

The Basophil Activation Test Can Be of Value for Diagnosing Immediate Allergic Reactions to Omeprazole



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What is already known about this topic? Incidence of hypersensitivity reactions to proton pump inhibitors has risen, likely because of increased consumption. Their diagnosis is difficult, with skin tests (STs) showing low sensitivity and drug provocation tests being complex and not risk-free.

What does this article add to our knowledge? Basophil activation test with omeprazole shows a sensitivity of 73.8%, a complete specificity (100%), and a negative predictive value of 67%. By combining ST and BAT we can diagnose 85.7% of patients with immediate allergy to omeprazole.

How does this study impact current management guidelines? BAT represents a complementary tool for inclusion in the allergological workup for omeprazole allergy. When combined with ST, it can be of value to guide the clinician as to whether to perform a drug provocation test.

BACKGROUND: Proton pump inhibitors (PPIs) are commonly used to treat gastrointestinal diseases. Incidence of hypersensitivity reactions to PPIs has risen, likely because of increased consumption. Their diagnosis is difficult, with skin tests (STs) presenting low sensitivity, making it necessary to perform drug provocation tests (DPTs). The value of *in vitro* tests for the diagnosis of immediate reaction to PPI is unclear. **OBJECTIVE:** To analyze the diagnostic value of the basophil activation test (BAT) in a group of patients diagnosed with immediate allergy to omeprazole.

METHODS: The study included 42 patients with confirmed immediate allergic reactions to omeprazole confirmed by positive ST results or DPT results and 22 age- and sex-matched subjects tolerant to PPIs. BAT was performed with omeprazole, pantoprazole, and lansoprazole using CD63 and CD203c as activation markers.

RESULTS: ST sensitivity was 66.7% with a specificity of 100%. BAT using CD63 with a stimulation index of more than 2 as positive revealed a sensitivity of 73.8%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value

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The present study has been supported by the Institute of Health “Carlos III” of the Ministry of Economy and Competitiveness (grants cofunded by European Regional Development Fund: grant nos. PI12/02529, PI15/01206, CP15/00103, RETICS ARADyAL RD16/0006/0001, and RD16/0006/0033) and by the Andalusian Regional Ministry of Economy and Knowledge (grants cofunded by European Regional Development Fund: grant no. CTS-06603); Andalusian Regional Ministry Health (grant nos. PI-0699-2011, PI-0352-2012, PI-0179-2014, and PI-0241-2016); Merck-Serono Research Grant from Fundacion Salud 2000; and Allergology and Clinical Immunology Spanish Society Foundation and Alfonso X el Sabio University Foundation. M.S. holds a “Rio Hortega” research contract (grant no. CM13/00029) supported by the Institute of Health “Carlos III” of the Ministry of Economy and Competitiveness (grants cofunded by European Social Fund [ESF]); I.D. holds a “Juan Rodes” contract (grant no. JR15/00036) supported by the Institute of Health “Carlos III” of the Ministry of Economy and Competitiveness (grants cofunded by ESF; C.M. holds a “Nicolas Monardes” research contract by Andalusian Regional Ministry of Health (grant no.

C-0044-2012); A.A. holds an Andalucía Talent Hub Fellowship (grant no. TAHUB/II-004) cofunded by the Junta de Andalucía and the UE, VII Framework Programme of the European Commission (grant agreement no. 291780); and T.D.F. holds a “Ramon y Cajal” research contract by the Ministry of Economy and Competitiveness cofunded by ESF (grant no. RYC-2013-01283).

Conflicts of interest: None of the authors have any conflict of interest and nor have they received any money for the present study. Research is part of their daily activities. All the authors had full access to all the data and take responsibility for the integrity of the data and the accuracy of the data analysis.

Received for publication August 21, 2017; revised November 13, 2017; accepted for publication December 6, 2017.

Available online January 12, 2018.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2017.12.001>

Abbreviations used

BAT- Basophil activation test
DPT- Drug provocation test
IDT- Intradermal test
PPI- Proton pump inhibitor
SI- Stimulation index
SPT- Skin prick test
ST- Skin test

of 66.7%. BAT was positive in 57.1% of patients with negative ST result, and thus by combining ST and BAT we can correctly diagnose 85.7% of patients with immediate allergy to omeprazole.

CONCLUSION: BAT represents a complementary tool for inclusion in the allergological workup for patients allergic to omeprazole. When combined with ST, it can be of value to guide the clinician as to whether to perform a DPT. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1628-36)

Key words: Basophil activation test; Drug provocation test; Omeprazole; Proton pump inhibitors; Skin test

INTRODUCTION

Proton pump inhibitors (PPIs) are drugs commonly used in clinical practice for the treatment of gastrointestinal diseases such as gastroesophageal reflux disease, gastrointestinal ulcers, or *Helicobacter pylori* infections. They are generally well tolerated, with a low incidence of adverse effects (between 1% and 3%),¹ although in the last decade there has been an increase in hypersensitivity reactions, probably related to their increasing consumption.² Most hypersensitivity reactions appear to be immediate, accounting for 86% of all immunological reactions to PPI,^{3,4} with the most common clinical manifestations being urticaria (54%-65%), pruritus (33%-52%), angioedema (38%-42%), hypotension (22%-23%), and dyspnea (20%-22%), with half of the reactions considered to be anaphylactic.^{3,4}

Immediate hypersensitivity reactions to PPI are difficult to diagnose. Skin testing (ST) has a sensitivity ranging from 22.6% to 62.5%.⁵⁻⁷ However, few studies^{6,7} have compared the results of standardized skin prick testing (SPT) and intradermal testing (IDT) with those of drug provocation testing (DPT) for patients with PPI-induced immediate hypersensitivity and for healthy controls. Although these studies gave different values for sensitivity (22.6%-58.8%) and negative predictive value (NPV; 70.8%-91.9%), they obtained the same specificity and positive predictive value (PPV; 100%). Therefore, DPT is still necessary to diagnose immediate allergic reactions to PPI, although it is not risk-free, especially for severe reactions and older patients.

