

Received Date:

Revised Date:

Accepted Date:

Article Type: Letter

Title: Platelet-activating factor acetylhydrolase: a biomarker in Hymenoptera venom allergy?

Manuscript Acceptance Date: 18-Oct-2021

*Maria Beatrice Bilò^{1,2,3}, MD

Alice Corsi³, MD

Matteo Martini⁴, MD

Maria Giovanna Danieli^{1,3,5}, MD

Sonila Alia⁶

Alice Di Paolo⁶

Gianluca Moroncini¹, MD

Arianna Vignirri¹, PhD

¹ Department of Clinical and Molecular Sciences – Marche Polytechnic University, Ancona, Italy

² Allergy Unit, Department of Internal Medicine, Ospedali Riuniti Ancona University Hospital, Italy

³ Postgraduate School of Allergy and Clinical Immunology, Marche Polytechnic University, Ancona, Italy

⁴ Global Clinical Development, Chiesi Farmaceutici S.p.A., Parma, Italy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/all.15161](#)

This article is protected by copyright. All rights reserved

⁵ Clinica Medica, Department of Internal Medicine, Ospedali Riuniti Ancona University Hospital, Italy

⁶ Department of Clinical Sciences, Section of Biochemistry, Biology and Physics, Marche Polytechnic University, Ancona, Italy

Corresponding Author: Maria Beatrice Bilò, Department of Clinical and Molecular Sciences – Marche Polytechnic University, Ancona, Italy - Allergy Unit, Department of Internal Medicine, Ospedali Riuniti Ancona University Hospital, Italy - Via Tronto 10/A, 60126 Ancona, Italy. Tel: 0715963804 Fax: +390715963253 E-mail: m.b.bilo@univpm.it

No funding was received for the present study. The authors declare no conflict of interests.

Word count: 943

Acknowledgments

The authors would like to thank Valerio Pravettoni for his contribution to the discussion of the results of the paper.

Keywords

1. Anaphylaxis
2. Asthma
3. Biomarkers
4. Hymenoptera venom allergy
5. Platelet-activating factor acetylhydrolase

Authors' contribution

This article is protected by copyright. All rights reserved

M.B.Bilò planned and designed the study. A.Corsi extracted, collected data, and wrote the manuscript. M.Martini conducted data analysis. A.Vignini, S.Alia and A. Di Paolo performed the assay of the enzymatic activity of PAF-AH.

All the authors contributed to the interpretation of data and revision of the article. All authors had access to the data, read, and approved the final manuscript.

Text

To the Editor

Anaphylaxis is a rapid, potentially fatal, immediate hypersensitivity reaction. Preformed and newly formed biochemical mediators, including histamine, tryptase, carboxypeptidase A, prostaglandin D₂, leukotrienes, and platelet-activating factor (PAF) are released systematically during the degranulation of mast cells and basophils. PAF is a proinflammatory phospholipid, synthesized and secreted by mast cells, monocytes and fixed tissue macrophages whose binding to its receptor on target cell-platelet, monocytes, macrophages, and neutrophils results in many of the manifestation of acute allergic reaction and anaphylaxis. Circulating levels of PAF are controlled by the activity of Platelet-activating factor-acetylhydrolase (PAF-AH), which is the enzyme that degrades PAF. Due to the quick elimination of PAF (half-time: 3-13 minutes), PAF-AH has been proposed as a surrogate biomarker for PAF activity correlated with the severity of anaphylaxis¹⁻⁵. However, it was investigated in Hymenoptera venom allergy (HVA) only in one previous study, in a real-world population⁴.

The objectives of this study were to investigate the role of PAF-AH as a predictive biomarker for the severity of the reaction in a HVA selected population (HVA-alone population), without confounding factors, and to compare their PAF-AH values with healthy subjects and patients with allergic asthma or rhinitis. We also investigated the correlation of PAF-AH activity with other factors (demographic factors, concomitant diseases and medications, serum tryptase values, venom sIgE levels, culprit insect), in a larger HVA real-world population.

HVA patients were consecutively enrolled at the Allergy Unit of the “Ospedali Riuniti” of Ancona, in Italy, with a history of systemic reaction after hymenoptera sting, according to Mueller classification, or of large local reaction⁶. A blood sample was taken before the start of venom immunotherapy.

Participants signed an informed consent. No formal approval by the Ethics Committee was needed, as all the interventions were part of routine clinical practice.

