REFERENCES

1. Di Marzo V. New approaches and challenges to targeting the endocannabinoid system. *Nat Rev Drug Discov*. 2018;17:623-639.
2. Angelina A, Perez-Diego M, Lopez-Abente J, Palomares O. The role of cannabinoids in allergic diseases: Collegium Internationale Allergologicum (CIA) Update 2020. *Int Arch Allergy Immunol*. 2020;181(8):565-584.
3. Martin-Fontecha M, Eiwegger T, Jartti T, et al. The expression of cannabinoid receptor 1 is significantly increased in atopic patients. *J Allergy Clin Immunol*. 2014;133(3):926-929.e2.
4. Angelina A, Martin-Fontecha M, Ruckert B, et al. The cannabinoid WIN55212-2 restores rhinovirus-induced epithelial barrier disruption. *Allergy*. 2020;76:1900-1902.
5. Angelina A, Pérez-Diego M, López-Abente J, et al. Cannabinoids induce functional Tregs by promoting tolerogenic DCs via autophagy and metabolic reprogramming. *Mucosal Immunol* 2021. https://doi.org/10.1038/s41385-021-00455-x
6. Palomares O, Ruckert B, Jartti T, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol*. 2012;129(2):510-520.e9.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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

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 an enzyme that degrades PAF. Due to the quick elimination of PAF (halftime, 3–13 min), PAF-AH has been proposed as a surrogate biomarker for PAF activity correlated with the severity of anaphylaxis.[1–5] However, it was investigated in Hymenoptera venom allergy (HVA) only in one previous study, in a real-world population.[4]

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, and culprit insect), in a larger HVA real-world population.

HVA patients were consecutively enrolled at the Allergy Unit of the "Ospedali Riuniti" hospital of Ancona, in Italy, with a history of systemic reaction after hymenoptera sting, according to Mueller classification, or of large local reaction.[6] 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 File).

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, among 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, $p < .001$), patients with allergic rhinitis (36.7 nmol/mL/min, $p < .001$), and asthma too (30.7 nmol/

TABLE 1 Baseline features

	Overall HVA (*N* = 103)	HVA-alone group (*N* = 49)	Allergic asthma (*N* = 10)[a]	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[b]					
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 reaction					
LLR	10%	20%	n.a.	n.a.	n.a.
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.

Abbreviations: IQR, Interquartile range; n.a., not applicable.

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

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

mL/min, unadjusted analysis only, $p = .046$; Table S2). However, no correlation with the severity of reaction was observed (Figure 1A). PAF-AH activity was lower in asthma, compared to healthy subjects ($p = .001$) and patients with rhinitis (unadjusted analysis only, $p = .014$) (Figure 1B). 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, $p = .015$), and in patients allergic to vespid venom, compared to bee venom (+3.6 nmol/mL/min, $p = .0133$), 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

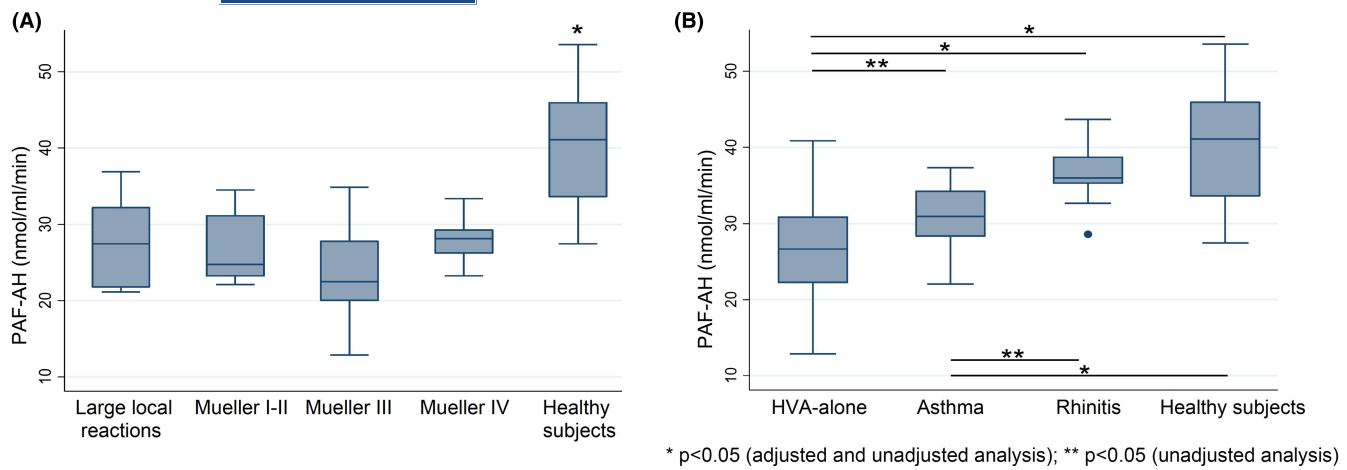

FIGURE 1 PAF-AH activity in patients with different reaction grades due to Hymenoptera venom allergy compared with healthy subjects (A), and in the overall patients with Hymenoptera venom allergy compared with the control groups (B). (HVA-alone population)

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 the 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.