Regarding *in vitro* tests, to our knowledge, only 2 case reports have studied the role of the basophil activation test (BAT) in the diagnosis of immediate reactions to PPIs,^{8,9} obtaining positive results for the patients analyzed and negative results in healthy controls. Given these promising initial results, further studies are needed to assess the usefulness of BAT for diagnosing immediate reactions to PPIs. It is of note that to perform BAT, 2 basophil activation markers related to degranulation and histamine release can be used—CD63^{10,11} and CD203c.^{12,13} CD203c is constitutively expressed in basophils and increases in expression after

activation, whereas CD63 is expressed only after activation and usually shows a bimodal expression, although it is also expressed in other cell types such as platelets.¹⁴ Moreover, the upregulation of one or the other may depend on the drug and the clinical entity,^{13,15} making it important to determine which is the best activation marker for PPIs.

The PPIs most frequently involved in immediate hypersensitivity reactions are omeprazole and lansoprazole, although reactions with pantoprazole, esomeprazole, and ransoprazole have also been reported.^{3,4,16} Incidence varies by country and is probably related to consumption patterns. In Spain, the intake of PPIs has increased in recent years, with almost 10% of the Spanish population consuming this type of drug daily,¹⁷ and omeprazole being the most highly consumed compared with other antiulcer drugs,^{18,19} which is likely why it is the most frequent inducer of immediate reactions to PPI in Spain.^{20,21}

PPIs consist of a benzimidazole and a pyridine ring, but vary in the specific side-ring substitution.²² Omeprazole, esomeprazole, and pantoprazole are substituted on the benzimidazole ring, whereas rabeprazole and lansoprazole are not.²³ Different patterns of immunological recognition have been described, possibly because of their chemical structures.^{6,24} It has been shown that patients with positive ST results to pantoprazole also give positive results to omeprazole.^{6,7,20,21,24-27} However, patients reacting to lansoprazole or rabeprazole, which have a different side chain, can give negative ST results with omeprazole, pantoprazole, and esomeprazole.^{6,7,24,26,28} Nevertheless, cross-reactivity among the whole group of PPIs has also been reported, probably because of the recognition of the shared pyridine ring.^{24,25}

In our opinion, the underlying mechanisms and immunological responses have not been sufficiently investigated in patients with immediate reactions to PPIs. In this study, we have evaluated the use of BAT to diagnose omeprazole hypersensitivity in a group of well-characterized patients.

METHODS

Patients and controls

All patients referred to the Allergy Service of the Regional University Hospital of Malaga and the Allergy Unit of Cruz Roja Hospital of Madrid, over a 3-year period (2013-2015) and with a clear clinical history of an immediate hypersensitivity reaction after administration of omeprazole, were prospectively eligible for inclusion in the study. Of these, those who were confirmed as allergic were finally included. Patients were confirmed as allergic on the basis of a compatible clinical history and the presence of a positive ST or DPT result with omeprazole. Different clinical categories were established: anaphylaxis and anaphylactic shock, defined according to the criteria of Sampson et al²⁹; urticaria when manifestations were limited to the skin and consisted of pruritic, erythematous cutaneous elevations that blanched with pressure at various sites on the body; and generalized pruritus.

BAT was performed for all patients, as well as in a control group of 22 age- and sex-matched subjects, who reported previous intake of omeprazole, and with negative ST results and confirmed tolerance by DPT. All the individuals tested as possible controls were included. Blood extraction was always performed in absence of any drug therapy.

The study was conducted according to the Declaration of Helsinki and all patients and controls participating in the study gave their informed consent. Protocols were approved by an institutional ethical committee (Ethical Committee of Malaga).

Atopic status

Atopic status was assessed by SPT using a battery of 20 common inhalant allergens, including pollens, house dust mites, molds, and animal dander, and 31 common food allergens that included animal, fruit, and vegetable allergens (ALK, Madrid, Spain). A positive SPT response was defined as a wheal diameter of 3 mm or larger to at least 1 of these allergens, and any patient producing such a wheal was considered atopic.

Skin testing

SPTs and IDTs were carried out, as previously described,⁶ in patients and in 22 control subjects with confirmed tolerance to omeprazole. SPT was performed with omeprazole (Normon, Madrid, Spain) using an undiluted commercial preparation (40 mg/mL); IDT was performed with 0.04, 0.40, and 4.0 mg/mL dilutions that were prepared daily. SPT results were read after 20 minutes and considered positive if a wheal of 3 mm or larger surrounded by erythema appeared, with a negative response to the control saline. IDTs involved the injection of 0.03 mL of the solution, the wheal area was marked initially and 20 minutes after testing, and an increase in diameter larger than 3 mm surrounded by erythema was considered positive. Histamine (0.01%) and saline were the positive and negative controls, respectively.

Drug provocation test

Single-blind placebo-controlled DPT was carried out using omeprazole (Normon) if the ST result was negative. It was performed under strict hospital surveillance, as described.³⁰ Briefly, placebo capsules were given at different times on the first day; on the second day, omeprazole was administered at 30-minute intervals in escalating doses (5, 5, 10, and 20 mg) until reaching the full therapeutic dose (40 mg). If cutaneous and/or respiratory symptoms or alterations in vital signs (rhythm alterations, decrease in peak expiratory flow, or hypotension) appeared, the procedure was stopped and the symptoms were evaluated and treated. If no symptoms appeared during drug administration, the therapeutic dose of omeprazole was followed by a 2-day course at maximum dose, after a gap of 24 hours.

