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Diagnostic approach of hypersensitivity reactions to cefazolin in a large prospective cohort.

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TITLE: Diagnostic approach of hypersensitivity reactions to cefazolin in a large prospective cohort.

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50 AUTHORS CONTRIBUTION

51 GB, ID, JD, TDF, CB, MIM and CR have made substantial contributions to acquisition of data, or
52 analysis and interpretation of data.

53 EM, JLL, MJT have made substantial contributions to conception and design of the study.

54 GB, ID, TDF, CM, MIM, MA and MJT have been involved in drafting the manuscript and revising
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57

58 CONFLICT OF INTEREST

59 None author has any conflicts of interest to disclose.

60

ABSTRACT

Background: Cefazolin is a common trigger of perioperative anaphylaxis. The diagnostic approach is controversial as the optimal concentration for skin testing is uncertain, drug provocation tests (DPTs) are contraindicated in severe reactions, and *in vitro* tests are not thoroughly validated.

Objective: We aimed to characterize a large number of patients reporting cefazolin allergic reactions and to analyze *in vivo* and *in vitro* tests' diagnostic role.

Methods: We prospectively evaluated patients with suspicion of allergic reactions to cefazolin by clinical history, skin tests (STs), and, if negative, DPT. In a subgroup of patients, basophil activation test (BAT) and radioallergosorbent test (RAST) were done before the allergological work-up was performed and the final diagnosis achieved.

Results: 184 patients were evaluated, being 76 (41.3%) confirmed as allergic, 90 (48.9%) as non-allergic, and 18 (9.8%) as non-confirmed. All cases reporting anaphylactic shock and most patients reporting anaphylaxis were confirmed as allergic ($p < 0.001$). 40 (52.6%) allergic cases were confirmed by STs, 22 (28.9%) by DPT, and 14 (18.4%) by clinical history. All subjects manifesting exanthemas and pruritus were non-allergic. BAT sensitivity was 66.7% when combining CD63 and CD203c as activation markers. Six out of eight patients with negative STs and positive DPT had a positive BAT.

Conclusions: Allergic patients to cefazolin often reported severe immediate-type reactions. STs allowed diagnosing half of patients when using cefazolin at 20 mg/ml. Unfortunately, DPT could not be performed in all cases due to reaction severity, making BAT a promising diagnostic tool. Further researches are needed to clarify the underlying mechanisms, especially in severe reactions.

86 **What is already known about this topic?**

87 The diagnosis of cefazolin-induced reactions is complex due to uncertainties concerning the
88 optimal concentration for skin tests, *in vitro* tests are not commercially available/validated and
89 provocation tests can be contraindicated due to the reaction severity.

90

91 **What does this article add to our knowledge?**

92 Skin tests using cefazolin at 20 mg/ml allowed diagnosing half of the patients. Basophil
93 activation test sensitivity was 66.7%, being positive in the 75% of patients with negative skin
94 tests.

95

96 **How does this study impact current management guidelines?**

97 Skin tests allow diagnosing half of the patients when using cefazolin at 20 mg/ml. Basophil
98 activation test is a promising diagnostic tool, especially in patients with severe reactions and
99 negative skin tests.

100

101

102 **KEYWORDS:** Anaphylaxis, basophil activation test, cefazolin, *in vivo* diagnosis, *in vitro* test.

103

104 **ABBREVIATIONS:** Basophil activation test (BAT); Drug provocation tests (DPTs); European
105 Academy of Allergy and Clinical Immunology (EAACI); Immediate reactions (IRs); intradermal
106 test (IDT); Non-immediate (NIRs); Radioallergosorbent test (RAST); Skin prick test (SPT); Skin
107 tests (STs).

INTRODUCTION

Cefazolin, a first generation cephalosporin, is a commonly prescribed antibiotic used for surgical prophylaxis [1-3]. It can induce allergic reactions, which can be immediate (IRs) or non-immediate (NIRs) depending on whether the interval time between the drug administration and the onset of the reaction is within 1 hour or longer, respectively [4]. IRs are the most frequent [5-7], being NIRs uncommon and only reported in small series and case reports [8-12]. Most IRs induced by cefazolin occur in perioperative setting [5-7]. Indeed, some studies indicate that betalactam antibiotics are a leading cause of perioperative anaphylaxis [13-17], being cefazolin the most common agent inducing these reactions in many countries [13, 17-22].

As cefazolin-induced allergic reactions are often severe [5-7], their correct diagnosis is extremely necessary to prevent recurrences [15, 17, 23, 24]. Moreover, a confirmed diagnosis is crucial for avoiding alternative medications, which are usually costlier and associated with increasing resistant bacterias [23-26]. The diagnostic approach of drug-induced allergic reactions is primarily based on a detailed clinical history [27, 28] and *in vivo* procedures, such as skin tests (STs) and drug provocation tests (DPTs) [23, 24, 29, 30]. *In vitro* methods such as determination of serum sIgE by immunoassay, and basophil activation test (BAT) are accepted as complementary tests for diagnosis [7, 23, 24, 31]. However, although cefazolin hypersensitivity constitutes a potential lifethreatening condition, its correct diagnosis is not straightforward. First of all, DPTs can be contraindicated due to the severity of the reactions; and secondly, although several studies have demonstrated the usefulness of STs for diagnosing IRs to cefazolin, there are uncertainties concerning the optimal concentration [32, 33]. The European Academy of Allergy and Clinical Immunology (EAACI) recommends a maximal non-irritating ST concentration of 2 mg/ml for all cephalosporins [34], but some studies have confirmed that 20 mg/ml concentration is non-irritant and might improve sensitivity, without affecting specificity [35]. In fact, it has been reported an additional 27% of patients giving positive results in STs when using a concentration of 20 mg/ml compared with 2 mg/ml [15]. Regarding *in vitro*

methods, no reliable cefazolin-sIgE antibody assay is commercially available [36, 37] and although the utility of BAT for diagnosing antibiotic-induced anaphylaxis, including cefazolin, has been reported [38-40], it has not been thoroughly validated.

One of the reasons that hamper the improvement of sensitivity of *in vivo* and *in vitro* tests is the fact that the chemical structure of cefazolin antigenic determinants has not been identified. Several studies suggest that the immune response to cefazolin is determined mostly by specificity of antibodies against the side chains rather than against the common nuclear determinants, with tolerance for alternative betalactam antibiotics [41-43]. The R1 side chain of cefazolin contains a heterocyclic ring, tetrazole, constituting a unique R1 group that is structurally different from other cephalosporins [36, 41]. However, although the R1 side chain seems to play a crucial role, it is not enough to explain the existence of cross-reactivity between cephalosporins and other betalactams [7, 44].

Given these concerns, we aim to perform a detailed clinical characterization of patients with an unequivocal clinical history of allergic reactions and to analyze the role of *in vivo* tests (STs and DPTs) and *in vitro* tests (BAT and radioallergosorbent test (RAST)) in diagnosis.

METHODS

We prospectively evaluated patients referred to the Allergy Unit of three different Spanish Hospitals (Regional University Hospital of Málaga, Cruz Roja Hospital, and University Hospital of Salamanca) between January 2000 and December 2019 with unequivocal suspicion of allergic reactions to cefazolin. Reactions were classified into IRs or NIRs [4]. The three participant centers followed the same protocol for testing and diagnosing patients throughout the study period.

