

Letter to the Editor

Basal platelet-activating factor acetylhydrolase: Prognostic marker of severe Hymenoptera venom anaphylaxis

To the Editor:

Platelet-activating factor (PAF) is a biologically active phospholipid¹ able to induce severe hypotension during anaphylaxis² and is quickly degraded by PAF-acetylhydrolase (PAF-AH).³ PAF and PAF-AH levels have correlated with cardiovascular-dysmetabolic diseases, asthma, and some drugs.^{4,5} In murine models, PAF-AH administration has been shown to prevent severe hypotension and death during anaphylaxis.² In humans, children with fatal anaphylaxis have a low level of PAF-AH, indicating massive PAF release.⁶

Our study investigated the basal plasma PAF-AH level in patients with Hymenoptera venom allergy (HVA) and its correlation with different grades of anaphylaxis, demographic factors, venom-specific IgE, tryptase, chronic diseases, and drug use. Initially, we wanted to evaluate the basal values in patients who had a risk of anaphylaxis during immunotherapy and search for a correlation with the PAF-AH level during anaphylaxis. On analyzing the preliminary data, we found unexpected results, and so we decided to evaluate basal PAF-AH levels in a large number of patients with HVA.

In all, 169 outpatients who were consecutively referred to our Allergy Unit over 30 months and presented with a history of a systemic reaction to Hymenoptera sting and positive diagnostic test results were classified according to the Mueller grade⁷ (Table I). Cardiovascular-dysmetabolic diseases and treatments (platelet antiaggregants, anticoagulants, statins, and antihypertensive drugs) were recorded. Patients with a tryptase value of more than 20 ng/mL, most likely indicating a mast cell disorder, were excluded to avoid a statistical bias. Sixty healthy blood donors with a negative history of atopy and anaphylaxis were included as controls. The study was approved by our foundation's Ethical Committee; all the subjects signed an informed consent form. All patients underwent the routine protocol for HVA, at

least 3 weeks after the anaphylactic reaction, comprising skin tests⁸ with commercial venom extracts, serum-specific IgE, and basal serum tryptase levels using ImmunoCAP (Thermo Fisher Scientific, Milan, Italy). For the basal plasma PAF-AH activity assay, blood samples were collected during the first outpatient visit in anticoagulant-containing test tubes, cool-centrifuged at 2200 rpm for 10 minutes, and stored at -80°C until use. The test was performed by using a colorimetric kit (Cayman Chemical Company, Ann Arbor, Mich). The final absorbance was registered at 405 nm every minute for at least 6 repetitions. The PAF-AH activity (nmol/mL/min) was calculated as described in the manufacturer's instructions; positive and negative controls were added to each analysis. The lower normal limit was 20 nmol/mL/min (manufacturer's instructions and study by Vadas et al⁵). We used univariate ANOVA for repeated measures to evaluate the differences among the PAF-AH values in the different groups, and multivariate ANOVA was used to evaluate the differences in the PAF-AH values among the groups and the effects of age, Mueller reaction grade, diseases, drugs, tryptase, and specific IgE levels. A logistic regression was calculated, with the grade levels as a dependent variable and other values as predictors of the grade in the model. A *P* value of less than .05 was considered to be statistically significant (no adjustment for multiple comparisons was made to the statistical significance level). A receiver operating characteristic (ROC) analysis was performed to determine the optimal PAF-AH cutoff value for severe reactions (Mueller grade II-III-IV). The data were analyzed by using the SPSS program, version 17.0 (SPSS, Inc, Chicago, Ill).