Basophil activation test

BAT was performed, as previously described,³¹ with omeprazole, pantoprazole, and lansoprazole at 2.0, 0.2, and 0.02 mg/mL (all from Sigma Aldrich, Saint Louis, Mo). Cells were stained with mAbs, anti-CCR3-APC, CD63-FITC, and CD203c-PE (Caltag Laboratories, Burlingame, Calif), and analyzed using a FACSCalibur flow cytometer (Becton-Dickinson Bioscience, San Jose, Calif) by acquiring at least 500 to 1000 basophils per sample, selected as CCR3+ cells (see [Figure E1](#) in this article's Online Repository at www.jaci-inpractice.org). Results were analyzed using FlowJo software (FlowJo, LLC, Ashland, Ore) and activation expressed as percentage of cells expressing CD63 or percentage of cells with increased expression of CD203c and as stimulation index (SI). The SI was calculated as the ratio between the percentage of activated basophils (CD63+ or CD203c+ cells) in samples stimulated with the different PPIs and the unstimulated sample. The percentage of spontaneously activated basophils was required to be equal to or greater than 5% to calculate the SI, as previously described.³² To confirm that basophil activation was IgE-mediated, we analyzed the inhibitory effect of wortmannin at 1 μ M, a specific inhibitor of phosphatidylinositol 3-kinase, which is activated by Fc ϵ RI receptor cross-linking.

Statistical analysis

Comparisons of quantitative variables without a normal distribution were performed using the Mann-Whitney, Kruskal-Wallis, and Wilcoxon matched-pairs signed rank tests. Comparisons of qualitative variables were performed using the chi-square test. All reported *P* values represent 2-tailed tests, with values less than .05 considered statistically significant. The optimal cutoff threshold was determined as the point on the receiver operating characteristic (ROC) curve at which (sensitivity + specificity – 100%) was maximal. Sensitivity and specificity were calculated with this cutoff value. The sensitivity and specificity of BAT, as well as the NPV and PPV, were calculated using standard formula.

RESULTS

The study included 42 patients with confirmed immediate hypersensitivity reactions to omeprazole ([Table I](#)). Of these, 64.3% (*n* = 27) were female, the median age was 41.5 years (interquartile range [IQR], 36–48.75 years), the median time interval between the reaction and study was 180 days (IQR, 120–360 days), and the median time interval between drug administration and onset of the reaction was 30 minutes (IQR, 20–50 minutes). Regarding the clinical entities, 10 (23.8%) patients suffered anaphylactic shock, 19 (45.2%) anaphylaxis, 10 (23.8%) urticaria, 2 (4.8%) intense pruritus, and 1 (2.4%) isolated angioedema. The median number of reported reactions was 2 (IQR, 1–2), with 17 (40.58%) cases developing 1 episode, 15 (35.7%) developing 2 episodes, 5 (11.9%) developing more than 2 episodes, and 5 (11.9%) developing an unknown number of episodes. Remarkably, some of these patients suffered a high number of episodes even including severe reactions as anaphylaxis. The explanation for this could lie in the fact that these drugs are usually not thought to induce anaphylaxis by general practitioners and therefore reactions are attributed to other drugs or even foods. As a result, in some patients the drug is not avoided, and they can suffer multiple episodes of anaphylaxis until an accurate allergological workup is performed.

In terms of other drugs involved in the reaction, in 16 (38.1%) cases there was no other drug involved, in 25 (59.5%) omeprazole was administered simultaneously with other drugs (21 cases with nonsteroidal anti-inflammatory drugs [NSAIDs], 7 with antibiotics, and 1 with metoclopramide), and in 1 (2.4%) patient it was impossible to know if there was another drug involved in the reaction. Interestingly, in patients who took omeprazole together with NSAIDs, 81% (17 out of 21) suffered anaphylaxis versus 55% (11 out of 20) of patients taking omeprazole alone or with other drugs. In 40 (95.2%) patients, omeprazole was administered by the oral route and in 2 (4.8%) intravenously.

Results of the allergological workup are presented in [Table II](#). Twenty-eight (66.67%) patients were ST positive—8 (28.57%) by SPT and 20 (71.43%) by IDT. All SPT results were positive at 40 mg/mL, and for IDT 11 cases were positive at 4.0 mg/mL and 9 at 0.4 mg/mL. None of the patients developed systemic symptoms with ST and none of the 22 control cases with confirmed good tolerance to omeprazole gave a positive ST result (100% specificity). Among patients with negative ST result, the percentage of patients with anaphylaxis was higher in patients taking omeprazole together with NSAIDs (50%; 3 of 6) compared with patients taking omeprazole alone or with other drugs (0%; 0 of 7). The 14 (33.33%) cases with negative ST result needed a DPT to be confirmed. Considering the