For IRs, the grading system for generalized allergic reactions of Brown was retrospectively used [45].

The allergological workup [46] included an exhaustive clinical history followed by STs, and, if negative, by a DPT with cefazolin (see Online Repository Text). When reactions occurred in perianesthetic setting, all other drugs and substances used such as latex and chlorhexidine were tested. In severe reactions (grade 3 reactions), the diagnosis was achieved once other potential inducing causes of the reaction were excluded.

From 2012, BAT, using CD63 and CD 203c as activation markers, [7, 47, 48] and RAST [49-51] were done in a representative sample of randomly selected patients by a pre-specified protocol (selecting one patient every 4th patient) before the allergological work-up was performed and the final diagnosis achieved (Figure 1). The clinicians were blinded for the result until the diagnosis as allergic or non-allergic was confirmed by *in vivo* tests.

All statistical analyses were carried out using the software package GraphPad PRISM v7. A value of $p < 0.05$ was considered statistically significant.

The study was conducted according to the principles of the Declaration of Helsinki and approved by the institutional review board (PI18/00095). All the participants were informed about the study and gave the corresponding informed consent.

Detailed methods are available as online supporting information.

RESULTS

We evaluated a group of 184 patients with unequivocal clinical history of allergic reactions after cefazolin administration. The mean age was 49.91 ± 15.4 years, 67 (36.4%) were male and 117 (63.6%) female and, regarding the timing of the reactions, 158 (85.9%) were IRs and 26 (14.1%) NIRs. The mean time interval between drug intake and development of symptoms was 27.5 ± 21.4 minutes for IRs and 894.6 ± 1144.3 for NIRs ($P=0.001$). The clinical manifestations of patients with IRs were anaphylactic shock in 14 (8.9%) cases, anaphylaxis in 51 (32.2%), urticaria in 69 (43.7%), angioedema in 3 (1.9%), urticaria/angioedema in 11 (7%), exanthema in 5 (3.2%), generalized pruritus in 5 (3.2%); and, from patients with NIRs, they were urticaria in 9 (34.6%), angioedema in 1 (3.8%), urticaria/angioedema in 2 (7.7%), and exanthema in 14 (53.8%). The severity of the IRs was grade 1 in 93 patients (58.86%), grade 2 in 51 patients (32.27%), grade 3 in 14 (8.9%). All NIRs were mild to moderate skin eruptions often treated with either antihistamines and/or corticosteroids, with no hospitalization needed. From all patients included 138 (75%) were perianesthetic reactions. The remaining 46 patients received cefazolin during hospital admissions for bones and joints infections ($n=22$), skin and soft tissues infections ($n=12$), sepsis ($n=7$), endocarditis ($n=3$), genitourinary system infections ($n=1$), and biliary tract infections ($n=1$) (data not shown in tables). The mean time interval between the reaction and the study was 29.46 ± 71.7 months for IR and 39.27 ± 69.2 months for NIR ($P=0.517$).

Allergological workup

After an allergological workup, 76 (41.3%) were confirmed as allergic to cefazolin, 90 (48.9%) as non-allergic to cefazolin, and 18 (9.8%) were not confirmed (12 refused to continue the study and 6 abandoned for unknown reasons) (Figure 1). The clinical characteristics of patients with confirmed allergic and non-allergic reactions to cefazolin are shown in Table 1. There were statistical differences between both groups in the type of reaction, being all IRs in the group of allergic, and also in the clinical manifestations, with all exanthemas and generalized pruritus included in the non-allergic, and all anaphylactic shock and most anaphylaxis in the allergic

group. There were also differences in the time interval of the reaction and the study and of the drug administration and the reaction, being both intervals shorter in allergic patients. However, there were no differences in age and gender between both groups. In all patients from these both groups, other agents that were administered simultaneously to cefazolin were tested, finding that in 34 who tolerated the cefazolin in DPT were confirmed as allergic to other agents: 15 to NSAIDs, 8 to latex, 3 to cisatracurium, and 1 to radio contrast media, heparin, proton pump inhibitors, midazolam, quimotripsy, quinolones, ranitidine, or remifentanyl. When we analyzed the year of evaluation of patients we found an increase in the percentage of patients confirmed as allergic and non-allergic to cefazolin over years, especially in allergies to cefazolin, although not significant (Figure 2): in the period 2000-2011 the percentage of allergic patients ranged from 1.3% to 5.3% and of non-allergic from 2.2% to 7.8%; and in the period 2012-2019 it ranged from 5.3% to 10.5% and 3.3% to 10.0%, respectively.

There were no statistical differences between allergic and not confirmed as allergic patients in gender, age, time between reaction and study, interval between drug intake and reaction, and type of reaction (IR and NIR) (data not shown in tables). However, allergic patients had more severe reactions than those not confirmed: anaphylactic shock 15.8% vs 0%, and anaphylaxis 36.8% vs 16.7% ($P<0.001$).

In vivo diagnostic methods

Regarding the diagnostic method, 14 (18.4%) patients were confirmed by clinical history, 40 (52.6%) by STs, and 22 (28.9%) by DPT. The clinical characteristics of patients diagnosed by clinical history are described in Table 2: 4 (28.6%) patients were male, the mean age was 47.9 ± 20.5 years, the mean time interval between the reaction and the study was 10.5 ± 18.1 months and between drug administration and development of symptoms 17.3 ± 10.6 minutes, most of them developed severe reactions (12 (85.71%) reported grade 3 reactions), and 2 (14.2%) cases with grade 2 reactions (anaphylaxis) in whom STs and DPTs were not performed

due to cardiovascular contraindications (both patients suffered from recent severe coronary heart disease).

From the 40 (52.6%) patients diagnosed by STs (Table 3), 17 (42.5%) were male, the mean age was 50.1 ± 18.1 years, the mean time interval between the reaction and the study was 14.8 ± 36.4 months and between drug administration and development of symptoms 19.0 ± 17.9 minutes. Regarding the original clinical symptoms, 18 (45%) patients developed anaphylaxis, 18 (45%) urticaria, and 4 (10%) urticaria/angioedema. Concerning the severity of the original reaction, 22 (55%) were grade 1 reactions and 18 (45%) grade 2 reactions. Five (12.5%) patients were positive by skin prick test (SPT) and 35 (87.5%) by intradermal test (IDT) to cefazolin, all of them at 20 mg/ml. No differences were found comparing those cases who give positive results in SPT and those positive in IDT (data not shown). All cases were negative to PLL/BP-OL, MDM/MD, AX and cefazolin at concentrations other than 20 mg/mL in SPT and IDT.

