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ORIGINAL ARTICLE

Diagnosis of immediate reactions to amoxicillin: Comparison of basophil activation markers CD63 and CD203c in a prospective study

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Abstract

Background: Amoxicillin (AX) combined or not with clavulanic acid (CLV) is frequently involved in IgE-mediated reactions. Drug provocation test (DPT) is considered as the gold standard for diagnosis, although contraindicated in high-risk patients. Basophil activation test (BAT) can help diagnose immediate reactions to beta-lactams, although controversy exists regarding the best activation marker. We have performed a real-life study in a prospective cohort to analyze the real value of BAT as diagnostic tool and the best activation marker, CD63 and CD203c, for the evaluation of immediate reactions to these drugs.

Methods: We prospectively evaluated patients with a clinical suspicion of immediate reactions after AX or AX-CLV administration during a 6-year period. The allergological work-up was done following the EAACI recommendations. BAT was performed in all patients using CD63 and CD203c as activation markers.

Results: In AX-allergic patients, both activation markers, CD63 and CD203c, showed similar SE values (48.6% and 46.7%, respectively); however, specificity was of 81.1% and 94.6%, respectively, with CD203c showing good positive predictive value and like-hood ratio. In CLV-allergic patients, CD203c showed higher SE (50%) than CD63 (42.9%), maintaining the same value of SP (80%). Combining the results of both markers can slightly increase the sensitivity (51.4% for AX and 54.8% for CLV), although decreasing the specificity (79.7% and 73%, respectively). Interestingly, all patients with an anaphylactic shock showed a positive BAT to CLV using CD203c.

Abbreviations: ANA, anaphylaxis; AUC, area under the curve; AX, amoxicillin; BAT, basophil activation test; BLs, beta-lactams; CLV, clavulanic acid; DPT, drug provocation test; FcεRI, high-affinity IgE receptor; IRs, immediate reactions; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; SE, sensitivity; SHOCK, anaphylactic shock; sIgE, specific IgE; SP, specificity; ST, skin test; ROC, receiver operating curves; URT/ANG, urticaria/angioedema.

Jose A. Céspedes, Rubén Fernández-Santamaría and Adriana Ariza equally contributed to this work; and, Cristobalina Mayorga, María J. Torres and Tahia D. Fernández Duarte equally contributed to this work.

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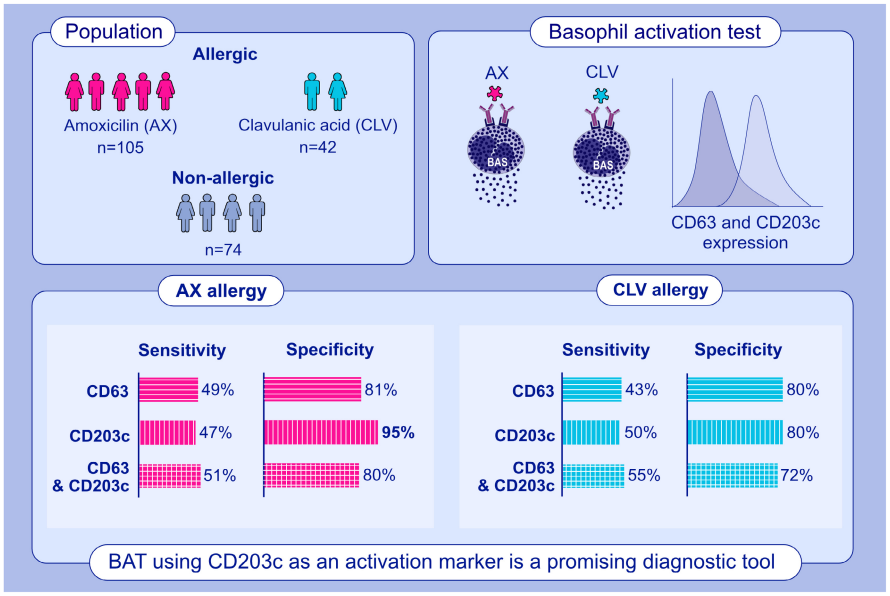
Conclusions: BAT using CD203c showed a good confirmatory power, especially for AX allergy. Placing BAT as a first step in the diagnostic procedure can help reduce the need of performing a complete allergological work-up in 46.6% of patients, diminishing the risk of reinducing allergic reactions.

KEYWORDS
amoxicillin, basophils, clavulanic acid, drug allergy, immediate reactions



GRAPHICAL ABSTRACT
Patients suffering from an immediate reaction after AX or AX-CLV intake have been prospectively recruited. BAT with CD63 and CD203c was performed. BAT using CD203c in IRs to AX has shown high specificity and good confirmatory power. BAT to AX with CD203c as first step in the diagnostic algorithm could avoid the performance of in vivo tests in some patients. Abbreviations: AX, amoxicillin; BAT, basophil activation test; CLV, clavulanic acid; IRs, immediate reactions

1 | INTRODUCTION

Antibiotics are widely prescribed to treat bacterial infections.[1] However, allergy to these drugs is nowadays generating a worldwide alarm due to their prevalence, affecting up to 10% of the general population, and to the impact on infection management.[2] In Europe, the most consumed antibiotics are beta-lactams (BLs), and more specifically those from the group of penicillins,[3] such as amoxicillin (AX) combined or not with the beta-lactamase inhibitor clavulanic acid (CLV).[4,5] These are frequently involved in reactions induced by specific immunological mechanisms[2] in both adults and children.[6,7] AX-CLV has been reported as the most frequently involved in allergic reactions to BLs in south Europe, increasing from 10%–35% between 2008 and 2010[8] to 60%–80% in 2014.[9] IgE-mediated reactions, or immediate reactions (IRs), are the most frequently elicited by AX-CLV,[8] resulting in clinical symptoms ranging from mild reactions, such as urticaria, to more severe and life-threatening, such as anaphylaxis or anaphylactic shock.[10] These reactions can be induced by both AX and CLV,[11,12] being 30% of cases after treatment with AX-CLV induced by CLV.[12–14] There is very low cross-reactivity between AX and CLV, probably due to differences in the chemical structure and the determinants derived from their metabolism[14]; thus, most CLV-allergic patients can be safely treated with AX. However, it should be noted that few cases of patients sensitized independently to both AX and CLV have been reported,[15,16] showing that specific reactions to both drugs can occur even if no cross-reactivity exists.