TABLE I. Clinical and demographic data of patients included in the study

Patient	Age (y)	Sex	Reaction	Interval between reaction and study (d)	Interval between drug and reaction (min)	Episodes	Associated drugs	Atopy	Administration route
1	29	Male	Anaphylactic shock	120	15	1	None	No	Oral
2	38	Female	Anaphylaxis	120	45	1	None	Yes	Oral
3	54	Male	Anaphylaxis	180	2	2	NSAID	No	Oral
4	51	Female	Anaphylactic shock	360	30	2	Quin-NSAID	NK	Oral
5	37	Female	Anaphylaxis	720	20	3	NSAID	No	Oral
6	45	Female	Anaphylaxis	360	30	3	None	No	Oral
7	56	Male	Anaphylaxis	360	60	1	NSAID	NK	Oral
8	38	Male	Urticaria	360	30	2	None	No	Oral
9	41	Male	Anaphylactic shock	360	50	2	None	No	Oral
10	21	Male	Anaphylactic shock	120	60	3	NSAID	No	Oral
11	30	Female	Anaphylaxis	360	30	4	None	No	Oral
12	45	Female	Anaphylaxis	NK	10	5	NK	No	Oral
13	66	Female	Urticaria	120	15	2	None	No	Oral
14	24	Female	Anaphylaxis	150	90	2	NSAID	No	Oral
15	43	Female	Anaphylaxis	15	30	1	NSAID	NK	Oral
16	36	Female	Anaphylaxis	548	20	2	NSAID	NK	Oral
17	38	Male	Anaphylactic shock	90	50	1	NSAID	No	Oral
18	48	Male	Anaphylaxis	30	20	1	NSAID	Yes	Oral
19	51	Female	Urticaria	210	50	2	NSAID	No	Oral
20	38	Female	Urticaria	60	60	NK	NSAID	Yes	Oral
21	36	Female	Anaphylaxis	80	60	1	Peni-NSAID	Yes	Oral
22	42	Male	Anaphylaxis	60	20	NK	Peni-NSAID	No	Oral
23	42	Female	Urticaria	3,650	30	NK	None	Yes	Oral
24	36	Female	Urticaria	1,465	NK	NK	NSAID	No	Oral
25	36	Female	Anaphylaxis	1,460	30	NK	None	Yes	Oral
26	31	Male	Anaphylactic shock	240	10	2	Peni	Yes	Oral
27	34	Female	Anaphylactic shock	30	15	1	NSAID	No	IV
28	50	Male	Anaphylactic shock	120	45	2	None	Yes	Oral
29	60	Female	Anaphylaxis	120	30	1	None	NK	Oral
30	42	Male	Anaphylaxis	180	30	1	NSAID	No	Oral
31	64	Male	Urticaria	150	60	2	NSAID	No	Oral
32	40	Female	Anaphylaxis	730	60	2	NSAID	Yes	Oral
33	22	Male	Urticarial-angioedema	90	60	2	Clind	Yes	Oral
34	31	Female	Anaphylaxis	425	15	1	Met	Yes	IV
35	46	Female	Pruritus	250	45	1	None	No	Oral
36	43	Female	Urticaria	200	30	2	None	Yes	Oral
37	42	Female	Anaphylaxis	156	50	2	Peni-NSAID	Yes	Oral
38	36	Female	Angioedema	180	10	1	Peni-Quin	No	Oral
39	71	Female	Pruritus	120	10	1	None	No	Oral
40	49	Female	Urticaria	NK	60	1	None	NK	Oral
41	56	Female	Anaphylactic shock	60	20	1	NSAID	Yes	Oral
42	41	Male	Anaphylactic shock	180	45	1	None	No	Oral

Clind, Clindamycine; *IV*, intravenously; *Met*, metoclopramide; *NK*, not known; *NSAID*, nonsteroidal anti-inflammatory drug; *Peni*, penicillin; *Quin*, quinolones.

cumulative dose of omeprazole that induced a positive result in the DPT, 1 case responded to 5 mg, 2 to 10 mg, 4 to 20 mg, and 7 to 40 mg. Nine cases (patients 8, 13, 20, 23, 24, 31, 33, 35, 38, and 39) developed immediate urticaria accompanied in 2 cases by angioedema (patients 33 and 38) that was resolved after the administration of intravenous antihistamines and corticosteroids. Patients 2, 15, 30, and 37 developed more severe symptoms, including generalized erythema, dyspnea, and hypotension (1 case, patient 15) that needed treatment with adrenaline. Of the 25 patients who took omeprazole with another drug, tolerance to the other drug was confirmed by DPT.

BAT was performed in all patients and in 22 sex- and age-matched tolerant subjects using CCR3 as a marker to select basophils. To select the concentration that best differentiates patients from controls, we produced ROC curves using the SI obtained for the activation markers CD63 and CD203c, and the 3 different concentrations of omeprazole. We observed that the best areas under the ROC curve were obtained with a concentration of 2 mg/mL and using CD63 as activation marker (area: 0.8765; $P < .0001$; 95% CI, 0.7892-0.9638) (Figure 1). Because of these results, only the drug concentration of 2 mg/mL was included for the following analyses. BAT results for this

TABLE II. Results for the allergological workup and cross-reactivity in *in vivo* and *in vitro* tests

Patient	Reaction	ST			BAT (SI)*		
		SPT	IDT	DPT	Omeprazole	Pantoprazole	Lansoprazole
1	Anaphylactic shock	—	+ (0.4 mg/mL)	ND	7.08	8.74	1.44
2	Anaphylaxis	Negative		+ (10 mg)	0.38	0.27	0.30
3	Anaphylaxis	+ (40 mg/mL)	ND	ND	6.55	2.18	0.56
4	Anaphylactic shock	—	+ (0.4 mg/mL)	ND	2.09	1.01	0.86
5	Anaphylaxis	—	+ (4.0 mg/mL)	ND	2.54	6.03	0.46
6	Anaphylaxis	—	+ (0.4 mg/mL)	ND	0.06	0.84	0.40
7	Anaphylaxis	—	+ (0.4 mg/mL)	ND	4.39	0.29	0.78
8	Urticaria	Negative		+ (40 mg)	6.19	0.40	0.56
9	Anaphylactic shock	+ (40 mg/mL)	ND	ND	0.67	4.14	0.87
10	Anaphylactic shock	+ (40 mg/mL)	ND	ND	3.81	1.29	0.38
11	Anaphylaxis	+ (40 mg/mL)	ND	ND	7.73	2.09	0.18
12	Anaphylaxis	—	+ (4.0 mg/mL)	ND	2.38	0.35	0.08
13	Urticaria	Negative		+ (20 mg)	1.09	1.02	0.38
14	Anaphylaxis	—	+ (0.4 mg/mL)	ND	8.31	2.12	0.34
15	Anaphylaxis	Negative		+ (5 mg)	2.99	3.45	0.75
16	Anaphylaxis	+ (40 mg/mL)	—	ND	8.59	3.72	0.26
17	Anaphylactic shock	—	+ (4.0 mg/mL)	ND	2.42	0.60	0.26
18	Anaphylaxis	—	+ (0.4 mg/mL)	ND	2.68	0.26	0.23
19	Urticaria	—	+ (4.0 mg/mL)	ND	2.35	0.40	0.21
20	Urticaria	Negative		+ (40 mg)	4.14	4.64	1.28
21	Anaphylaxis	—	+ (0.4 mg/mL)	ND	8.30	4.61	0.82
22	Anaphylaxis	+ (40 mg/mL)	ND	ND	0.44	0.75	0.82
23	Urticaria	Negative		+ (20 mg)	7.57	0.91	0.98
24	Urticaria	Negative		+ (40 mg)	3.28	0.78	1.19
25	Anaphylaxis	—	+ (4.0 mg/mL)	ND	2.09	0.35	0.43
26	Anaphylactic shock	—	+ (0.4 mg/mL)	ND	3.34	0.13	0.19
27	Anaphylactic shock	—	+ (4.0 mg/mL)	ND	3.62	5.21	0.30
28	Anaphylactic shock	+ (40 mg/mL)	ND	ND	1.62	7.25	0.79
29	Anaphylaxis	—	+ (4.0 mg/mL)	ND	9.63	3.90	0.54
30	Anaphylaxis	Negative		+ (20 mg)	1.70	2.13	1.47
31	Urticaria	Negative		+ (40 mg)	1.17	0.24	1.31
32	Anaphylaxis	—	+ (4.0 mg/mL)	ND	13.41	7.16	2.24
33	Urticarial-angioedema	Negative		+ (20 mg)	3.00	1.25	0.87
34	Anaphylaxis	—	+ (4.0 mg/mL)	ND	1.74	0.68	0.64
35	Pruritus	Negative		+ (40 mg)	4.43	0.96	1.40
36	Urticaria	—	+ (0.4 mg/mL)	ND	5.01	0.70	1.33
37	Anaphylaxis	Negative		+ (10 mg)	2.04	0.36	0.72
38	Angioedema	Negative		+ (40 mg)	1.18	1.36	0.13
39	Pruritus	Negative		+ (40 mg)	0.41	0.26	0.72
40	Urticaria	—	+ (4.0 mg/mL)	ND	8.80	0.58	0.70
41	Anaphylactic shock	+ (40 mg/mL)	ND	ND	15.51	12.06	10.61
42	Anaphylactic shock	—	+ (4.0 mg/mL)	ND	2.51	1.05	1.43