From the 22 (28.94%) patients diagnosed by DPT (Table 4), 7 (31.8%) were male, the mean age was 49.3 ± 11.6 years, the mean time interval between the reaction and the study was 9.3 ± 20.5 months and between drug administration and development of symptoms 23.5 ± 21 minutes. Concerning the severity of the original reaction, 14 (63.6%) reported grade 1 reactions and 8 (36.4%) grade 2 reactions. Regarding the original clinical symptoms, 12 patients (54.5%) developed urticaria, 8 (36.4%) anaphylaxis, and 2 (9.1%) urticaria/angioedema. Regarding the DPT results, 17 (77.3%) developed urticaria, 3 (13.6%) symptoms compatible with anaphylaxis, 1 (4.5%) urticaria/angioedema, and 1 (4.5%) generalized erythema and pruritus. The mean total cumulative dose of response was 290 ± 207.9 mg and the mean time interval between first drug administration and development of symptoms was 71.6 ± 40.3 minutes. Comparisons between patients diagnosed by STs and those diagnosed by DPT showed no statistical differences in age, interval between reaction and study, interval of time between drug administration and development of symptoms and clinical manifestations (data not shown in tables).

In vitro diagnostic methods

BAT was performed in 46 patients, 3 were eliminated of the study for being non responders. Comparisons between patients in which *in vitro* tests were performed with those in which they were not carried out showed no statistical differences in age, interval between reaction and study, interval of time between drug administration and development of symptoms and clinical manifestations (data not shown in tables). From the 43 patients finally included, 23 were diagnosed as allergic to cefazolin by clinical history, ST or DPT and 20 as non-allergic. BAT was performed using CD63 as activation marker, and also with CD203c, in 18 patients confirmed as allergic and 15 subjects confirmed as non-allergic (patients evaluated from 2013) (Table E1). Activation with both markers was analyzed and SI values showed a significant positive correlation ($p=0.002$; $R^2=0.472$) (Figure E1). The mean average of SI values was 2.93 and 0.96 with CD63 in allergic and non-allergic patients, respectively, and 3.45 and 1.06 with CD203c in allergic and non-allergic patients, respectively. BAT was positive in 14 allergic cases but negative in all non-allergic individuals. Therefore, sensitivity of BAT using CD63 was 43.47% ($n=10$) and using CD203c was 50% ($n=9$). Combining the results for both markers, the sensitivity increased up to 66.7% ($n=12$ of the 18 patients). The specificity was 100% independently of the activation marker used. Positive predictive value was 100% for both markers. However, BAT negative predictive value was greater using CD63 (60.6%) than using CD203c (40%) or the combination of both markers (50%). Interestingly, among the 18 patients in whom BAT with both markers was performed, 8 (44.44%) had negative STs and were finally diagnosed by DPT and 3 (16.66%) by clinical history. From the 8 patients diagnosed by DPT, 6 (75%) showed positive results with BAT. In the 3 patients confirmed by clinical history, all developing anaphylactic shock, BAT was positive in one of them with both markers (Table E1). Most of these patients (82.6%; $n=19$) were studied within the first 6 months after the reaction, and no correlation was found between the time interval between the reaction and the study and BAT results.

Determination of specific IgE to cefazolin was performed by RAST in 29 patients confirmed as allergic and in 17 non-allergic subjects (Table E2). The mean average of RAST percentage was

276 1.42 for allergic patients and 1.07 for those non-allergic. Serum sIgE to cefazolin mean average
277 was 1.42, and positive levels were detected in only one out of the 29 patients with confirmed
278 cefazolin allergy in whom BAT was not performed. This patient was studied three months after
279 the reaction, which was manifested as urticaria five minutes after the administration of cefazolin
280 as prophylaxis in a surgery for trauma following a road traffic accident. The diagnosis was
281 confirmed by positive SPT. Serum sIgE mean average was 1.07 and positive values were not
282 detected in any of those confirmed as non-allergic.

DISCUSSION

In this study, we included a total of 166 well-characterized patients reporting allergic reactions to cefazolin, from which 76 (45.78%) were confirmed as allergic and 90 (54.21%) as non-allergic to this drug. This high frequency of allergy might be due to the frequent use of this first-generation cephalosporin, mainly in prophylactic surgical protocols, which has increased in the last years [1-3, 13, 17-21]. In fact, analyzing the year of evaluation of allergic patients to cefazolin, we have detected in the last two decades an increase in the percentage of cases: from 1.3% in the year 2000 to 10.5% in the year 2019.

It has been reported that allergic reactions induced by cefazolin are often severe, immediate-type, with a significant proportion of anaphylactic shocks and anaphylaxis [5-7]. In our study, cases finally confirmed as allergic were all IRs, and those reporting anaphylactic shock and most of cases reporting anaphylaxis were confirmed as allergic. This can be partly explained by the mode of administration as an intravenous bolus before the surgery. It is of note that 13 cases reported anaphylaxis and were finally confirmed as non-allergic to cefazolin. However, they were confirmed as allergic to other agents that were administered simultaneously to cefazolin. Clinical suspicion of cefazolin allergy is generally confirmed by STs [15, 36, 52]. In our study, we detected 52.6% of positive cases by STs. Dilutions of cefazolin used for STs are controversial. The recommendation of a maximal nonirritating concentration up to 2 mg/ml [34, 53, 54] can induce false-negative results in STs [42]. On the contrary, it has been reported that ST concentrations up to 20 mg/ml are not irritant, even a concentration of cefazolin up to 33 mg/ml has been described as being non-irritant in some studies [35, 41, 55]. Moreover, the increasing cefazolin concentration for skin testing up to 20 mg/ml has been reported to improve the sensitivity of the test without affecting its specificity, as it allows identifying an additional 30% of patients [15, 35, 41, 55]. Based on this evidence, in our study we consider performing STs at 20 mg/ml dilutions. Indeed, all tested patients were negative for cefazolin at concentrations other than 20 mg/ml, despite the characteristics of our population not differing from previously published

studies. It has been described that false negative results in STs could be obtained after a long time interval between the allergic reaction and the test performance [56]. This may be the reason for the lower percentage of positive STs found in other studies. However, in our study both negative and positive STs groups were evaluated in a short period of time after the reaction (12.8 ± 31.6 months), and when comparing patients diagnosed by STs and those with negative results in STs and diagnosed by DPT no statistical differences were found. However, although we only found positive STs at 20 mg/ml, we recommend initiating skin testing at higher dilutions, especially in patients reporting severe reactions.

The negative predictive value of STs to cephalosporins has not been established, therefore DPT with the culprit cephalosporin is recommended in order to confirm or rule out the diagnosis of allergy [30, 57]. However, DPT cannot distinguish the underlying mechanism, as the appearance of symptoms may be similar in immunologically and non-immunologically mediated reactions. In our study, DPT with cefazolin was performed in 112 cases with negative STs, being positive in 22 (19.64%). We can not confirm if this group of patients belongs to a distinct phenotype/endotype. Although only in 6 out of 8 cases in whom BAT was performed were positive, we can not confirm if the activation of basophils was or not IgE-mediated.

The fact that we performed DPT when STs were negative is an important strength of our study as in most published manuscripts, despite STs being negative, DPT is not performed or is carried out with alternative cephalosporins, but not with the culprit [15, 41, 42]. This is due to the fact that reactions induced by cefazolin are often severe and DPT may be hazardous. However, in our study only 3 out of 22 cases with positive DPT developed symptoms compatible with anaphylaxis during its performance, which is lower than previously reported [42]. This low percentage of anaphylaxis may be due to the fact that in DPT the drug is administered in escalating-controlled doses, inducing milder reactions after the administration of initial low ones. Indeed, 2 out of 3 cases inducing anaphylaxis in DPT reacted to the lowest dose (TCD 50 mg). Moreover, DPT was not carried out in patients reporting severe reactions.