In general, less than 30% of adults and 10% of children reporting BLs reactions are confirmed as allergic after an allergological work-up.[8,17] This is important since the label of "allergy to BLs," whether real or not, leads to the prescription of alternative broad-spectrum antibiotics that have been associated with the development of more bacterial resistance and a higher rate of severe infections, resulting in longer hospitalizations.[18] Ultimately, this represents a greater risk to the patient's health and higher costs for the health system. Therefore, it is crucial to perform an allergological evaluation to achieve a precise diagnosis.

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The allergological work-up includes clinical history, skin test (ST), and drug provocation test (DPT).[19] This work-up is time-consuming, with procedures including many steps in different days, needs to be done in a specialized setting, is not risk-free, especially in anaphylaxis, and is far from being standardized.[19] Moreover, ST should be performed not only with AX but also with CLV, which, although commercially available, is not approved in all countries. DPT is the gold standard; however, tolerance to CLV must be assessed evaluating tolerance to AX and reaction with AX-CLV, hampering diagnosis. In vitro assays are quicker and safer and seem the most reasonable diagnostic step before DPT, especially in high-risk patients. Basophil activation test (BAT), with moderate sensitivity (around 50%), has demonstrated utility for diagnosing IRs to BLs,[20] especially with recently identified culprits such as CLV[11] and cefazolin,[21] which lack other in vitro diagnosis tests,[11,12,22] and in high-risk patients with negative STs.[12,21] Blood basophils are leukocytes that become activated through a cross-linking process driven by the binding of allergens/drugs to at least two adjacent specific IgE (sIgE) bound on high-affinity IgE receptor (FcεRI) on cell surface. Basophil activation triggers cell degranulation and release of cytokines and inflammatory mediators (mainly histamine and leukotrienes).[23,24]

Activated basophils are usually identified by CD63 expression on their surface. CD63 is located in the membrane of secretory granules[25,26] whose expression is directly correlated with basophil degranulation and histamine release.[25,27,28] Another option is the analysis of upregulation in CD203c, which is constitutively expressed on resting basophils, but overexpressed upon activation.[29] However, controversy exists regarding if both markers are necessary or which can display better sensitivity/specificity, with studies showing CD203c lower sensitivity[30,31] although with protein allergens. In the evaluation of BL allergy, most studies used CD63 as activation marker with sensitivity around 50% and specificity around 90%.[11,12,20,32,33] Only one study has evaluated both markers, CD63 and CD203c, finding that CD203c showed better results in the study of IRs to AX, with a sensitivity of 52% vs a sensitivity of 22% found with CD63.[34] However, this low sensitivity with CD63 could be explained because basophils were not primed with IL-3 in this study, cytokine that has been proved to be necessary to induce CD63 but not CD203c expression upon stimulation.[31]

Nevertheless, all the studies have been made using well-phenotyped patients in case–control studies; thus, values about the efficacy of BAT in diagnosing AX-CLV allergy in real life are needed. In this study, we have performed a prospective analysis of BAT with two activation markers, CD63 and CD203c, as a complementary diagnostic tool during 6 years. This study has shown that sensitivity of BAT in real life is similar to data reported in case–control studies. Moreover, BAT using CD203c has been revealed as a promising complementary tool to diagnosed IRs after AX-CLV administration, especially IRs to AX, with a high specificity and good confirmatory power; thus, a patient with a positive result could be considered as allergic without further studies.

2 | METHODS

We prospectively evaluated patients older than 14 years old referred to the Allergy Unit of the Regional University Hospital of Málaga, between January 2014 and December 2019 with clinical suspicion of IRs after AX or AX-CLV administration. Brown's grading system for generalized allergic reactions was used[35]: grade 1 (mild: skin and subcutaneous tissues), classified as urticaria/angioedema (URT/ANG); grade 2 (moderate: features suggesting respiratory, cardiovascular, or gastrointestinal involvement), classified as anaphylaxis (ANA); grade 3 (severe: hypoxia, hypotension, or neurologic compromise) classified as anaphylactic shock (SHOCK). The study was conducted according to the principles of the Declaration of Helsinki and approved by the institutional review board (PI1800095). All participants were informed about the study and signed the corresponding informed consent.

The allergological work-up (Figure 1) was done following the EAACI recommendations[19] and included an exhaustive clinical history according to the EAACI questionnaire[36] followed by STs, and, if negative, by DPT. Blood for performing BAT was obtained in all evaluated patients before STs, and the clinicians were blinded for BAT results until diagnosis was confirmed by in vivo tests. BAT was performed as described previously.[12]

Statistical analysis was carried out using the software package GraphPad PRISM v7. A value of $p < .05$ was considered statistically significant.

For further details, see the Methods section of this article's Online Repository.

3 | RESULTS

3.1 | Clinical results

We evaluated 467 patients with IRs after AX or AX-CLV administration (Figure 1). We excluded 246 patients because they reported no clear suspicion of IRs ($N = 184$) and because they had contraindications for STs and/or DPTs ($N = 47$: 21 had cardiac diseases, 10 had uncontrolled asthma, seven took beta-blockers with impossibility of suspending, four had chronic urticaria, three were pregnant, and two had psychosomatic disorders). Fourteen patients who had negative STs and reported grade III reactions were also excluded.

Two hundred and twenty-one patients with clinical history of IRs after AX or AX-CLV administration and with a complete allergological work-up were included. The mean age was 43.96 ± 14.16 years, and 114 (51.6%) were male. The mean time interval between drug intake and symptoms development was 32.02 ± 55.62 minutes, and the clinical manifestations were SHOCK in 39 (17.64%) cases (severity grade III), ANA in 84 (38%; severity grade II), and URT/ANG in 98 (44.34%; severity grade I). The mean time interval between reaction and study was 23.93 ± 37.42 months.