PAF-AH activity was measured blindly by the Department of Clinical Sciences, Section of Biochemistry, Biology and Physics of the Marche Polytechnic University using a colorimetric kit (Methods available on the Online Supplementary space).

A sample size of 54 subjects has been calculated to detect the minimum clinically significant difference (MCSD) in PAF-AH activity of 10 nmol/mL/min, amongst healthy subjects and the different grades of severity of the **HVA-alone group** (power=80%, $\alpha=0.05$), and between severe reactions and the control groups of rhinitis and asthma (power=94%, $\alpha=0.05$). Additional HVA patients with concomitant diseases/medications (**HVA real-world group**) were enrolled to complete the total available 140 PAF-AH assessments. ANOVA test with Bonferroni adjustment was performed using STATA v.13 (StataCorp-College Station, Texas, USA). From 2017 to 2019 PAF-AH activity was measured in 103 consecutive HVA patients, 12 healthy controls, 13 patients with allergic rhinitis, and 10 patients with allergic rhinitis and asthma, who were controlled with maintenance medium to high dose of inhaled corticosteroids plus long-acting beta agonists. No asthmatic patients were in treatments with oral steroids and/or biologics. (Figure S1, Table 1). In the **HVA-alone population** the mean PAF-AH activity (24.5-27.9 nmol/mL/min) was significantly lower, compared to healthy subjects (40.4 nmol/mL/min, $p<0.001$), patients with allergic rhinitis (36.7 nmol/mL/min, $p<0.001$) and asthma too (30.7 nmol/mL/min, unadjusted analysis only, $p=0.046$) (Table S2). However, no correlation with the severity of reaction was observed (Figure 1-A). PAF-AH activity was lower in asthma, compared to healthy subjects ($p=0.001$) and patients with rhinitis (unadjusted analysis only, $p=0.014$) (Figure 1-B). These results were confirmed when the analyses were extended to the **HVA real-world population** (Table S3). PAF-AH activity was not correlated with gender, age, hypertension, dyslipidemia, diabetes, or baseline serum tryptase levels. A markedly decreased PAF-AH activity was found in the only HVA patient with asthma (PAF-AH: 10.9 nmol/mL/min). A significant but modest PAF-AH increase was found in patients taking beta-blockers (+4 nmol/mL/min, $p=0.015$), and in patients allergic to vespid venom, compared to bee venom (+3.6 nmol/mL/min, $p=0.0133$), but no correlation was found between the PAF-AH values and the sIgE values of the whole extract of the culprit insect.

This is the first study assessing the role of PAF-AH as a biomarker in an HVA-alone selected population, compared to both healthy subjects and patients with respiratory allergic diseases.

Our results apparently disagree with those of Pravettoni et al. about the observed difference across different degrees of reaction severity. However, the difference in the enzyme values in the Mueller-III (M-III) and Mueller-IV (M-IV) and Mueller-II (M-II) reactions, in Pravettoni et al, is lower (about 7.5 nmol/mL/min) than the MCSD that we have estimated (10 nmol/mL/min); hypotension is a feature present in M-IV grade only, and it is not clear the reason why subjects who have experienced M-III reactions have the same values as those with M-IV reactions. It should be also noted that the two studied populations are different in terms of their place of origin and potential concomitant diseases/treatments, possibly reflecting genetic differences and different confounding effects of comorbidities and medications, as demonstrated by our results in asthmatics and beta-blocker users.

Our results do not support the role of PAF-AH as a biomarker of reaction severity, for HVA. However, since PAF-AH values in HVA population were lower than those of the control groups, we can assume that PAF-AH was able to characterize HVA patients as a separate population from healthy subjects and patients with allergic asthma and/or rhinitis.

PAF-AH is not a reliable predictor biomarker of the severity of allergic reactions, to be used in clinical practice, possibly because it is not an adequate surrogate biomarker of PAF activity. It is also possible that the PAF alone is not sufficient to determine the reaction severity, as several mediators and factors, both biochemical and clinical, take part in the onset and severity of the anaphylactic reaction.

Moreover, standardization of the methods to measure PAF-AH activity is needed before its implementation in clinical practice.