ND, Not determined.

*BAT results are considered positive if the SI is higher than 2.

concentration are presented in Table II. On analyzing the sensitivity/specificity tables, the cutoff for assessing positivity was established as an SI of more than 2. Because of the requirement of a minimum spontaneous activation of 5%, a drug activation of at least 10% was needed.

Comparisons between both markers, CD63 versus CD203c, showed a significantly higher expression of CD63 in patients analyzing the results as percentage ($P = .0221$) and as SI ($P = .0080$) (Figure 2, A). Regarding the different clinical entities,

anaphylactic shock, anaphylaxis, and urticaria, we did not find significant differences between CD203c and CD63 expressions, calculated as either percentage (Figure 2, B) or SI (Figure 2, C), although the expression of CD63 was higher in all groups. Moreover, no case of positive CD203c ($SI > 2$) and negative CD63 was observed. Given these findings, we used only the results obtained using activation marker CD63 for further analyses.

After the analysis of BAT results using CCR3 to select basophils, CD63 as activation marker, and stimulating the cells with

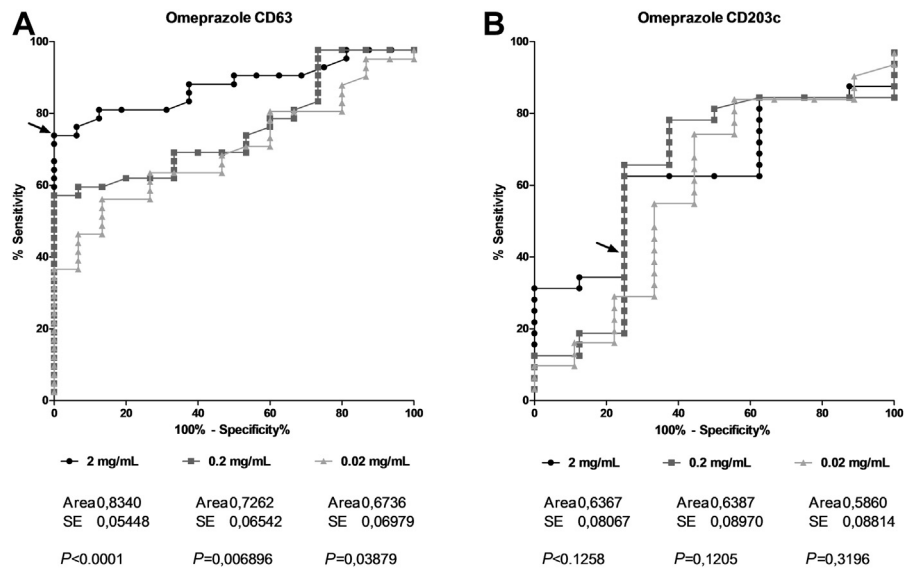


FIGURE 1. ROC curves comparing the area under the curve and statistical significance for (A) the 3 concentrations of omeprazole, using CD63 as activation marker, and (B) the 3 concentrations of omeprazole, using CD203c as activation marker. P values less than .05 were considered statistically significant. The cutoff point for a concentration of 2 mg/mL is marked with an arrow. *ROC*, Receiver operating characteristic; *SE*, standard error.