After allergological work-up, 147 were confirmed as allergic: 105 (47.7%) to AX and 42 (18.9%) to CLV. From AX-allergic patients, 32

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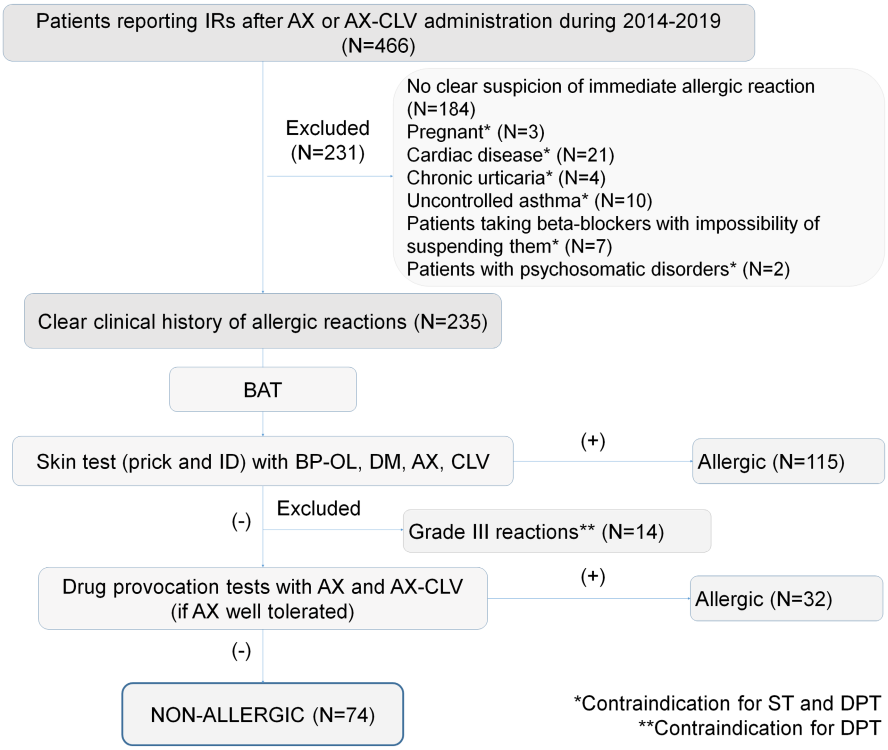

FIGURE 1 Study flow chart. From the 466 patients attended with suspicion of immediate allergic reaction to AX or CLV, BAT was performed in 235. From these, 115 were diagnosed as allergic by ST, 32 by DPT, 72 were diagnosed as nonallergic, and 14 were excluded due to present Grade III reactions and negative STs.

TABLE 1 Clinical characteristics of allergic patients to AX or CLV and nonallergic patients.

	Allergic	Nonallergic	*p* Value
N	147	74	
Age (median, IR, years old)	46 (35.7–55)	41.55 (33.6–51.24)	.099
Gender (*N*, % of females)	93 (63.3)	44 (59.5)	.742
Atopy (*N*, %)	42 (28.6)	26 (35.1)	.318
Allergy to other drugs (*N*, %)	19 (12.92)	24 (32.43)	**.0005**
N° of episodes (median, IR)	1 (1–2)	1 (1–1)	.104
Symptoms manifested in reaction (*N*, %)			
Anaphylactic shock	39 (26.5)	–	**.0001**
Anaphylaxis	74 (50.3)	10 (8.1)	**<.0001**
Urticaria/angioedema	34 (23.1)	64 (86.48)	**2.2 e-16**
Interval between the first drug administration and the onset of the reaction (median, IR, min)	15 (10–30)	15 (10–90)	**.034**
Interval time reaction—allergological study (median, IR, months)	11.25 (4.5–123.97)	21.78 (5–264.06)	.420

Abbreviations: IR, interquartile range; min, minutes; *N*, number.
Significant differences are indicated in bold.

(30.48%) were selective to AX, 33 (31.43%) cross-reactors to penicillin, and in 39 (37.14%) selectivity could not be confirmed. From the 147 allergic patients, 115 (78.23%) were confirmed by STs and 32 (21.76%) by DPTs. The clinical characteristics of patients with confirmed allergic and nonallergic reactions are shown in Table 1. Comparison of clinical data between AX- and CLV-allergic patients (Table 2) showed statistical differences in the clinical manifestations ($p = .025$), with equal proportion of ANA in both groups (around 50%), a greater proportion of SHOCKs in AX group than in CLV group (31% vs. 14%, respectively), and a greater proportion of URT in CLV group (36% vs. 18% in AX group). There were no significant differences in age, gender, and time interval between reaction and study and between drug administration and reaction.

3.2 | BAT for diagnosing AX allergy

A dose–response curve was performed using stimulation index (SI) for both activation markers, CD63 and CD203c, to select the best AX concentration to distinguish allergic and nonallergic subjects. The 1.25 mg/ml concentration was selected for both markers ($p < .001$ for CD63 and $p < .001$ for CD203c; Figure 2A). No differences were

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TABLE 2 Clinical characteristics of AX- and CLV-allergic patients.

	Allergic to AX	Allergic to CLV	*p* Value
N	105	42	
Age (median, IR, years old)	47.5 (35.75–56)	45.5 (35.7–51.29)	.398
Gender (*N*, % of females)	55 (52.4)	29 (69)	.065
Atopy (*N*, %)	27 (25.7)	15 (35.7)	.225
Allergy to other drugs (*N*, %)	14 (13.3)	5 (11.9)	.816
N° of episodes	1 (1–2)	1 (1–1.75)	.882
Symptoms manifested in reaction (*N*, %)			
Anaphylactic shock	33 (31.4)	6 (14.3)	**.033**
Anaphylaxis	53 (50.5)	21 (50)	1
Urticaria/angioedema	19 (18.1)	15 (35.7)	**.015**
Interval between the first drug administration and the onset of the reaction (median, IR, min)	10 (10–23.75)	17.5 (10–37.5)	.077
Interval time reaction—allergological study (median, IR, months)	6.78 (3.45–14.58)	10.87 (4.13–36.5)	.589
Methods of diagnosis (*N*, %)			
STs	79 (75.2)	36 (85.7)	.164
DPT	26 (24.8)	6 (14.3)	

Abbreviations: DPT, drug provocation test; IR, interquartile range; min, minutes; N, number; STs, skin tests.

Significant differences are indicated in bold.

found between CD63 and CD203c in terms of SI values for either allergic or nonallergic subjects (Figure 2B). After this, receiver operating curves (ROCs) of both markers were used to assess the discriminative power of BAT and to find the SI cutoff that provides the best balance between sensitivity (SE) and specificity (SP), favoring an SP over 80%. As it is used as a confirmatory test, BAT requires high SP to minimize false-positive results. Thus, we must favor SP over SE. The discriminative power of BAT was good for both markers with an area under the curve (AUC) = 0.691 for CD63 ($p < .001$) and an AUC = 0.722 for CD203c ($p < .001$). Two different cutoffs (SI > 1.2 and SI > 1.4) were studied, being a 1.2 cutoff with the concentration of 1.25 mg/ml selected as the best value for both markers to perform the following analysis (Figure 2C). Comparing the results obtained for each marker and also using both, an SE of 48.6% and an SP of 81.1% were obtained for CD63, and an SE of 46.7% and an SP of 94.6% for CD203c, whereas results for combining both markers slightly increased SE (51.4%) although with a decrease in SP (79.7%; Figure 2D). Best results in terms of predictive values were observed using CD203c as activation marker, with a positive predictive value (PPV) of 92.5%, higher than CD63 (78.5%) or the combination of both markers (78.3%); a negative predictive value (NPV) of 55.6%, slightly higher than with CD63 (52.6%) and the combination (53.6%); and better positive like-hood ratio (PLR) (8.63) and negative like-hood ratio (NLR) (0.56) (Figure 2D bottom). Moreover, this test showed a good diagnostic accuracy calculated using Fagan's nomogram, with a prevalence pretest of 58.7% and a probability post-test, after a positive test, which reached 78.5% using CD63 and 92.5% using CD203c. Similar results have been obtained using the % raw data to analyze the results (Figure S1).