A limitation of this study could be that 14 cases were diagnosed by clinical history. They were 12 cases reporting grade 3 reactions and 2 cases reporting grade 2 reactions with cardiovascular diseases in which the performance of the tests was contraindicated after the risk-benefit balance. In these cases, the imputability of the cefazolin as the culprit drug is very high due to the severity of the reactions, the short time interval between the cefazolin administration and the onset of the reaction and that other potential causes were discarded.

In the cases in which it is not possible to perform the *in vivo* tests, *in vitro* tests are crucial in the diagnostic approach. Novel approaches, such as BAT, are recently gaining importance [24, 31, 57, 58]. In our study, we analyzed the role of the *in vitro* tests in the diagnosis of patients reporting allergic reactions to cefazolin. We performed the *in vitro* tests before the patient was tested *in vivo* in order to avoid the BAT and RAST results being influenced by exposure to cefazolin in the STs or in the DPT. The clinicians were blinded for the result until the diagnosis as allergic or non-allergic was confirmed by *in vivo* tests. As the blood sample was taken prior the start of the allergological work-up and randomly, *in vitro* tests were not performed in all patients not undergoing STs and DPTs. As not all patients underwent all *in vitro* tests and this may be considered as a bias, the analysis of the clinical characteristics of the patients in which the *in vitro* tests were performed did not show clinical differences compared to those cases in which the tests were not carried out. In our study, BAT sensitivity ranges from 41% using CD63 as activation marker to 50% using CD203c. Results for CD63 are similar to those observed in previous studies [38], although results observed with CD203c are lower. These differences could be due to the different populations analyzed in both studies. In Uyttebroek study [38] all patients were diagnosed by STs, while in our case 52% of patients were diagnosed by DPT (the gold standard), 39% of patients were diagnosed by STs, and 9% by clinical history. Our study showed that, as expression of both markers, CD63 and CD203c, the sensitivity increased up to 67%, same results that were reported in Uyttebroek study using only CD203c [38]. Although we are aware that these sensitivity values are not optimal, we found that the specificity was 100%,

leading to a very high positive predictive value of the test (100%). On the contrary, negative predictive value ranged from 40% to 60%, depending on the activation marker used, being BAT performed only with CD63 the one that obtained the best results. These results confirm that a patient with a positive BAT to cefazolin has a high probability of being allergic to this drug, whilst a negative result cannot be used to rule out allergy. The results of BAT in patients with negative STs are very interesting. BAT was positive in 7 (64%) out of 11 patients with negative STs, 8 of them were diagnosed by DPT and 3 by clinical history due to the development of life threatening reactions. In these patients, BAT could be useful to avoid a risky procedure such as DPT, as it has been observed for other antibiotics as amoxicillin and clavulanic acid [48], or other drugs such as proton pump inhibitors [59]. However, in the other two patients with negative STs that were diagnosed by clinical history due to the development of life threatening reactions (anaphylactic shock), BAT with both markers was negative. Further research is needed to find out if these reactions could be driven by other mechanisms in which basophils and/or IgE are not involved [60]. We did not find a decrease in positive BAT results related to the period of time between the reaction occurrence and the test performance, probably because most of the patients were studied within the first 6 months after the reaction.

On the other hand, sIgE determination by RAST has shown a very low sensitivity (3.4%) to diagnose cefazolin allergic patients, with only one positive result out of 29 confirmed patients in whom it was performed. As far as we are concerned, only a couple of previous studies had carried out cefazolin-sIgE determination. RAST was used for evaluation of two patients with allergic reactions to cefazolin, in which specific anti-cefazolin IgE was found only in one of them [61]. More recently another study employed the cefazolin ImmunoCAP, for research purposes only, for evaluating 43 allergic and 30 non-allergic whose levels of cefazolin-sIgE resulted in no significant differences between patients and controls. However, sIgE/tIgE ratio revealed a cutoff ratio of 1.42×10^{-3} to be most discriminative between patients and control individuals, showing a sensitivity of 49% and a specificity of 94% [62]. The infrequently reported *in vitro* IgE detection

to cefazolin is probably due to the lack of standardized methods and the inconvenience of manipulating radioactive materials for RAST, as commercial straightforward immunoassays are not available. An effective methodology/system to detect sIgE implies the inclusion of the antigenic determinants of the drug, which is impeded by the lack of knowledge of these structures [63].

Summarizing, we found a high incidence of allergic patients to cefazolin in those reporting reactions after this antibiotic administration, being often severe immediate-type, with a significant proportion of anaphylactic shocks and anaphylaxis. STs allowed diagnosing half of the patients when using cefazolin at 20 mg/ml. Unfortunately, DPT could not be performed in all patients due to the severity of reactions, making BAT a promising complementary diagnostic tool. However, further researches are needed in order to clarify the underlying mechanisms of these reactions, especially in patients with severe reactions and negative STs and BAT.

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TABLES

Table 1.- Comparisons of patients with confirmed allergic and non-allergic reactions to cefazolin.

	Allergic to cefazolin	Non-allergic to cefazolin	P value
N	76	90	NA
Age (median, SD, years old)	49.4±16.8	50.2±14.9	0.659
Gender (N, % of males)	28 (36.8)	31 (34.4)	0.748
Type of reaction (N, %)			<0.001
Immediate	76 (100)	64 (71.1)	
Non-Immediate	0 (0)	26 (28.9)	
Symptoms manifested in reaction (N, %)			<0.001
Anaphylactic shock	12 (15.8)	2 (2.2)	
Anaphylaxis	28 (36.8)	17 (18.8)	
Urticaria	30 (39.5)	41 (45.6)	
Angioedema	0 (0)	3 (3.3)	
Urticaria/angioedema	6 (7.9)	4 (4.4)	
Exanthema	0 (0)	18 (20)	
Generalised pruritus	0 (0)	5 (5.6)	
Interval between the first drug administration and the onset of the reaction (mean±SD, min)	20.01±17.8	285.29±721.6	0.001
Interval time reaction - allergological study (mean± SD, months)	12.41±29.5	46.67±85.7	0.001

Table 2.- Clinical characteristics of the cefazolin allergic patients diagnosed by clinical history. BAT results are expressed as stimulation index and RAST results as %. Positive results are highlighted in bold.