We enrolled 169 patients (107 men, 62 women; mean age, 47.9 ± 18.1 years) and 60 controls (34 men, 26 women; mean age, 46.6 ± 14.8 years). Table I reports the descriptive data for patients classified by Mueller grade. A significant difference in the IgE level was found between patients with grades I and II ($P = .001$), between patients with grades I and III ($P = .01$), and between patients with grades II and IV ($P = .026$). Patients with grade IV had greater tryptase values than did those with

TABLE I. Patients' descriptive data and PAF-AH activity (mean $\pm$ SD, median and range), classified by Mueller's grade*

Mueller grade: symptoms and signs	No. of patients (men; women)	Age (y)	Specific IgE level† (kU _A /L)	Tryptase (ng/mL)	PAF-AH activity (nmol/mL/min)
Grade I: Generalized urticaria, sickness, and anxiety	38 (26; 12)	39.0 ± 16.6 Median: 36 Range: 9-68	6.2 ± 13.2 Median: 1.6 Range: 0.1-68	5.4 ± 2.6 Median: 5 Range: 1-12	28.2 ± 6.3 Median: 27.1 Range: 19.7-42.9
Grade II: Previous symptoms + angioedema, gastrointestinal symptoms (nausea, vomiting, diarrhea, and abdominal pain), dizziness, and breathlessness	37 (17; 20)	48.5 ± 18.0 Median: 47 Range: 17-79	22.6 ± 26.0 Median: 12.5 Range: 0.1-87.9	5.5 ± 3.0 Median: 5 Range: 2.3-15	24.1 ± 3.9 Median: 23.3 Range: 13.1-32.5
Grade III: Previous symptoms + dyspnea, laryngeal stridor, raucousness and glottis edema, dysarthria, asthenia, mental confusion, and feeling of impending death	57 (37; 20)	49.0 ± 19.2 Median: 50 Range: 15-75	16.8 ± 27.5 Median: 5.1 Range: 0.1-100	7.7 ± 10.1 Median: 5 Range: 1-19	16.4 ± 5.0 Median: 17.5 Range: 6.6-26.3
Grade IV: Previous symptoms + hypotension or shock with loss of consciousness, incontinence, and cyanosis	37 (27; 10)	53.8 ± 15.2 Median: 57.0 Range: 24-77	11.6 ± 18.9 Median: 4.1 Range: 0.1-100	7.7 ± 4.3 Median: 7 Range: 1.2-17	16.6 ± 3.9 Median: 18.0 Range: 8.0-23.3
Controls	60 (34; 26)	46.6 ± 14.8 Median: 49 Range: 12-66	Not tested	Not tested	30.2 ± 6.3 Median: 29.8 Range: 20.4-59.8

*Specific IgE positive cutoff, 0.10 kU_A/L; tryptase positive cutoff, 5 ng/mL.

†For the responsible Hymenoptera venom.

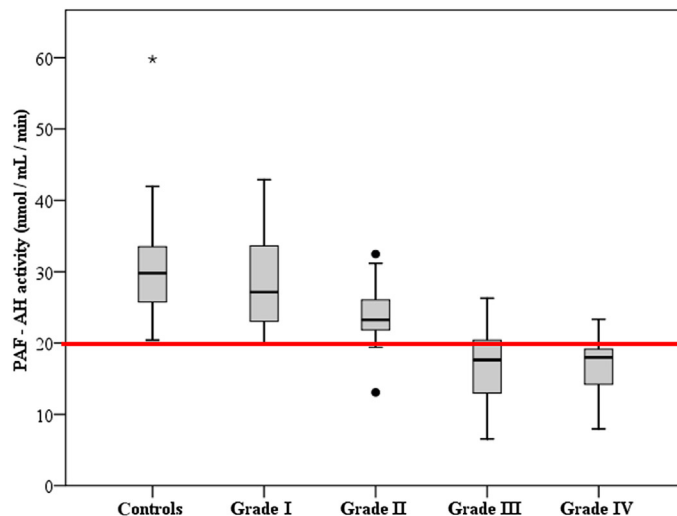


FIG 1. PAF-AH activity among patients with different systemic reaction grades. The red line shows the lower normal limit (20 nmol/mL/min). The bold lines inside boxes indicate the median values. The vertically extending lines from the boxes indicate the upper and lower quartiles; dots indicate outliers. Significant differences are reported in the right side of the figure. *59.8 nmol/mL/min.