3.3 | BAT for diagnosing CLV allergy

A dose–response curve was performed using SI for both activation markers, CD63 and CD203c, to select the CLV concentration able to better distinguish allergic from nonallergic subjects. The 0.25 mg/ml concentration was selected for both markers ($p < .001$ for CD63 and $p < .001$ for CD203c; Figure 3A). Similar to AX, no differences were found between both markers concerning SI values for either allergic or nonallergic subjects (Figure 3B). The ROC analysis results with the selected concentration showed a good discriminating power of BAT for both markers (AUC = 0.705; $p < .001$ for CD63 and AUC = 0.757; $p < .001$ for CD203c). Two different cutoffs were studied for each marker (SI > 1.5 and SI > 2.2 for CD63 and SI > 1.25 and SI > 1.5 for CD203C) to select the one that provides the best SE/SP balance. A 1.5 cutoff with the 0.25 mg/ml concentration was selected as the best value for both markers to perform the following analysis (Figure 3C). Comparing the results obtained for each marker and also using both, an SE of 42.9% and an SP of 80% were obtained for CD63 and an SE of 50% and an SP of 80% for CD203c, whereas the results for combining both markers showed a slightly increase in SE (54.8%) although decreasing SP (73%; Figure 3D). Interestingly, and similar to AX, best results regarding predictive values were observed using CD203c as activation marker, with a PPV of 58.3%, slightly higher than using CD63 (54.5%) or both markers (53.5%); an NPV of 73.8%, slightly higher than with CD63 (71.1%) and similar to the combination (74%); and positive (2.47) and negative (0.63) LRs (Figure 3D bottom). This test showed a moderate diagnostic accuracy calculated using Fagan's nomogram, with a 36.2% pretest prevalence and a probability post-test, after a positive test, of 54.5%

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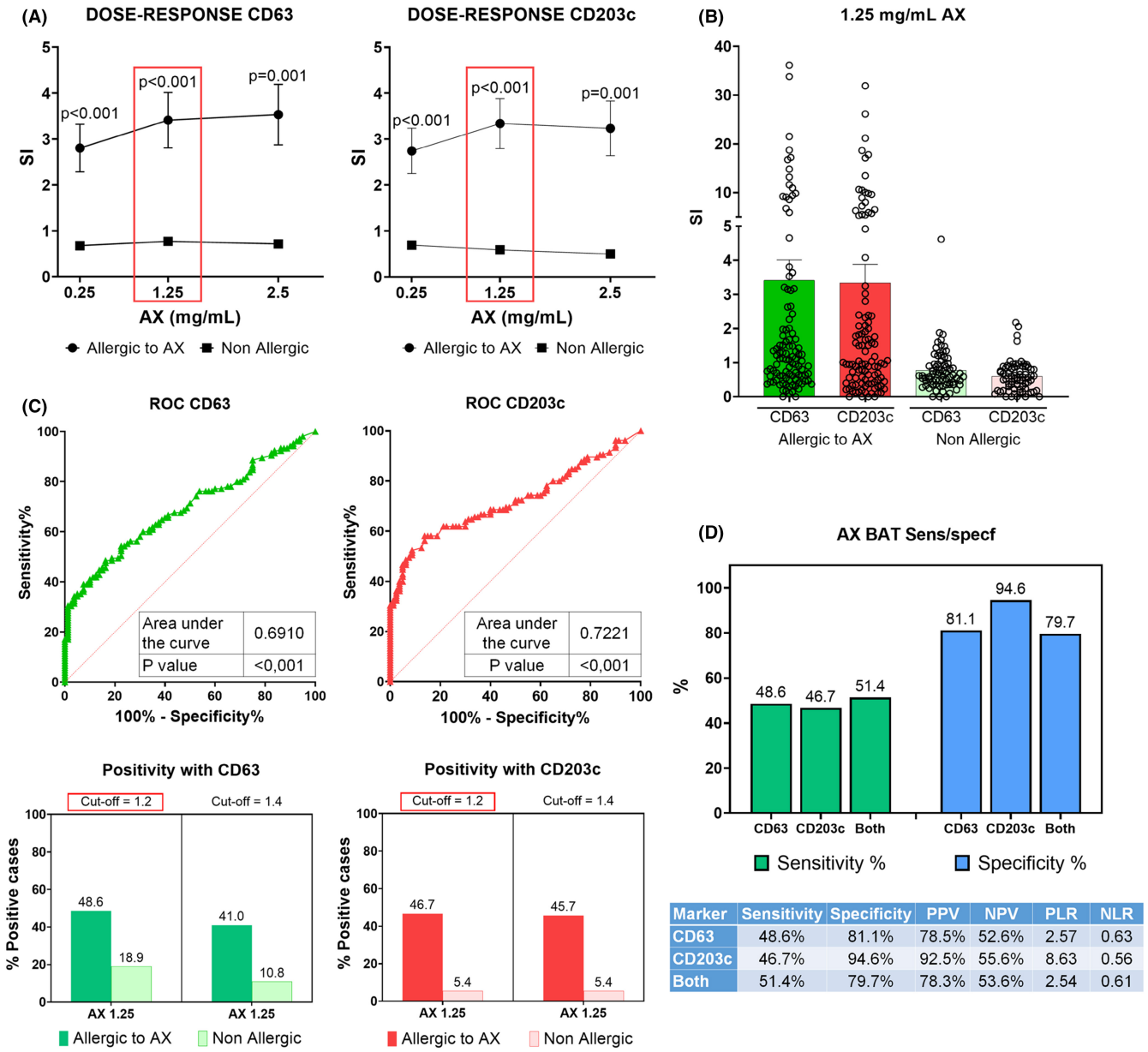