Pat	Age	Sex	Interval R-S (months)	Interval D-R (min)	Severity grade [45]	Clinical Symptoms	Risk stratification	BAT		RAST
								CD63	CD203c	
116	69	M	9	10	2	Anaphylaxis	Cardio-vascular contraindications	ND	ND	ND
117	20	F	5	5	3	Anaphylactic shock	2 episodes with cefazolin and cefadrine	ND	ND	ND
112	45	F	7	30	2	Anaphylaxis	Cardio-vascular contraindications	ND	ND	ND
113	39	F	72	5	3	Anaphylactic shock	Severe reaction	ND	ND	ND
175	67	M	4	5	3	Anaphylactic shock	Severe reaction	14.53	2.49	1.3
176	24	F	9	15	3	Anaphylactic shock	Severe reaction	ND	ND	ND
177	58	F	9	20	3	Anaphylactic shock	Severe reaction	0.54	1.01	ND
178	79	F	3	25	3	Anaphylactic shock	Severe reaction	0.9	1.51	0.8
179	48	F	3	2	3	Anaphylactic shock	Severe reaction	ND	ND	1.2
180	62	M	1	30	3	Anaphylactic shock	Severe reaction	ND	ND	0
181	18	M	3	15	3	Anaphylactic shock	Severe reaction	ND	ND	0.7
182	30	F	15	30	3	Anaphylactic shock	Severe reaction	ND	ND	ND
183	38	F	1	30	3	Anaphylactic shock	Severe reaction	2.49	ND	1.4
184	73	F	6	20	3	Anaphylactic shock	Severe reaction	ND	ND	ND

Pat: Patient identification. F: Female. Interval R-S: Interval between the reaction and the study. Interval D-R: Interval between the first drug administration and the onset of the reaction. M: Male. ND: Not done.

416 **Table 3.-** Clinical characteristics of patients with positive ST results

Pat	Age	Sex	Severity grade [45]	Symptoms	Interval R-S (months)	Interval D-R (min)	Skin Test (Prick/ID)			
							CEF	PPL or BP-OL	MDM or MD	AX
134	30	F	1	Urticaria	72	30	-/+	-/-	-/-	-/-
135	51	M	2	Anaphylaxis	1	5	-/+	-/-	-/-	-/-
136	48	F	1	Urticaria	180	2	-/+	-/-	-/-	-/-
137	55	M	1	Urticaria	2	5	-/+	-/-	-/-	-/-
138	43	F	2	Anaphylaxis	8	50	-/+	-/-	-/-	-/-
139	78	F	1	Urticaria	3	1	-/+	-/-	-/-	-/-
140	53	F	1	Urticaria	2	5	-/+	-/-	-/-	-/-
141	50	F	2	Anaphylaxis	2	10	-/+	-/-	-/-	-/-
142	53	F	1	Urticaria	10	5	-/+	-/-	-/-	-/-
143	80	F	2	Anaphylaxis	2	25	-/+	-/-	-/-	-/-
144	70	M	1	Urticaria	4	1	-/+	-/-	-/-	-/-
145	35	M	1	Urticaria	5	15	-/+	-/-	-/-	-/-
146	70	F	2	Anaphylaxis	4	5	-/+	-/-	-/-	-/-
147	62	F	1	Urticaria	144	15	-/+	-/-	-/-	-/-
148	37	M	1	Urticaria	4	25	-/+	-/-	-/-	-/-
149	76	M	2	Anafilaxia	1	30	-/+	-/-	-/-	-/-
150	75	F	1	Urticaria/Angioedema	2	30	-/+	-/-	-/-	-/-
151	65	F	2	Anaphylaxis	2	45	-/+	-/-	-/-	-/-
152	20	M	1	Urticaria	10	15	-/+	-/-	-/-	-/-
153	38	F	1	Urticaria/Angioedema	13	35	-/+	-/-	-/-	-/-
154	49	F	1	Urticaria/Angioedema	2	60	-/+	-/-	-/-	-/-
155	28	F	1	Urticaria	11	2	-/+	-/-	-/-	-/-
156	38	M	2	Anaphylaxis	1	5	-/+	-/-	-/-	-/-
157	35	F	1	Urticaria	24	60	-/+	-/-	-/-	-/-
158	59	F	2	Anaphylaxis	1	3	-/+	-/-	-/-	-/-
159	62	F	1	Urticaria	24	60	-/+	-/-	-/-	-/-
160	74	M	2	Anaphylaxis	2	10	-/+	-/-	-/-	-/-
161	43	F	2	Anaphylaxis	2	5	-/+	-/-	-/-	-/-
162	59	F	2	Anaphylaxis	1	20	-/+	-/-	-/-	-/-
163	51	M	2	Anaphylaxis	3	2	-/+	-/-	-/-	-/-
164	35	F	1	Urticaria/Angioedema	6	5	-/+	-/-	-/-	-/-
165	22	M	2	Anaphylaxis	1	5	-/+	-/-	-/-	-/-
166	69	M	2	Anaphylaxis	9	30	-/+	-/-	-/-	-/-
167	74	F	1	Urticaria	6	15	-/+	-/-	-/-	-/-
169	40	F	1	Urticaria	9	15	+/-ND	-/-	-/-	-/-
170	66	M	2	Anaphylaxis	4	10	+/-ND	-/-	-/-	-/-
171	34	M	2	Anaphylaxis	2	35	+/-ND	-/-	-/-	-/-
172	16	M	1	Urticaria	3	5	+/-ND	-/-	-/-	-/-

173	44	M	2	Anaphylaxis	1	15	-/+	-/-	-/-	-/-
174	15	M	1	Urticaria	10	45	+ /ND	-/-	-/-	-/-

417 Pat: Patient identification. Interval R-S: Interval between the reaction and the study. Interval D-
418 R: Interval between the first drug administration and the onset of the reaction. CEF: Cefazolin.
419 PPL: benzylpenicilloyl-octa-L-lysine/benzylpenicilloyl-poly-L-lysine. BP-OL: benzylpenicilloyl-
420 octa-L-lysine (BP-OL). MDM: minor determinant mixture. MD: Minor determinant. AX:
421 amoxicillin. ND: Not done.

Table 4. Comparison of clinical data of original reaction and during DPT with intravenous cefazolin.

Pat	Age	Sex	Route	Original reaction			DPT			
				Severity grade (45)	Symptoms	Interval R-S (months)	Interval D-R (min)	TCD (mg)	Symptoms	Interval first DPT D-R (min)
91	32	F	IV	2	Anaphylaxis	2	15	50	Generalized erythema and pruritus and dyspnea	20
92	46	F	IV	1	Urticaria/Angioedema	1	10	150	Urticaria	45
93	56	M	IV	2	Anaphylaxis	12	30	300	Urticaria	70
94	50	F	IV	1	Urticaria	1	30	500	Urticaria	110
95	60	F	IV	2	Anaphylaxis	2	20	150	Palm and sole pruritus and sore throat	35
114	59	M	IV	1	Urticaria	4	50	50	Urticaria/angioedema	30
119	54	F	IV	1	Urticaria	6	5	300	Urticaria	80
120	58	M	IV	2	Anaphylaxis	1	5	300	Generalized erythema and pruritus	65
121	73	M	IV	1	Urticaria	6	15	300	Urticaria	15
122	34	F	IV	2	Anaphylaxis	7	20	50	Generalized erythema, isolated urticaria, and dyspnea	10
123	44	F	IV	1	Urticaria	3	60	1000	Urticaria	180
124	42	F	IV	1	Urticaria	3	20	300	Urticaria	90
125	40	M	IV	1	Urticaria	0.2	2	150	Urticaria	50
126	19	F	IV	2	Anaphylaxis	1	5	300	Urticaria	90
127	51	F	IV	2	Anaphylaxis	3	60	300	Urticaria	85
128	55	F	IV	1	Urticaria	72	10	300	Urticaria	100
129	50	F	IV	1	Urticaria/Angioedema	3	5	150	Urticaria	45
130	57	F	IV	1	Urticaria	1	60	300	Urticaria	110
131	53	F	IV	2	Anaphylaxis	1	60	500	Urticaria	120

132	42	F	IV	1	Urticaria	72	30	300	Urticaria	80
133	61	M	IV	1	Urticaria	2	10	150	Urticaria	55
168	49	M	IV	1	Urticaria	1	15	500	Urticaria	90

Pat: Patient identification. Interval R-S: Interval between the reaction and the study. Interval D-R: Interval between the first drug administration and the onset of the reaction. TCD: Total cumulative dose. DPT: Drug provocation test. Interval first DPT D-R: Interval between the drug administration and the onset of the reaction during the DPT.