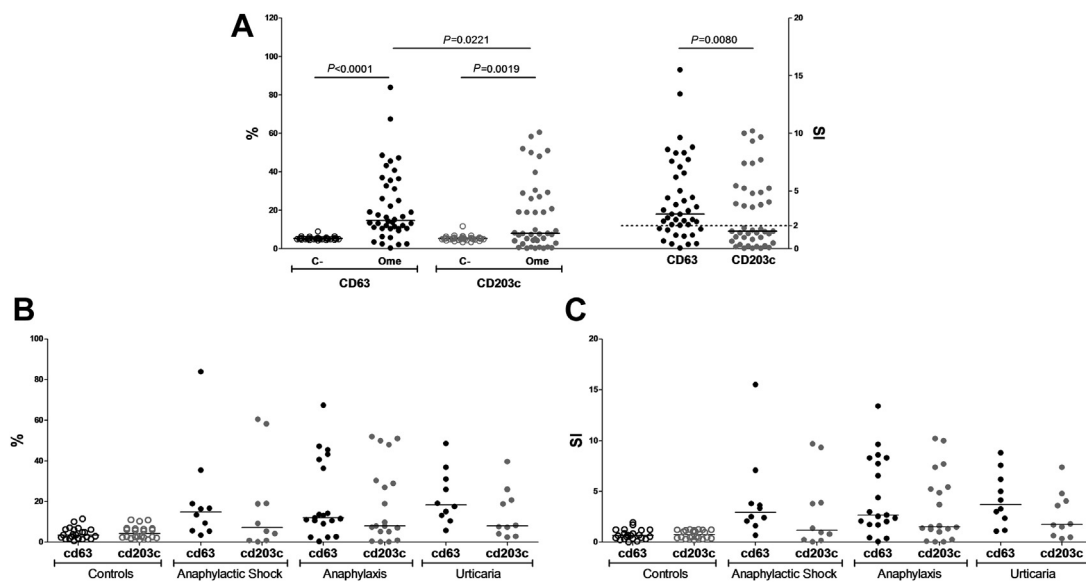


FIGURE 2. Differences in BAT results performed with omeprazole at 2 mg/mL between the 2 different activation markers, CD63 and CD203c (A) the whole group of allergic patients to omeprazole, expressed as percentage (left axis) or SI (right axis); (B) between the 3 different clinical entities (anaphylactic shock, anaphylaxis, and urticaria), expressed as percentage; and (C) between the 3 different clinical entities (anaphylactic shock, anaphylaxis, and urticaria), expressed as SI. P values of less than .05 were considered statistically significant.

omeprazole at 2 mg/mL, we observed a significant increase in CD63 expression for patients compared with controls, measured as percentage and SI ($P < .0001$ for both; Figure 3, A). We found that both %CD63 and SI were significantly higher compared with controls for the 3 clinical entities (%CD63: $P = .0009$ in anaphylactic shock, $P = .0003$ in anaphylaxis, and $P < .0001$ in urticaria; SI: $P = .0002$ in anaphylactic shock

and $P < .0001$ in both anaphylaxis and urticaria). However, we did not find differences between the clinical entities (Figure 3, B). No correlation was found between BAT results and the time interval between the last reaction and the BAT performance.

The study of positive cases using CD63 revealed a sensitivity of 73.8%, with a specificity of 100% (Figure 4, A), a PPV of 100%, and an NPV of 66.7%. BAT result was positive in 8 of 14

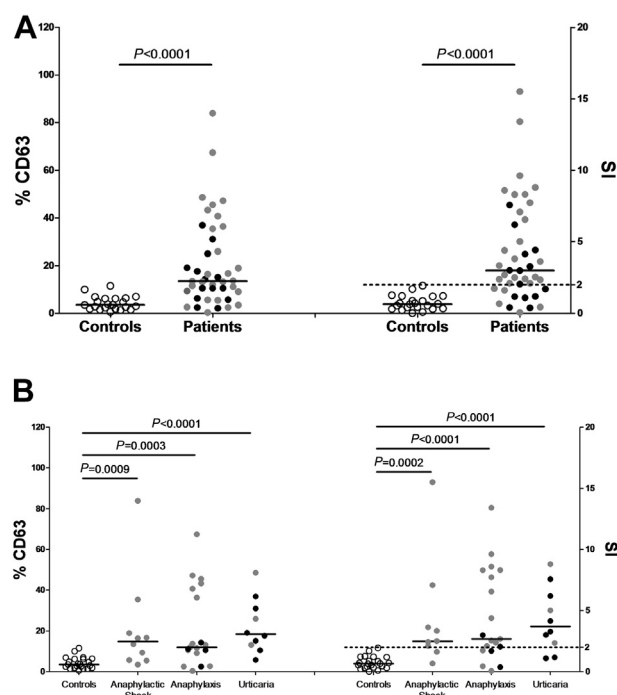


FIGURE 3. Comparison of the expression of CD63 expressed as absolute percentage (left axis) or SI (right axis) after BAT performance with omeprazole at 2 mg/mL between (A) allergic patients to omeprazole and controls and (B) 3 different clinical entities (anaphylactic shock, anaphylaxis, and urticaria) and controls. Patients with positive ST results are marked in gray.

patients with negative ST result; thus, by combining ST and BAT to omeprazole we can diagnose 85.7% ($n = 36$) of patients (Figure 4, B). Moreover, to prove that the activation observed was IgE-mediated, BAT was performed after incubating cells with wortmannin, a potent inhibitor of the IgE signaling pathway,³² in 10 patients showing positive results. This treatment significantly reduced basophil activation ($P = .0313$) (Figure 4, C), demonstrating the involvement of IgE in the stimulation of basophils by omeprazole.

BAT was performed using other PPIs such as pantoprazole and lansoprazole, with similar and different side-chain chemical structures to omeprazole, respectively. Interestingly, BAT result with pantoprazole was positive in 16 (38.1%) patients, including 3 patients with negative results to omeprazole. When BAT was performed with lansoprazole, only 2 (4.8%) patients showed positive results (Table II). Combining the results obtained with omeprazole and pantoprazole, the sensitivity can be increased to 81.0%; however, specificity decreases to 86.4% and PPV to 91.9%, with an NPV of 70.4%.

DISCUSSION

PPIs are among the most highly prescribed drugs worldwide and although they are generally well tolerated, especially for short-term use, hypersensitivity reactions have been known. The PPIs involved in the reactions vary among countries: lansoprazole in studies from Turkey,^{7,16} esomeprazole and lansoprazole for Italy,⁶ and omeprazole for Spain.^{5,8,20,21,24} This is probably related to PPI consumption, because in 2012 omeprazole represented 79.1% of all consumed PPIs in Spain.² This

increased consumption of omeprazole in the last decades has occurred jointly with increased reports of hypersensitivity reactions to this drug.^{3,4} However, there is a lack of studies investigating the mechanisms of these reactions and potential involvement of IgE. In this work, we have analyzed the value of BAT as a diagnostic tool for diagnosing immediate hypersensitivity to omeprazole; we have also indirectly assessed the involvement of specific IgE. We have found that BAT with omeprazole is useful for diagnosis, with a sensitivity of 73.8%, a complete specificity (100%), and an NPV of about 67%. These values show that BAT could be very useful as part of the allergological workup, because it can help the clinician to decide whether to perform a DPT, especially in patients with severe reactions,³³ as is the case of most patients with allergy to PPI.^{5,7,16,21} In fact, in our study about 70% of patients suffered severe reactions, with 45.2% experiencing anaphylaxis and 23.8% anaphylactic shock.