Patients	Controls	Grade I	Grade II	Grade III
Grade I	$P = .39$	/	/	/
Grade II	$P < .001$	$P = .001$	/	/
Grade III	$P < .001$	$P < .001$	$P < .001$	/
Grade IV	$P < .001$	$P < .001$	$P < .001$	$P = .09$

grade I ($P = .033$) or II ($P = .048$), whereas there were no differences among the other groups. The prevalence of cardiovascular-dysmetabolic diseases (hypertension, chronic ischemic heart disease, ischemic stroke, dyslipidemia, and diabetes) was low (7% to 15%) and similar among all the groups, and no significant difference in drug use was detected.

The basal PAF-AH activity was normally distributed and decreased according to the anaphylaxis severity. Patients with grade III or IV had the lowest basal enzyme activity: 73.7% for grade III and 89.2% for grade IV presented with enzyme activity below 20 nmol/mL/min; in contrast, no healthy controls and only 1 patient with grade I and 2 patients with grade II had a PAF-AH below this level (Fig 1). There were significant differences in PAF-AH activity between patients with grades I and II ($P < .001$), between patients with grades II and III ($P < .001$), and between patients with grades II and IV ($P < .001$), but there were no significant differences between controls and patients with grade I ($P = .39$) or between patients with grades III and IV ($P = .09$). The correlation between continuous variables and PAF-AH activity was statistically significant only for age ($R = -0.184$, $P = .006$). Multivariate ANOVA revealed the anaphylaxis grade as the only variable with a significant effect on the PAF-AH level ($F = 23.917$; $P < .001$). There was no significant effect of age ($F = .835$; $P = .36$), sex ($F = 2.511$; $P = .12$), venom-specific IgE ($F = 1.002$; $P = .32$), tryptase ($F = .049$; $P = .83$), or the interaction between the anaphylaxis grade and sex ($F = .676$; $P = .57$). Diseases and drugs were not considered because of their low prevalence. Logistic regression revealed that only the PAF-AH level was significantly associated with the anaphylaxis grade ($\chi^2 = 68.066$; $P < .001$). On analyzing the ROC curves for the PAF-AH level for all the patients (area under the ROC curve, 0.862; 95% CI, 0.815-0.909) and in patients with grades II to IV (area under the ROC curve, 0.938; 95% CI, 0.906-0.970), we detected that the best cutoff (sensitivity, 89.3%; specificity, 81.7%) for grades II to IV was 25 nmol/mL/min.

We analyzed the basal PAF-AH activity in 169 patients with HVA and 60 healthy controls; interestingly, we did not find

significant differences between patients with grades III and IV, generally joint in other anaphylaxis grading. Moreover, patients with grade I and controls had similar PAF-AH activity, possibly because grade I presents with only a systemic skin reaction. The prevalence of cardiovascular diseases was low in our population, and no statistical correlation between these pathologies, investigated drugs, and PAF-AH levels was detected. In our population, none of the patients with grade IV anaphylaxis and low PAF-AH levels had fatal anaphylaxis; most of them were treated emergently with corticosteroids (79%), whereas only a few were treated with epinephrine (16%). Epinephrine does not improve PAF-AH activity *in vivo*, as demonstrated in the literature.⁶ In mice,⁹ dexamethasone has been shown to induce a significant transient increase in PAF-AH after 1 day. Till now, no rapid effects on PAF-AH activity have been demonstrated for either epinephrine or corticosteroids.

In conclusion, this study is the first to examine the basal PAF-AH level in a large population of patients with HVA experiencing systemic reactions. The only factor that significantly correlated with the basal PAF-AH level was anaphylaxis grade. Future studies on PAF-AH, including an *in vivo* kinetic study, should help define the significance of a low baseline PAF-AH level. The level indicates the genetic background for an individual and its predictability for anaphylaxis severity in the future. Finally, in our population, the PAF-AH level was not affected by chronic drug use and/or cardiovascular-dysmetabolic diseases. Future investigations, including gene polymorphisms and human *in vivo* kinetic studies, are needed.

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