References

1. Iordan R, Tsoupras A, Zabetakis I, Demopoulos CA. Forty years since the structural elucidation of platelet-activating factor (PAF): historical, current and future research perspective. *Molecules* 2019 doi:10.3390/molecules24234414
2. McIntyre TM, Prescott SM, Stafforini DM. The emerging role of PAF acetylhydrolase. *J Lipid Res* 2009;50:255-9
3. Wadas P, Gold M, Perelman B et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008;358:28-35

4. Pravettoni V, Piantanida M, Primavesi L, Forti S, Pastorello EA. Basal platelet-activating factor acetylhydrolase: prognostic marker of severe Hymenoptera venom anaphylaxis. *J Allergy Clin Immunol* 2014;133:1218-20
5. Brown SGA, Stone SF, Fatovich DM, et al. Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin Immunol* 2013;132:1141-9
6. Hø MB, Rueff F, Mosbech H, et al. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;60:1339-49

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table 1 Baseline features

	Overall HVA (N=103)	HVA-alone group (N= 49)	Allergic Asthma (N= 10)†	Allergic Rhinitis (N= 13)	Healthy (N= 12)
Median Age (IQR)	54 (39-61)	43 (33-58)	47 (29-56)	43 (28-48)	52 (47-60)
M/F	71/32 (69/31%)	34/15 (69/31%)	2/8 (20/80%)	6/7 (46/54%)	3/9 (25/75%)
Allergic comorbidities					
Allergic asthma	1%	0	100%	0	0
Allergic rhinitis	5%	0	100%	100%	0
Airborne sensitization	8%	0	100%	100%	0
Other comorbidities					
Cardiac disease	14%	0	0	0	0
Hypertension	24%	0	10%	0	0
Diabetes	4%	0	0	0	0
Dyslipidemia	9%	0	10%	0	0
Concomitant treatments‡					
Beta-blockers	11%	0	0	0	0
Statins	10%	0	10%	0	0
Sartans	12%	0	10%	0	0

ACE-inhibitors	4%	0	0	0	0
Antiarrhythmics	4%	0	0	0	0
Anticoagulants	13%	0	0	0	0
Hymenoptera allergy					
Bee	27%	31%	n.a.	n.a.	n.a.
Vespid	73%	69%			
Type of action					
LLR	10%	20%			
Mueller I	7%	12%			
Mueller II	10%	8%			
Mueller III	37%	24%			
Mueller IV	36%	35%			
Median Serum tryptase levels, ng/nl (IQR)	4.6 (3.4-6.6)	4.9 (3.2-5.9)	n.a.	n.a.	n.a.

† One outlier patient was excluded by the analysis (PAF-AH: 68.4 nmol/mL/min)

‡ All the asthmatic patients were in treatment with medium-high doses of inhaled corticosteroids + long-acting beta agonists.

IQR: Interquartile range.

