactoid reactions to anesthetic or other drugs, 42% had elevated levels of both tryptase and histamine and 21% of the patients had normal levels of both mediators.²⁷ However, in these and numerous other studies, the detection of these mediators was highly dependent on the time of sampling because of the time to peak concentrations and the half-lives of the mediators involved.^{28,29}

It should be important for a marker or mediator of acute allergic reactions (and anaphylaxis) to show some evidence of dose-response. In other words, the test result should be greater or more frequently positive (or elevated) in severe as compared with milder disease. Thus, the fact that PAF was elevated in 100% of those with severe manifestations of the condition suggests that its operating characteristics (with respect to sensitivity, at least) appear to outperform histamine and tryptase. Consistent with this, for PAF, all 3 grades of allergic reaction were different from each other on comparisons between grades. Histamine would appear to perform poorest, with little evidence of an increase in the proportion of results elevated with increasing severity of the condition, and no significant differences between the groups or on proportions elevated, and lower amount of variation explained.

Mast cell and basophil activation during allergic reactions leads to synthesis and release of a number of mediators that contribute to the derangements seen in the target organ systems. These

mediators do not function in isolation but rather there are likely significant and possibly synergistic interactions among these chemical mediators. Nevertheless, the data from animal models suggest a predominant role for PAF in mediating the severe manifestations of anaphylaxis. Our data have shown that PAF may play a comparable role in the clinical setting and may contribute to morbidity and mortality in human anaphylaxis. These data provide the rationale for the development of drugs to selectively block the actions of PAF, both as rescue therapy for acute anaphylaxis and potentially as chronic treatment for those at the highest risk of life-threatening reactions.

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Clinical implications: PAF correlates better with the severity of acute allergic reactions than either histamine or tryptase. These data suggest an important contributory role for PAF in morbidity and mortality in human anaphylaxis.

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