KEYWORDS

anaphylaxis, asthma, biomarkers, Hymenoptera venom allergy, platelet-activating factor acetylhydrolase

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

1. Lordan 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;24:4414.
2. McIntyre TM, Prescott SM, Stafforini DM. The emerging role of PAF acetylhydrolase. *J Lipid Res*. 2009;50:255-259.
3. Vadas 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;133:1218-1220.
5. Brown SGA, Stone SF, Fatovich DM, et al. Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin Immunol*. 2013;132:1141-1149.
6. Bilo BM, Rueff F, Mosbech H, et al. Diagnosis of Hymenoptera venom allergy. *Allergy*. 2005;60:1339-1349.

SUPPORTING INFORMATION

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The NLRP3 inflammasome inhibitor, OLT1177®, ameliorates experimental allergic asthma in mice

To the Editor,

Despite differences, all types of asthma share a complex symptomatology that derives from chronic inflammation of the airways. Since a curative therapy is not yet available, standard of care treatment regimens aim to control symptoms by reducing this inflammation through inhaled corticosteroids. Though this approach has proved to be a reliable and effective treatment option in the majority of patients with mild-to-moderate asthma, patients with severe, neutrophilic, difficult-to-treat, or uncontrolled asthma frequently experience acute exacerbations and loss of symptom control despite permanent corticosteroid treatment. A safe and more tolerable treatment option for these patients remains an unmet medical need.[1] Consequently, evaluating selective NLRP3 inhibitor, OLT1177® (dapansutrile) on the pathologic features of experimental allergic asthma (EAA) in different mouse models, became the focus of our studies.

Upon activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3), the formation of the NLRP3 inflammasome occurs, leading to the release of caspase-1-dependent proinflammatory cytokines interleukin (IL) 1β and IL-18 as well as pyroptosis, which actually facilitates clearance of inhaled pathogens. However, overactivation of this axis has been implicated in the development and exacerbation of asthma.[2]

In our first model, intra-peritoneal treatment with 60 mg/kg body weight (bw) OLT1177® of mice with ovalbumin- (OVA-) induced asthma showed reduced NLRP3 expression and caspase-1 activation in lung tissue, and levels of activated IL-1β in broncho-alveolar lavage fluid (BAL) fluid (Figure 1A, B, G). All pathophysiologic hallmarks of this Th2-high, eosinophilic asthma endotype were also diminished, such as inflammatory cell counts in BALF and airway tissue, goblet cell hyperplasia, airway hyper-responsiveness (AHR), and BAL levels of T helper 2 (Th2) type (IL-4, IL-5, IL-13) and proinflammatory (IL-6, tumor necrosis factor [TNF]) cytokines (Figure 1C–G).

Next, we evaluated two models that mimic other asthma endotypes or diseases stages, namely HDM-induced EAA and poly(I:C)-triggered exacerbation of EAA. In mice with HDM-induced EAA i.p., OLT1177® diminished airway inflammation as evidenced by reduced eosinophil and neutrophil numbers in BAL and inflammatory cell infiltrate in lung tissue and significantly reduced AHR (Figure 2A–C). Comparable effects of OLT1177® were also observed in mice undergoing the induction of an acute exacerbation of OVA-induced EAA.[3] Consistent with the previous findings, OLT1177® treatment markedly reduced eosinophil and neutrophil numbers in BAL, inflammatory cell infiltrate in lung tissue, and lowered AHR (Figure 2D–F). Since drug administration requiring repeated injection is known to have low compliance in patients, we tested the effectiveness of OLT1177® in the oral route of administration in mice with OVA-induced EAA. OLT1177® delivered via enriched mouse feed reaching therapeutic exposure confirmed by pharmacokinetic evaluation (Figure S1A), demonstrated therapeutic benefit on the pathophysiologic hallmarks of EAA comparable to the findings using i.p. treatment (Figure 2G–I).

During the study, no signs of negative effects of OLT1177® were seen in any study animal. This is consistent with our previous findings from administration of OLT1177® in animals,[4] as well as the safety outcomes in all its six human trials to date, including in patients with acute gout flares[5] or with heart failure.[6]

Taken together, we have shown that systemic treatment, both i.p. and orally, with OLT1177® is consistently bioactive and