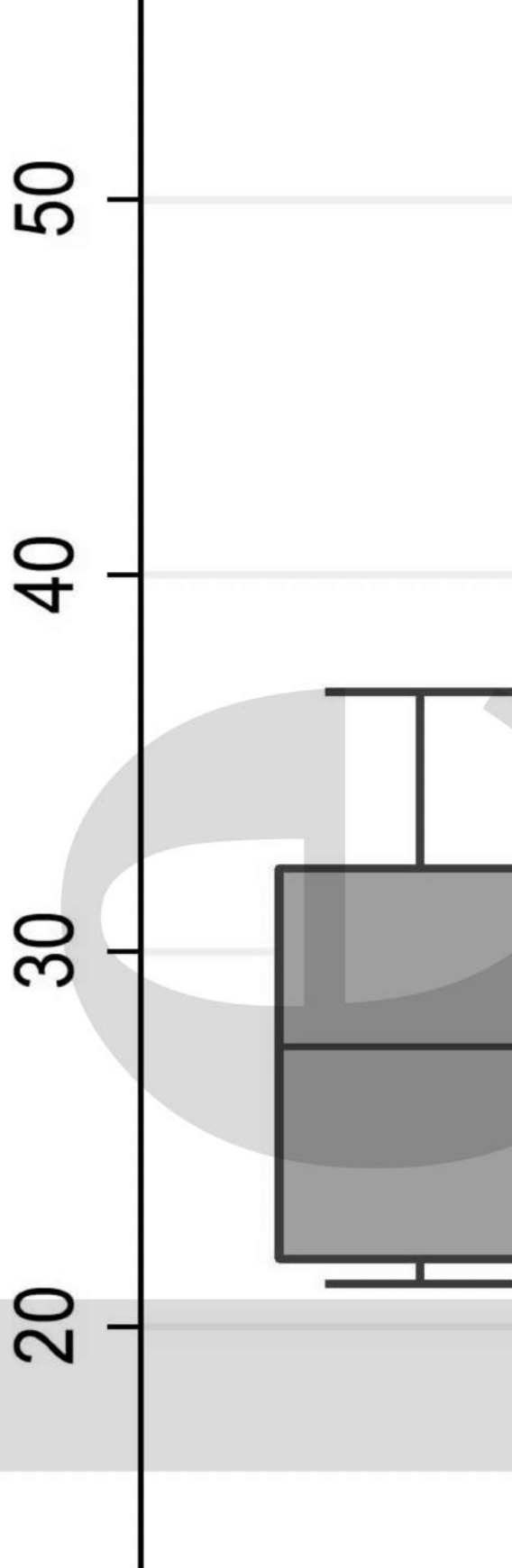
n.a. not applicable

Figure legends

Figure 1 - PAF-AH activity in patients with different reaction grades due to Hymenoptera venom allergy compared with healthy subjects (A), and in the overall patients with Hymenoptera venom allergy compared with the control groups (B). (HVA-alone population).

(A)

PAF-AH (nmol/ml/min)



10

Large local
reactions

M

AC

TO

DE

Q

4

R

e



This article is protected by copyright. All rights reserved

Supporting information section

Table S2 – Platelet-activating factor acetylhydrolase results (primary analyses)

	HVA-alone	Asthma	Rhinitis	Healthy subjects
Large Local Reaction	27.4 ± 5.6	30.7 ± 4.5*	36.7 ± 3.9**	40.4 ± 8.0**
Mueller 1	26.6 ± 4.6			
Mueller 2	27.3 ± 5.3			
Mueller 3	24.5 ± 7.6			
Mueller 4	27.9 ± 4.2			

Values are reported as means ± standard deviation nmol/mL/min

*p<0.05 (unadjusted comparison *versus* overall HVA-alone group)

**p<0.05 (adjusted and unadjusted comparison *versus* overall HVA-alone group)

HVA-alone: Hymenoptera venom allergic patients without concomitant diseases/treatments.

Table S3 – Impact of demographic and clinical characteristics on platelet-activating factor acetylhydrolase activity (secondary analyses in the total population with Hymenoptera venom allergy)

Factor	Mean change†	p-value
Age	n.a.	n.s.
Male gender	+2.8	n.s.
Hypotension	+1.5	n.s.
Anaphylaxis	-1	n.s.
Vespid allergy	+3.6	0.01
Specific IgE levels	n.a.	n.s.
Comorbidities		
Hypertension	+1.5	n.s.
Dyslipidemia	+0.7	n.s.
Cardiac	+0.6	n.s.
Diabetes	+0.6	n.s.
Allergy‡	-4.7	0.02
Concomitant treatments		
Beta-blockers	+4.1	0.01
Statins	+0.6	n.s.
Sartans	-1.1	n.s.
ACE-inhibitors	+3.1	n.s.
Antiarrhythmics	-4.8	n.s.
Anticoagulants	+1.1	n.s.