LEGENDS TO FIGURES

Figure 1. Flow-chart for diagnosis of patients. BAT: Basophil activation test. RAST: Radioallergosorbent test.

Figure 2. A) Number of patients with confirmed allergy and non-allergy to cefazolin, and not confirmed subjects. B) Percentage of patients studied in different time-periods and finally diagnosed as allergic and non-allergic, or not confirmed subjects. Evaluation years were grouped in 5-year periods.

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Figure 1.

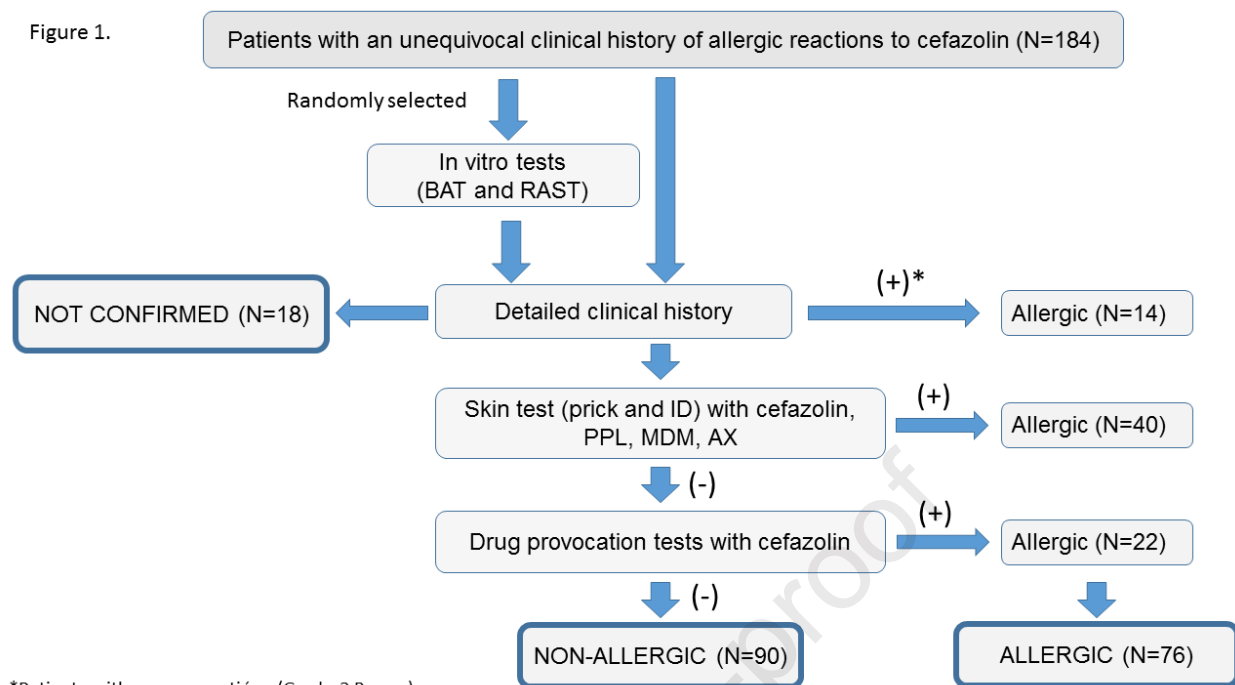
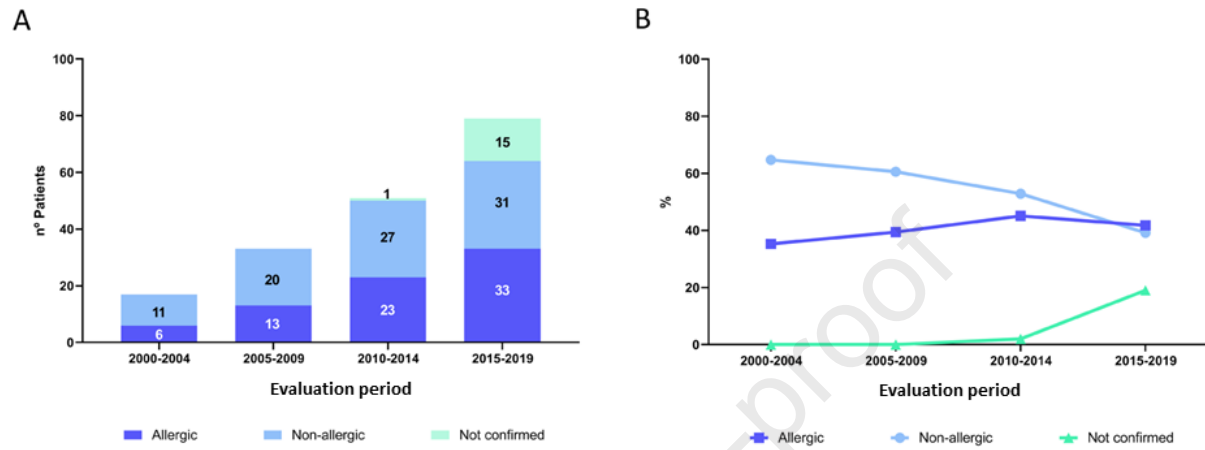
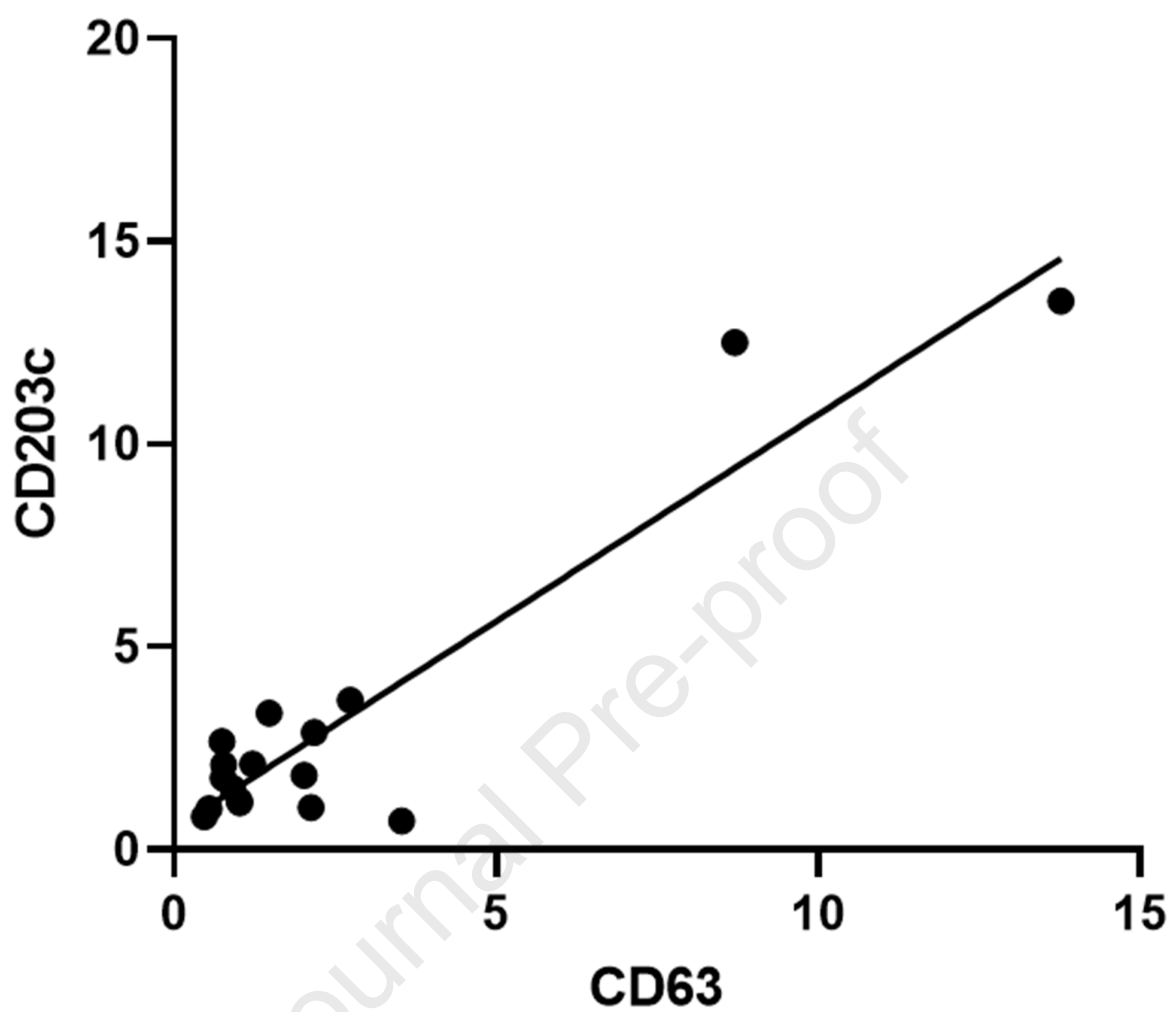