Marker	Sensitivity	Specificity	PPV	NPV	PLR	NLR
CD63	48.6%	81.1%	78.5%	52.6%	2.57	0.63
CD203c	46.7%	94.6%	92.5%	55.6%	8.63	0.56
Both	51.4%	79.7%	78.3%	53.6%	2.54	0.61

FIGURE 2 Basophil activation test results in AX-allergic patients. (A) Dose–response curves in BAT with AX using CD63 or CD203c as activation marker. The concentration of 1.25 mg/ml of AX was selected for both markers. (B) Expression of both activation markers in allergic and nonallergic subjects. No differences were found between the expression of CD63 (green) and CD203c (red) in allergic patients nor in nonallergic subjects. (C) ROCs for CD63 (green) and CD203c (red) showing the area under the curve (up); SE and SP using two different cutoffs. A cutoff of SI ≥ 1.2 was selected as the one with an SP over 80% and the best SE values (bottom). (D) Values of SE and SP, predictive values and likehood ratios of BAT with AX using CD63 or CD203c as activation markers, or combining the results of both.

using CD63 and 58.3% using CD203c. Similar results have been obtained using the % raw data to analyze the results (Figure S2).

3.4 | BAT results depending on the clinical entity

When patients were distributed by clinical entity, we observed that AX patients showed differences from nonallergic for CD63 (SHOCK: $p = .027$, ANA: $p = .001$, URT/ANG: $p = .004$) and CD203c (SHOCK: $p = .002$, ANA: $p = .001$, URT/ANG: $p = .002$). However, no differences were observed in SI between both markers for any of the clinical entities, nor among the three groups (SHOCK, ANA and URT/ANG) for each marker. (Figure 4A). Regarding CLV-patients, most groups showed differences from nonallergic for CD63 (SHOCK: $p = .001$, ANA: $p = .001$, URT/ANG: $p = .033$) and CD203c (SHOCK: $p = .001$, ANA: $p = .001$, but not for URT/ANG). No differences in SI were obtained between both markers for any clinical entity. Interestingly, comparing SI levels among the three clinical entities for each marker, differences were found between SHOCK and URT/ANG for CD63 ($p = .018$) and CD203c ($p = .001$) and between SHOCK and ANA only for CD203c ($p = .003$). (Figure 4B).

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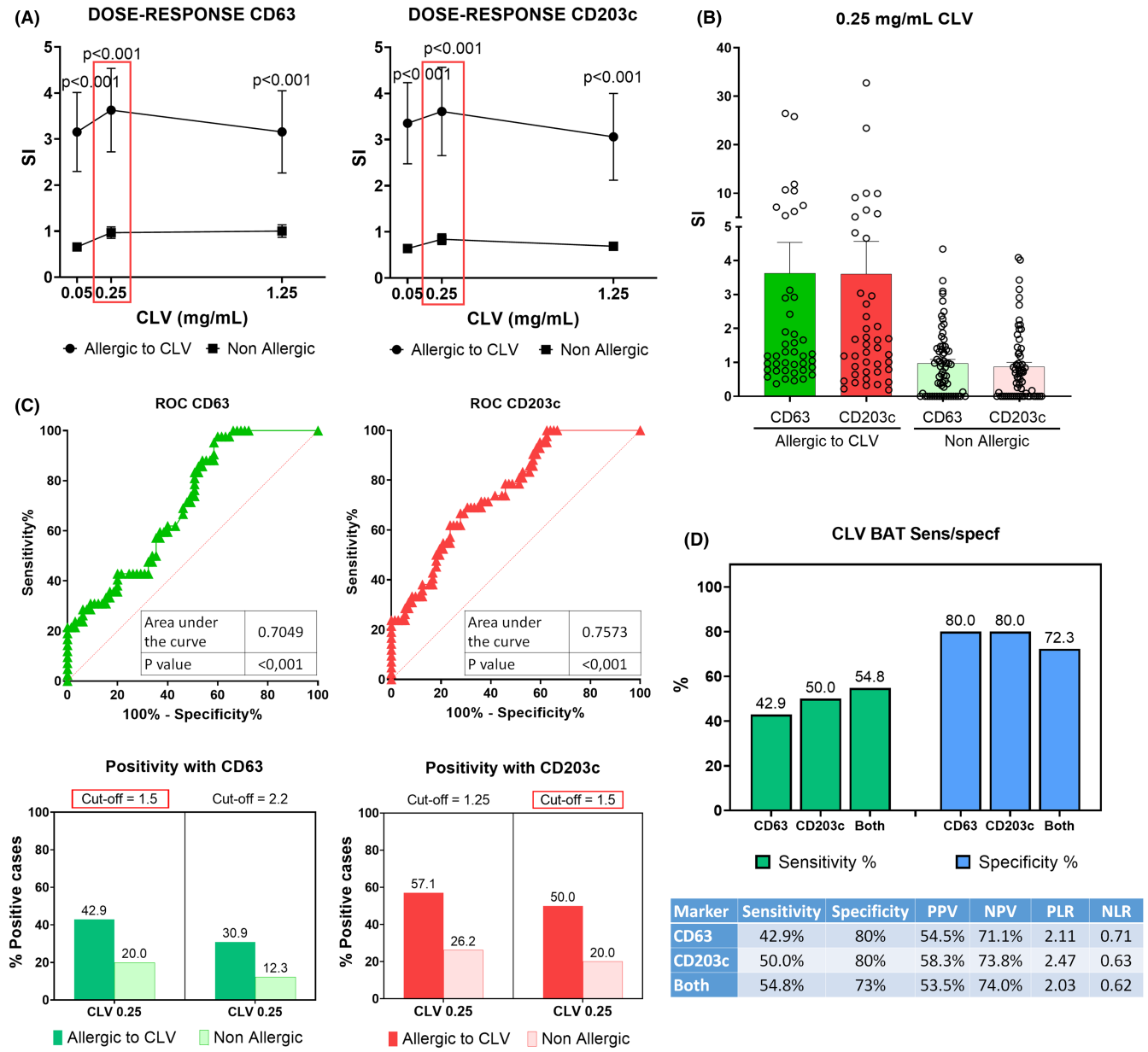

Marker	Sensitivity	Specificity	PPV	NPV	PLR	NLR
CD63	42.9%	80%	54.5%	71.1%	2.11	0.71
CD203c	50.0%	80%	58.3%	73.8%	2.47	0.63
Both	54.8%	73%	53.5%	74.0%	2.03	0.62

FIGURE 3 Basophil activation test results in CLV-allergic patients. (A) Dose–response curves in BAT with CLV using CD63 or CD203c as activation marker. The concentration of 0.25 mg/ml of CLV was selected for both markers. (B) Expression of both activation markers in allergic and nonallergic subjects. No differences were found between the expression of CD63 (green) and CD203c (red) in allergic patients nor in nonallergic subjects. (C) ROCs for CD63 (green) and CD203c (red) showing the area under the curve (up); SE and SP using two different cutoffs. A cutoff of SI ≥ 1.5 was selected as the one with an SP over 80% and the best SE values (bottom). (D) Values of SE and SP, predictive values and like-hood ratios of BAT with CLV using CD63 or CD203c as activation markers, or combining the results of both.