Several studies have demonstrated that STs are useful for the diagnosis of immediate reactions to PPIs^{5-7,16,21}; however, sensitivity is lower than 65%, independently of the PPI involved, making DPT the only alternative tool for diagnosis. Specifically, when omeprazole is the main culprit, ST sensitivity is 62.5%^{5,21}; when lansoprazole is the main culprit, sensitivity is 59%¹⁶; and when esomeprazole and lansoprazole are the main culprits, sensitivity is 50%.⁶ In the present study, we have performed SPT for all patients with the undiluted commercial preparation (40 mg/mL) of omeprazole, and IDTs at 0.04, 0.40, and 4.0 mg/mL, and confirmed previous results as we found an overall ST sensitivity of 67% (28.6% with SPT and 71.4% with IDT). This finding is important because previous studies have disagreed regarding the NPV of ST, with some authors indicating that it is higher than 92%,⁶ whereas others have found lower values (70.8%).⁷ In fact, in our study 33% of the patients were ST-negative and needed a DPT to be diagnosed, which is important if we take into account that most patients were studied in less than 1 year after the development of the reaction. Moreover, 57% of patients with negative ST result obtained positive results in BAT, meaning that by combining ST and BAT results we can diagnose almost 86% of patients. These results show BAT to be a promising tool to diagnose omeprazole allergy.

BAT has been widely used for the diagnosis of drug allergy.^{13,15,33-38} However, only isolated case reports can be found about its use in diagnosing PPI allergy^{8,9}; moreover, no studies have evaluated a series of both patients and controls. There are some factors that have been shown to influence BAT results.³⁴ One is the choice of activation marker. Both available activation markers, CD63 and CD203c, have proven to be reliable,¹⁰⁻¹³ although differences exist as has been shown for drugs such as amoxicillin and neuromuscular blocking agents^{13,36} and allergens.^{12,14} The upregulation of these markers seems to be related with 2 different degranulation mechanisms, anaphylactic or piecemeal. Anaphylactic degranulation is characterized by the fusion of the histamine granules to the plasma membrane, leading to the exposition of CD63.³⁹ Piecemeal degranulation is driven by the formation of small vesicles from the histamine-containing granules that are gradually shuttled to the plasma membrane.⁴⁰ It has been described that different drugs, even drugs from the same pharmacological group,¹⁵ interact with basophils in different ways, activating 1 of these 2 pathways specifically.^{13,36} In our case, omeprazole has been shown to be able to activate basophils by a mechanism that

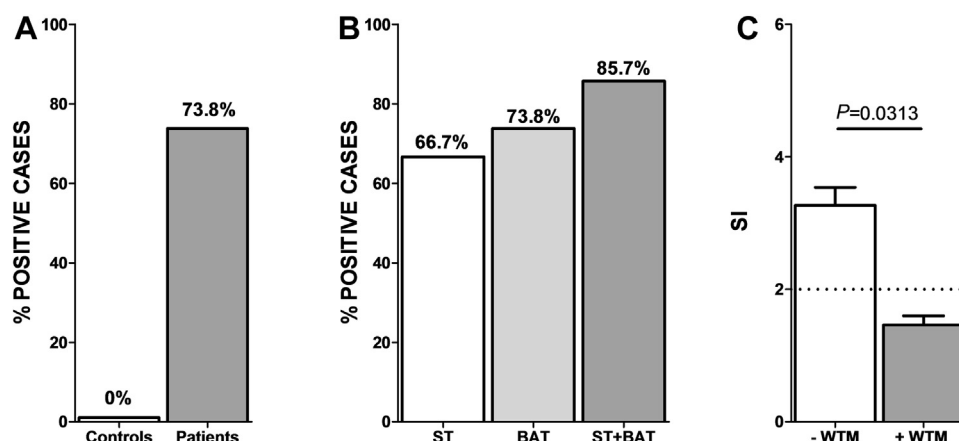


FIGURE 4. (A) Percentage of positive cases in BAT using a cutoff of more than 2, CD63 as activation marker, and a concentration of omeprazole of 2 mg/mL. (B) Sensitivity of ST, BAT, and combination of both diagnostic tests. (C) Inhibition of basophil activation after WTM treatment using a cutoff of more than 2, CD63 as activation marker, and a concentration of omeprazole of 2 mg/mL. WTM, Wortmannin.

induces a greater upregulation of CD63, with similar results for all patients, independently of the symptoms experienced. However, other studies of other drugs have found differences in the expressions of CD63 and CD203c related to the clinical entity involved.^{15,32,41}

Another factor that can influence BAT results is the time interval between the last reaction and the performance of the test.³⁴ It has been observed that, in the case of penicillins, tests can become less effective the longer this time interval is,⁴² as occurs with ST,^{43,44} probably because of the clearance of IgE from the serum. This is also true for other drugs such as neuromuscular blocking agents, although negativization of BAT seems to be slower.⁴⁵ In our study, we have not observed any correlation between this time interval and BAT or ST results. This may be explained by the short mean interval between the reaction and the study, which in our case is only 180 days. A longer follow-up study of these patients is necessary to study the negativization rates of BAT to omeprazole.