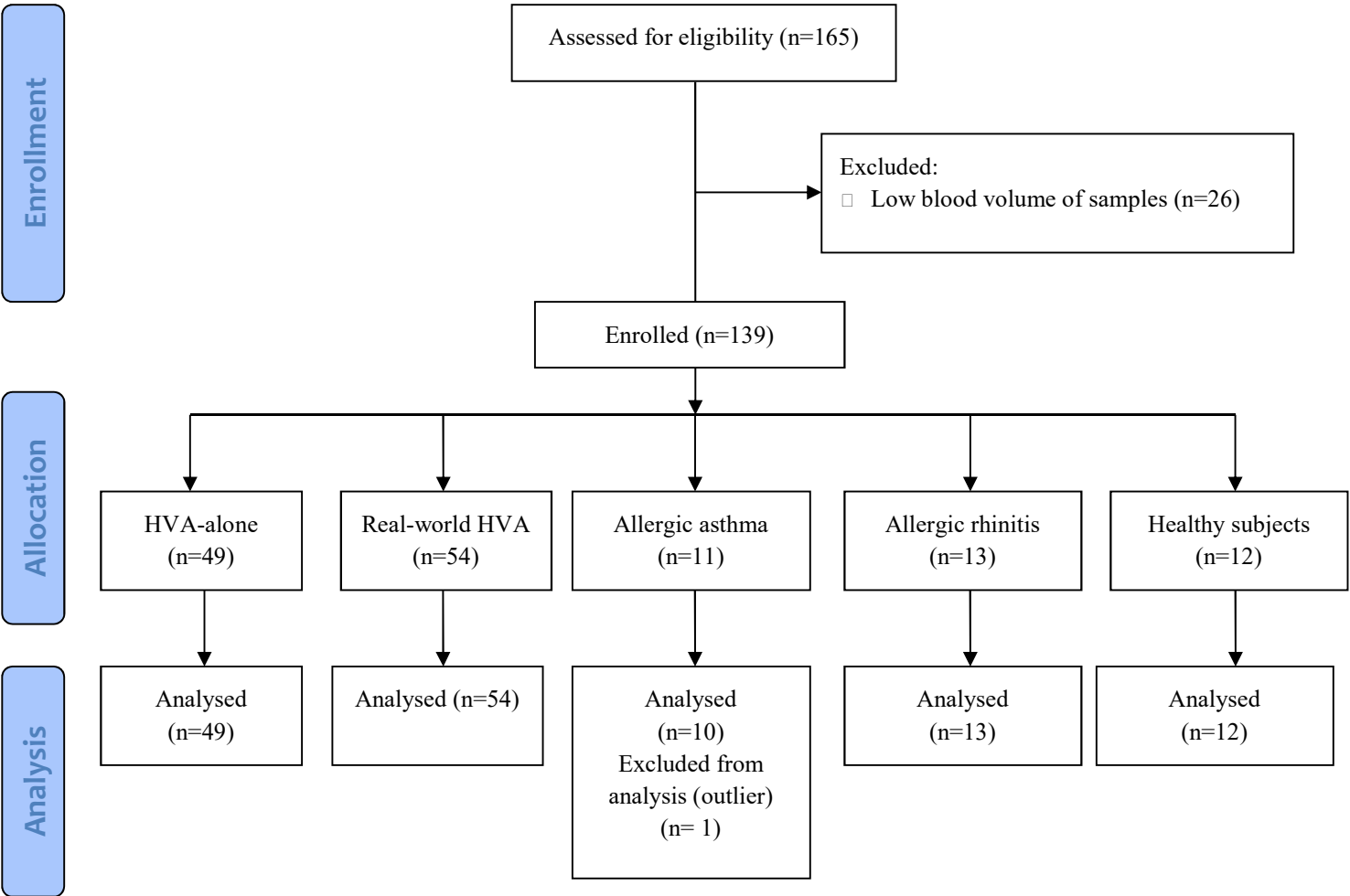
Values are reported as nmol/mL/min. T-test was performed if not otherwise specified.

n.a. not applicable (linear regression); n.s. not statistically significant.

† Presence *versus* absence of the factor, in the overall Hymenoptera venom allergy population.

‡Allergic asthma, rhinitis, and/or mild food allergy. The difference is not significant if the only patient with asthma is removed from the analysis.

Figure S1 – Flow diagram of the study (HVA-alone and real-world HVA: Hymenoptera allergy patients without and with concomitant diseases/treatments, respectively)



Method for sample collection and dosage of the enzymatic activity of PAF-AH

For the basal plasma PAF-AH activity assay, blood samples were collected in anticoagulant (Ethylenediamine tetraacetic acid)-containing test tubes and centrifuged at 1000 rpm for 15 min. The plasma was then separated and stored at -80°C until use. The assay of the enzymatic activity of PAF-AH was performed blindly by the Department of Clinical Sciences, Section of Biochemistry,

Biology and Physics of the Marche Polytechnic University. Before performing the analysis, the plasma was concentrated using Amicon® Ultra-4 concentrators (Millipore, Billerica, MA), with a molecular weight cut-off of 30 kda, by centrifuging at 7500 rpm for 15 minutes. Plasma levels of PAF-AH activity were determined with a colorimetric PAF-AH Assay Kit (Cayman Chemical Company, Ann Arbor, MI). Blank wells (no enzyme controls), positive control wells (human PAF-AH), and sample wells were prepared in duplicate. The final absorbance was registered at 405-414 nm once every minute for at least 5 minutes using a microplate reader (Synergy™ HTX, BioTek) to obtain at least five time points. The PAF-AH activity, expressed as nmol/mL/min was calculated as described in the manufacturer's instructions.