Figure 2.





Online Repository Text

TITLE: Diagnostic approach of hypersensitivity reactions to cefazolin in a large prospective cohort.

SHORT TITLE: Cefazolin hypersensitivity diagnosis

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35 **Supplemental Material**

36 **METHODS**

37 ***Patients***

38 We evaluated patients older than 14 years old with unequivocal suspicion of allergic reactions
39 to cefazolin. Reactions were classified into IRs or NIRs, if the symptoms appeared within one
40 hour after cefazolin administration or later [1]. IRs severity was graded as: grade 1 (mild: skin
41 and subcutaneous tissues); grade 2 (moderate: features suggesting respiratory, cardiovascular,
42 or gastrointestinal involvement); grade 3 (severe: hypoxia, hypotension, or neurologic
43 compromise) [2].

44 The allergological workup [3] started by carrying out an exhaustive clinical history, performed
45 according to the EAACI questionnaire [4], followed by STs, and, if negative, by a DPT with
46 cefazolin. STs and DPT with cefazolin were not performed in severe reactions (grade 3) [2] as
47 well as in patients with severe or uncontrolled pulmonary or cardiovascular diseases [5, 6].

48 None of the included patients in our study had dermatographism or any other disease that
49 might interfere with the assessment of the response to STs. Moreover, all included patients
50 underwent blood tests including hemogram, biochemistry with glucose, hepatic and renal
51 profile, autoimmunity, immunoglobulins and tryptase, in order to exclude other pathologies
52 that could induce symptoms that could confuse the diagnosis.

53

54 ***Skin test***

55 Skin prick test (SPT) and, if negative, intradermal test (IDT) were carried out as previously
56 described [3], using daily prepared solutions. The reagents were: benzylpenicilloyl-poly-L-lysine
57 (PPL, DAP, Diater, Leganés, Spain) at $1.07 \cdot 10^{-2}$ mol/L; minor determinant mixture (MDM:

benzylpenicillin, benzylpenicilloate, and benzylpenilloate) at 1.5 mol/L; amoxicillin (AX) (Diater laboratories, Madrid, Spain), and cefazolin (Reig Jofre Laboratorio SA, Barcelona, Spain) at 2 and 20 mg/ml in patients reporting only skin symptoms and 0.2, 2 and 20 mg/ml in patients reporting anaphylaxis. Since May 2011, the reagents composition of Diagnosis of Allergy to Penicillin (DAP) has changed and includes the major determinant benzylpenicilloyl-octa-L-lysine (BP-OL) at 0.04 mg/ml, equivalent to an $8.64 \cdot 10^{-5}$ molar (M) concentration of the benzylpenicilloyl (BPO) moiety, and the minor determinant (MD) at 0.5 mg/ml, equivalent to a concentration of $1.5 \cdot 10^{-3}$ M of sodium benzylpenilloate.

Readings were done after 20 minutes and considered positive: (i) in SPT, if a wheal larger than 3 mm surrounded by erythema appeared, with a negative response to the control saline; (ii) in IDT, if the diameter of the wheal area that was marked initially increased more than 3 mm and it was surrounded by erythema. Positive data are expressed as the mean diameter recorded by measuring the largest and the smallest diameters at angles right to each other [5].

Drug Provocation Test

In subjects with negative STs to PPL/BP-OL, MDM/MD, AX, and cefazolin, DPT with cefazolin (Reig Jofre) was performed by intravenous route at incremental dose (50, 100, 150, 200, 500 mg) with a minimum 30-min interval between each, reaching the total cumulative dose of 1000 mg. Patients were monitored during DPT procedures and for 2 hours after the last dose of DPT. Complete equipment for cardiopulmonary resuscitation was immediately available [6].