Taking into account the previously selected cutoffs for positivity, we found that for AX-allergic patients, positivity was similar for the three entities using both activation markers, CD63 and CD203c (42.4% and 39.34%, respectively, in SHOCK; 54.7% and 52.8 in ANA; and 42.1% for both markers in URT/ANG). Positivity was greater combining both markers, for the three clinical entities (48.5% for SHOCK, 62.3% for ANA, and 52.6% for URT/ANG; Figure 4C). In CLV-allergic patients, although positivity was similar for URT/ANG using both activation markers (33.3%), for SHOCK and ANA, results were better using CD203c compared with CD63 (100% vs. 83.3% in SHOCK and 47.6% vs. 38.1% in ANA) although not significant. Positivity was greater combining both markers, for ANA (52.4%) and URT/ANG (40%; Figure 4D).

3.5 | BAT results in excluded patients due to reaction severity

After evaluating the diagnostic value of BAT compared with in vivo diagnostic work-up, we analyzed BAT results obtained in the group of severe reactions (Grade III) excluded from the initial analysis due to negative ST and contraindication for DPT. From these 14 patients

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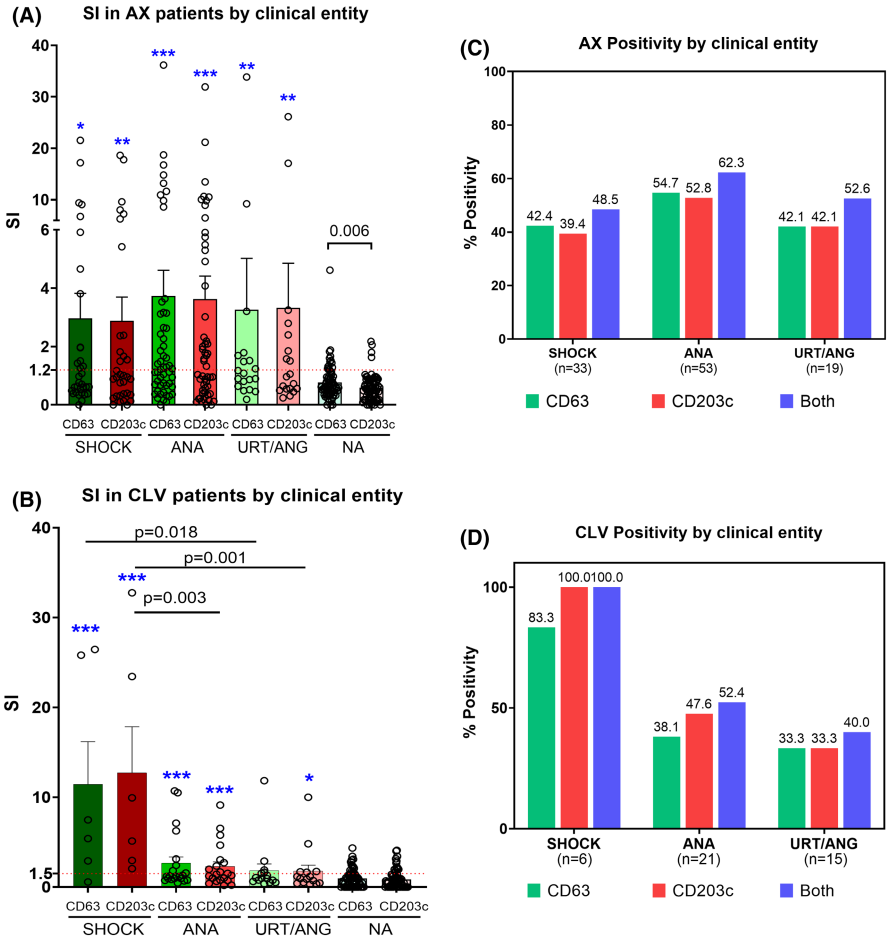

FIGURE 4 Basophil activation test results depending on the clinical entity in AX- and CLV-allergic patients. (A) Expression of CD63 (green) and CD203c (red) depending on the clinical manifestation in AX-allergic patients. In all different clinical entities, the expression of both markers was significantly higher than in nonallergic subjects (blue asterisks). The expression of CD63 was higher than the expression of CD203c in nonallergic subjects. (B) Expression of CD63 (green) and CD203c (red) depending on the clinical manifestation in CLV-allergic patients. In all different clinical entities, except for patients with URT/ANG using CD63, the expression of both markers was significantly higher than in nonallergic subjects (blue asterisks). (C) Positivity of BAT with AX in allergic patients depending on the clinical entities. No significant differences were found. The combination of the results obtained with both markers showed the best SE values (blue bars). (D) Positivity of BAT with CLV in allergic patients depending on the clinical entities. No significant differences were found although CD203c showed the best SE values in severe reactions (SHOCK and ANA). The combination of the results obtained with both markers showed the best SE values (blue bars).

undergoing BAT with both AX and CLV, and using CD203c as activation marker, 14.3% were positive to AX ($n = 2$), 14.3% to CLV ($n = 2$), 35.7% ($n = 5$) to both, and five patients negative to both. Using CD63 as activation marker, 14.3% were positive to AX ($n = 2$), 21.4% to CLV ($n = 3$), 28.6% ($n = 4$) to both, and 35.7% negative to both ($n = 5$). 28.6% showed different results analyzing BAT with CD63 or CD203c ($n = 4$), thus combining both markers, 21.4% were positive to AX ($n = 3$), 21.4% to CLV ($n = 3$), 35.7% to both drugs ($n = 5$), and only 21.4% ($n = 3$) were negative to both.

Further results about the correlation between the expression of CD63 and CD203c in basophils, BAT results depending on the selective response to AX, and the use of CD203c mean fluorescence intensity (MFI) to analyze BAT can be found in the Results section of this article's Online Repository.