Finally, we have found that most omeprazole-allergic patients with positive BAT result to omeprazole also recognized pantoprazole but not lansoprazole. This is in agreement with previous results that have found 3 main recognition patterns^{6,24}: patients who recognize omeprazole and pantoprazole,^{6,7,20,21,24-27} patients who recognize lansoprazole or rabeprazole,^{6,7,24,26,28} and patients who react to the whole group.^{24,25} These different patterns are likely to be related to the chemical structures of the different PPIs (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org), because omeprazole and pantoprazole have the same side chain, whereas lansoprazole is not substituted on the benzimidazole ring.^{22,23} However, we have found 2 patients showing BAT-positive results to the 3 PPIs (omeprazole, pantoprazole, and lansoprazole), probably because of recognition of the shared pyridine ring.^{24,25} Whether this *in vitro* cross-reactivity can predict clinical tolerance is currently unknown and should be investigated in further studies.

CONCLUSIONS

We have demonstrated for the first time that BAT represents a promising complementary tool for inclusion in the allergological

workup for patients allergic to omeprazole. When combined with ST it can be of great value to the clinician in guiding the decision of whether to perform a DPT. This is particularly important for PPIs, because they frequently cause severe reactions.

Acknowledgment

We thank James R. Perkins for help with the English version of the article.

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APPENDIX

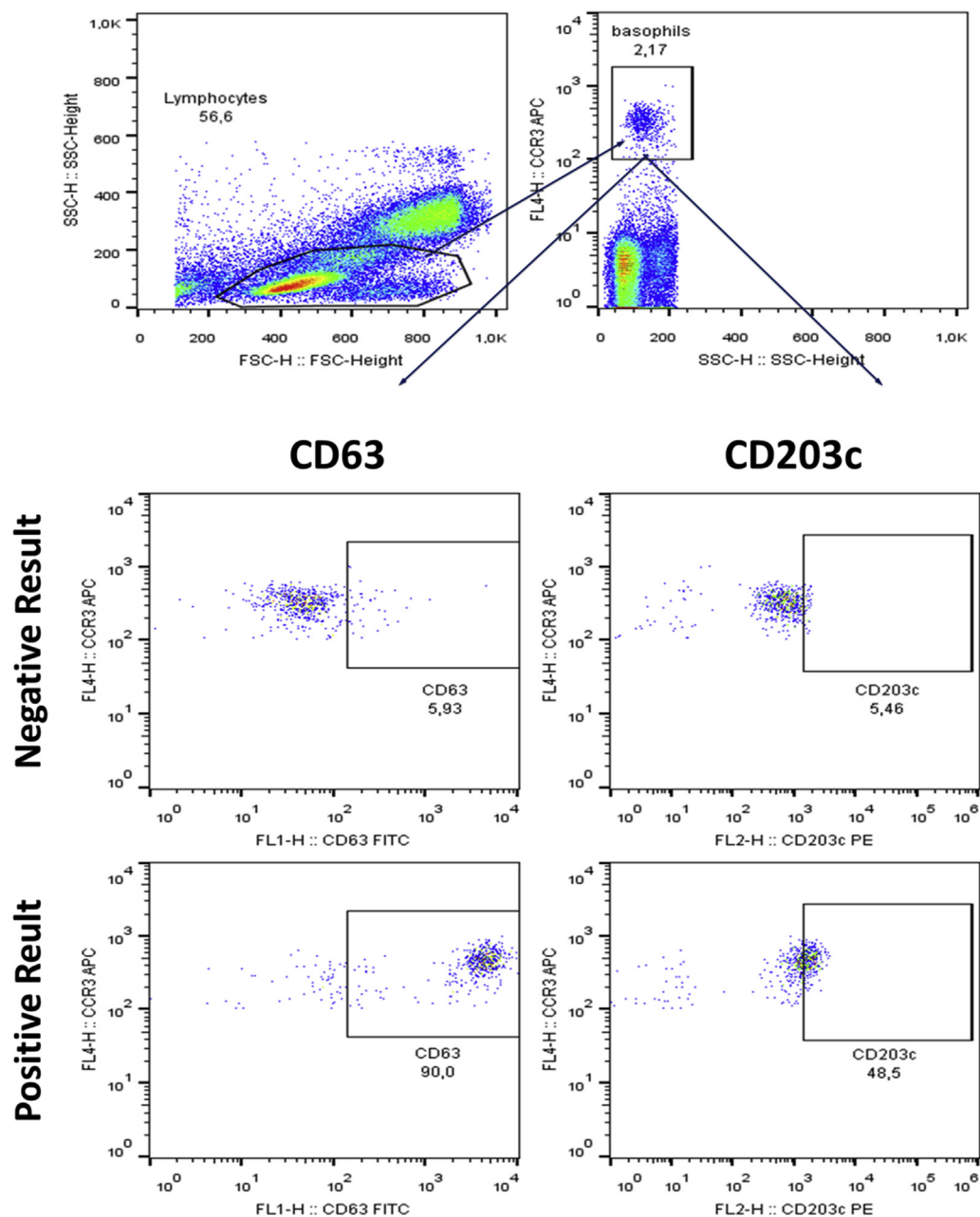


FIGURE E1. Flow cytometry gating strategies for the different subpopulations analyzed. Basophils were selected as CCR3-high cells from the lymphocyte population. The percentage of spontaneous expression of CD63 or CD203c was required to be equal to or greater than 5% to gate the positive population.

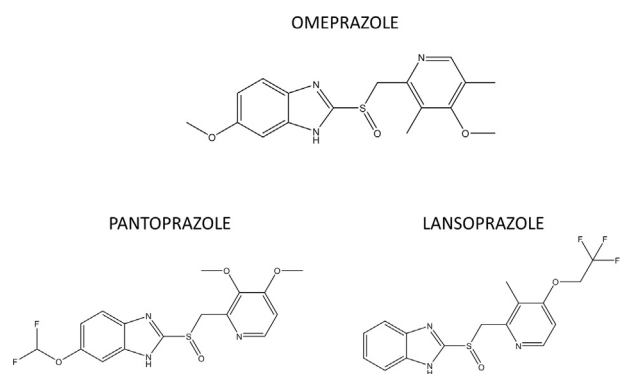


FIGURE E2. Chemical structures of omeprazole, pantoprazole, and lansoprazole.