4 | DISCUSSION

We have performed a prospective evaluation of BAT in patients reporting IR after AX or AX-CLV treatment. SE of BAT in real life, similar to data reported in case–control studies, is moderate. Nevertheless, BAT with CD203c could be a promising complementary tool to diagnose IRs to AX, with a high SP and PPV, thus, could be included into diagnostic algorithms as a first step.

Previous analysis of BAT as a potential in vitro diagnostic tool for AX- and AX-CLV-allergic patients has shown SE values ranging from 48%[20] to 55%[12] and SP ranging from 89%[12] to 93%.[20] However, all these studies have been performed in well-phenotyped retrospectively selected patients; thus, the real clinical value of BAT to diagnose BL-allergic patients was unknown.

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In our study, we have performed dose–response curves to select the drug concentration able to better discriminate allergic from nonallergic subjects (1.25 mg/ml for AX and 0.25 mg/ml for CLV). Cutoffs were selected using ROCs as the value that provided the best SE/SP balance, favoring an SP over 80%. The selected cutoff was similar for both drugs and markers (1.2 for AX and 1.5 for CLV) and similar to the one we calculated in a previous study using the same method.[12] We observed that, although BAT SE for AX was greater using both markers (51.4%), the best SE/SP balance was found using CD203c (46.7% and 94.6%, respectively). These results have been obtained analyzing the SI and are similar to those obtained analyzing the % of activated basophils observed using each activation marker.

Previous case–control study, performed in allergic confirmed patients, that had been done using CD63 as activation marker, showed higher SE than this prospective study,[12] which can be explained by the different study designs. However, results obtained using CD203c are in line with those obtained by Abuaf et al.[34] who found CD203c as a more sensitive activation marker for the diagnosis of AX allergy. After our study, this affirmation seems to be true also for CLV allergy diagnosis.

SE and SP values indicate the proportion of allergic and nonallergic subjects who are correctly diagnosed, but it cannot predict the probability of being allergic in an individual patient. For this, we must analyze the PPV and NPV of the test, which provide information about the probability of a subject who displayed a positive or negative result to have been correctly diagnosed. In BAT for AX, we also found the best PPV (92.5%) and NPV (55.6%) using CD203c. Nevertheless, it should be noted that the disease prevalence can vary depending on the population of the study, influencing predictive values.[37]

There are other parameters, such as PLR and NLR, able to directly relate pretest and post-test probability independently of disease prevalence,[38] summarizing diagnostic accuracy and providing a more powerful approach to clinical interpretation and decision-making. These parameters have shown a variable impact of BAT for AX in its clinical usefulness. PLR was of 8.65 with a probability post-test of 92.5% after a positive test using CD203c, situating BAT in a good range of clinical utility, with a high probability to be allergic to AX after having a positive test result. Regarding NLR, it was 0.56; thus, a negative BAT result is not enough to rule out allergy. These results confirm the high diagnostic value of BAT for identifying true positive patients, reducing the need of further allergological studies when it shows positive results, although a negative result does not confirm the absence of allergy and a complete allergological work-up should be done for them. Therefore, BAT could be done previously to in vivo test for reducing the number of patients that need to be evaluated. Moreover, BAT for AX using CD203c showed positive results in 50% of patients in which AX was implicated but was excluded from the study due to reaction severity. These patients might have benefited from the inclusion of this test in the diagnostic algorithm.

Similar results have been found in BAT to CLV, in which the best SE/SP balance was observed using CD203c (SE of 50%, SP of 80%, PPV of 61.8%, NPV of 71.2%). Nevertheless, LR values place BAT to CLV as a moderate tool to diagnose IR to this drug (PLR of 2.5, NLR of 0.65 and a probability post-test, after a positive test, of 58.3%.).

Importantly, the number of positive BATs (either to AX or CLV) among all the patients studied (allergic to AX, allergic to CLV, and nonallergic) was of 46.6%; thus, these individuals could be diagnosed as allergic without any other test (a 39.9% of real allergic subjects with a 92.3% of specificity).

Positivity was also analyzed depending on the clinical entity. For both AX- and CLV-allergic patients, similar or higher results were obtained with CD203c compared with CD63, for the three clinical entities (SHOCK, ANA, and URT/ANG). Combining both markers, positivity increased, but SP decreased. These results for AX patients are different from those obtained by Abuaf et al. These discrepancies could be due to the fact that Abuaf only defined two clinical entities, ANA and URT, and results with CD203c were 60% of positivity in ANA and only 29% in URT.[34] In our work, results are more similar between entities and markers, even if we grouped ANA and SHOCK in a unique category as severe reactions, with SE values ranging from 40% to 50%. These differences can be not only due to the different designs of the studies but also due to the different criteria used to define positivity in BAT, as Abuaf et al.[34] used a percentage value greater than two times the SD of the negative control. Regarding CLV-allergic patients, it is interesting the observation that all the patients with the most severe reactions (SHOCK) presented positive results in BAT using CD203c as activation marker, although we must be aware that the number of patients was low ($n = 6$). The rest of clinical entities showed similar percentages than those of AX patients, although with an increasing positivity respect to severity as it was observed in the previous study.[12]

In conclusion, we found that AX-induced reactions seem to be more severe than CLV-induced reactions and the performance of BAT using CD203c as activation marker is a promising diagnostic tool, especially for AX allergy, with a high confirmatory power. Therefore, this could place BAT as a first step in the diagnostic algorithm, helping reduce the need of a complete allergological work-up in around 46% of patients, with a specificity of 92.3%, and diminishing the risk of allergic reactions during the diagnostic procedure. However, a negative result in BAT does not rule out allergy to these BLs. Moreover, patients in which in vivo study is contraindicated, because of different comorbidities, could be beneficiated with the inclusion of BAT in the diagnostic algorithm.

AUTHOR CONTRIBUTIONS

The authors approved the final version of the manuscript as submitted and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Jeffres MN, Narayanan PP, Shuster JE, Schramm GE. Consequences of avoiding beta-lactams in patients with beta-lactam allergies. *J Allergy Clin Immunol*. 2016;137(4):1148-1153.
2. Doña I, Barrionuevo E, Blanca-Lopez N, et al. Trends in hypersensitivity drug reactions: more drugs, more response patterns, more heterogeneity. *J Investig Allergol Clin Immunol*. 2014;24(3):143-153.
3. Versporten A, Coenen S, Adriaenssens N, et al. European surveillance of antimicrobial consumption (ESAC): outpatient penicillin use in Europe (1997-2009). *J Antimicrob Chemother*. 2011;66(Suppl 6):vi13-vi23.
4. Solensky R. Penicillin allergy as a public health measure. *J Allergy Clin Immunol*. 2014;133:797-798.
5. ECDC. *Surveillance of Antimicrobial Consumption in Europe*. ECDC: European Centre for Disease Prevention and Control (ECDC); 2012 2014. Report No: TQ-AT-14-002-EN-N.
6. Rubio M, Bousquet PJ, Gomes E, Romano A, Demoly P. Results of drug hypersensitivity evaluations in a large group of children and adults. *Clin Exp Allergy*. 2012;42(1):123-130.
7. Gomes ER, Brockow K, Kuyucu S, et al. Drug hypersensitivity in children: report from the pediatric task force of the EAACI drug allergy interest group. *Allergy*. 2016;71(2):149-161.
8. Doña I, Blanca-Lopez N, Torres MJ, et al. Drug hypersensitivity reactions: response patterns, drug involved, and temporal variations in a large series of patients. *J Investig Allergol Clin Immunol*. 2012;22(5):363-371.
9. Barrionuevo E. EAACI meeting. 2015.
10. Gell PGH, Coombs RRA. *Clinical Aspects of Immunology*. Blackwell; 1963.
11. Torres MJ, Ariza A, Mayorga C, et al. Clavulanic acid can be the component in amoxicillin-clavulanic acid responsible for immediate hypersensitivity reactions. *J Allergy Clin Immunol*. 2010;125(2):502-505.e502.
12. Salas M, Fernandez-Santamaria R, Mayorga C, et al. Use of the basophil activation test may reduce the need for drug provocation in amoxicillin-clavulanic allergy. *J Allergy Clin Immunol Pract*. 2018;6(3):1010-1018.e1012.
13. Sanchez-Morillas L, Perez-Ezquerra PR, Reano-Martos M, Laguna-Martinez JJ, Sanz ML, Martinez LM. Selective allergic reactions to clavulanic acid: a report of 9 cases. *J Allergy Clin Immunol*. 2010;126(1):177-179.
14. Blanca-Lopez N, Perez-Alzate D, Ruano F, et al. Selective immediate responders to amoxicillin and clavulanic acid tolerate penicillin derivative administration after confirming the diagnosis. *Allergy*. 2015;70(8):1013-1019.
15. Cahen YD, Wuthrich B. Drug allergy to the beta-lactam antibiotics clavulanic acid and amoxicillin. *Allergy*. 1997;52(1):117-118.
16. Salas M, Laguna JJ, Doña I, et al. Patients taking amoxicillin-clavulanic can become simultaneously sensitized to both drugs. *J Allergy Clin Immunol Pract*. 2017;5(3):694-702.e693.
17. Zambonino MA, Corzo JL, Munoz C, et al. Diagnostic evaluation of hypersensitivity reactions to beta-lactam antibiotics in a large population of children. *Pediatr Allergy Immunol*. 2014;25(1):80-87.
18. Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "Allergy" in hospitalized patients: a cohort study. *J Allergy Clin Immunol*. 2014;133(3):790-796.
19. Romano A, Atanaskovic-Markovic M, Barbaud A, et al. Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper. *Allergy*. 2020;75(6):1300-1315.
20. Torres MJ, Padial A, Mayorga C, et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to beta-lactams. *Clin Exp Allergy*. 2004;34(11):1768-1775.
21. Bogas G, Dona I, Dionicio J, et al. Diagnostic approach of hypersensitivity reactions to cefazolin in a large prospective cohort. *J Allergy Clin Immunol Pract*. 2021;9:4421-4430.e4.
22. Barbero N, Fernandez-Santamaria R, Mayorga C, et al. Identification of an antigenic determinant of clavulanic acid responsible for IgE-mediated reactions. *Allergy*. 2019;74(8):1490-1501.
23. Arribas F, Falkencrone S, Sola J, et al. Basophil histamine release induced by Amoxicilloyl-poly-L-lysine compared with amoxicillin in patients with IgE-mediated allergic reactions to amoxicillin. *J Investig Allergol Clin Immunol*. 2017;27(6):356-362.
24. Pineda F, Ariza A, Mayorga C, et al. Role of histamine release test for the evaluation of patients with immediate hypersensitivity reactions to clavulanic acid. *Int Arch Allergy Immunol*. 2015;168(4):233-240.
25. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol*. 1991;88(3 Pt 1):328-338.
26. Hoffmann HJ, Frandsen PM, Christensen LH, Schiotz PO, Dahl R. Cultured human mast cells are heterogeneous for expression of the high-affinity IgE receptor FcepsilonRI. *Int Arch Allergy Immunol*. 2012;157(3):246-250.
27. Ebo DG, Bridts CH, Mertens CH, Hagendorens MM, Stevens WJ, De Clerck LS. Analyzing histamine release by flow cytometry (HistaFlow): a novel instrument to study the degranulation patterns of basophils. *J Immunol Methods*. 2012;375(1–2):30-38.
28. MacGlashan D Jr. Marked differences in the signaling requirements for expression of CD203c and CD11b versus CD63 expression and histamine release in human basophils. *Int Arch Allergy Immunol*. 2012;159(3):243-252.

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29. Fernandez TD, Ariza A, Palomares F, et al. Hypersensitivity to fluoroquinolones: the expression of basophil activation markers depends on the clinical entity and the culprit fluoroquinolone. *Medicine (Baltimore)*. 2016;95(23):e3679.
30. Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandene L. Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods*. 2007;320(1–2):40-48.
31. Sturm EM, Kranzelbinder B, Heinemann A, Groselj-Strele A, Aberer W, Sturm GJ. CD203c-based basophil activation test in allergy diagnosis: characteristics and differences to CD63 upregulation. *Cytometry B Clin Cytom*. 2010;78(5):308-318.
32. Sanz ML, Gamboa PM, Antepara I, et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy*. 2002;32(2):277-286.
33. De Week AL, Sanz ML, Gamboa PM, et al. Diagnosis of immediate-type beta-lactam allergy in vitro by flow-cytometric basophil activation test and sulfidoleukotriene production: a multicenter study. *J Investig Allergol Clin Immunol*. 2009;19(2):91-109.
34. Abuaf N, Rostane H, Rajoely B, et al. Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy*. 2008;38(6):921-928.
35. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol*. 2004;114(2):371-376.
36. Demoly P, Kropf R, Bircher A, Pichler WJ. Drug hypersensitivity: questionnaire. EAACI interest group on drug hypersensitivity. *Allergy*. 1999;54(9):999-1003.
37. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates of test accuracy are transferable. *BMJ*. 2002;324(7338):669-671.
38. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. *BMJ*. 2004;329(7458):168-169.

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