Basophil activation test

BAT was performed as previously described [7] with cefazolin (Reig Jofre Laboratorio SA, Barcelona, Spain) at 1.25 and 0.25 mg/ml, using as positive controls fMLP (4 μ M) and anti-IgE (0.05 mg/ml). Cells were incubated with stimulation buffer containing IL-3 (2ng/mL), stained with monoclonal antibodies, anti-CCR3-APC, CD63-FITC, and CD203c-PE, (Caltag Laboratories, Burlingame, CA) and acquired in a FACSCalibur flow cytometer (Becton-Dickinson Bioscience,

San Jose, CA) by obtaining at least 500-1000 basophils per sample, selected as $SSC^{low}CCR3^{+}$ cells. Results were analyzed using FlowJo® software (FlowJo LLC, Becton Dickinson, Ashland, OR), and activation was expressed as percentage of basophils expressing CD63 or percentage of basophils with increased expression of CD203c and as stimulation index (SI). SI was calculated as the ratio between the percentage of activated basophils ($CD63^{+}$ or $CD203c^{+}$ cells) in samples stimulated with cefazolin and in the unstimulated sample. The percentage of spontaneously activated basophils was required to be $>2.5\%$ to calculate the SI. Following previous publications about the use of BAT for betalactam allergy diagnosis [8, 9], it was considered positive with a SI greater than 2 for at least one of the tested concentrations of cefazolin. The same criterion was followed for both activation markers, CD63 and CD203c.

In vitro specific IgE determination

In vitro IgE determination was performed by RAST, using cefazolin (Reig Jofre Laboratorio SA, Barcelona, Spain) conjugated to Poly-L-Lysine (PLL) (Sigma, St. Louis, MO) (cefazolin-PLL) coupled to the cellulose solid phase, as described [10, 11]. Briefly, 30 μ L of the patient's sera was incubated with cefazolin-PLL solid phases. After washing, radiolabeled anti-IgE antibody (kindly provided by Thermo Fisher Scientific and radiolabelled in our laboratory) [12] was added and the mixture was incubated overnight. The solid phases were then washed and their radioactivity measured in a gamma counter, Multi Crystal LB 2111 (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany) with GMS LBIS gamma c-12 software. Results were calculated as percentage of the maximum counts. Samples were considered positive if they were higher than 2.5% of label uptake, which was the mean + 2 standard deviation of a negative control group.

Statistical analysis

Description of quantitative variable was performed including the median, mean, standard deviation, and interquartile range. Differences between qualitative variables were analyzed by

Chi-square test (no-related samples) and McNemar test (related samples). Comparisons between quantitative variables with normal/no-normal distribution were performed by Student t test or Mann-Whitney U test (no-related samples), and by Student t test or Wilcoxon test (related samples) respectively. All statistical analyses were carried out using the software package GraphPad PRISM v7. A value of $p < 0.05$ was considered statistically significant.

Table E1. Clinical characteristics of patients confirmed to be allergic to cefazolin in whom BAT was performed. BAT results are expressed as stimulation index. Positive results are highlighted in bold.

Pat	Age	Sex	Severity grade (45)	Symptoms	Interval R-S (months)	Interval D-R (min)	Diagnosed by	BAT	
								CD63	CD203c
91	32	F	2	ANA	2	15	DPT	8.70	12.50
92	46	F	1	URT/ANG	1	10	DPT	0.76	2.10
93	56	M	2	ANA	12	30	DPT	1.21	2.11
94	50	F	1	URT	1	30	DPT	3.53	0.71
95	60	F	2	ANA	2	20	DPT	1.47	3.36
129	50	F	1	URT/ANG	3	5	DPT	1.01	1.16
130	57	F	1	URT	1	60	DPT	1.00	1.21
132	42	F	1	URT	72	30	DPT	2.73	3.68
148	37	M	1	URT	4	25	ST	0.47	ND
151	65	F	2	ANA	2	45	ST	0.95	ND
152	20	M	1	URT	10	15	ST	0.75	1.76
158	59	F	2	ANA	1	3	ST	0.46	0.81
161	43	F	2	ANA	2	5	ST	2.17	2.89
162	59	F	2	ANA	1	20	ST	0.13	ND
163	51	M	2	ANA	3	2	ST	2.01	1.83
164	35	F	1	URT/ANG	6	5	ST	0.74	2.66
165	22	M	2	ANA	1	5	ST	2.12	1.04
170	66	M	2	ANA	4	10	ST	4.49	ND
171	34	M	2	ANA	2	35	ST	13.77	13.52
175	67	M	3	AS	4	5	CH	14.53	2.49
177	58	F	3	AS	9	20	CH	0.54	1.01
178	79	F	3	AS	3	25	CH	0.90	1.51
183	38	F	3	AS	1	30	CH	2.49	ND

Pat: Patient identification. Interval R-S: Interval between the reaction and the study. Interval D-R: Interval between the first drug administration and the onset of the reaction. BAT: basophil activation test. ANA: anaphylaxis. URT: urticaria. ANG: angioedema. AS: anaphylactic shock. DPT: drug provocation test. ST: skin test. CH: clinical history. ND: Not done.

Table E2. Clinical characteristics of patients confirmed to be allergic to cefazolin in whom RAST was performed. RAST results are expressed as %. Positive results are highlighted in bold.

Pat	Age	Sex	Severity grade (45)	Symptoms	Interval R-S (months)	Interval D-R (min)	Diagnosed by	BAT		RAST
								CD63	CD203c	
91	32	F	2	ANA	2	15	DPT	8.70	12.50	1.4
92	46	F	1	URT/ANG	1	10	DPT	0.76	2.10	0.8
95	60	F	2	ANA	2	20	DPT	1.47	3.36	1.4
114	59	M	1	URT	4	50	DPT	ND	ND	1.57
125	40	M	1	URT	0.2	2	DPT	ND	ND	1.6
126	19	F	2	ANA	1	5	DPT	ND	ND	1.36
127	51	F	2	ANA	3	60	DPT	ND	ND	1.45
128	55	F	1	URT	72	10	DPT	ND	ND	1.5
129	50	F	1	URT/ANG	3	5	DPT	1.01	1.16	0
148	37	M	1	URT	4	25	ST	0.47	ND	1.4
151	65	F	2	ANA	2	45	ST	0.95	ND	1.6
152	20	M	1	URT	10	15	ST	0.75	1.76	1.3
154	49	F	1	URT/ANG	2	60	ST	ND	ND	2.38
155	28	F	1	URT	11	2	ST	ND	ND	1.36
156	38	M	2	ANA	1	5	ST	ND	ND	1.1
158	59	F	2	ANA	1	3	ST	0.46	0.81	1.29
159	62	F	1	URT	24	60	ST	ND	ND	0
160	74	M	2	ANA	2	10	ST	ND	ND	1.13
161	43	F	2	ANA	2	5	ST	2.17	2.89	1.09
162	59	F	2	ANA	1	20	ST	0.13	ND	1.26
167	74	F	1	URT	6	15	ST	ND	ND	1.39
168	49	M	1	URT	1	15	DPT	ND	ND	1
172	16	M	1	URT	3	5	ST	ND	ND	9.1
174	15	M	1	URT	10	45	ST	ND	ND	0.8
175	67	M	3	AS	4	5	CH	14.53	2.49	1.3
178	79	F	3	AS	3	25	CH	0.90	1.51	0.8
179	48	F	3	AS	3	2	CH	ND	ND	1.2
180	62	M	3	AS	1	30	CH	ND	ND	0
181	18	M	3	AS	3	15	CH	ND	ND	0.7
183	38	F	3	AS	1	30	CH	2.49	ND	1.4

Pat: Patient identification. Interval R-S: Interval between the reaction and the study. Interval D-R: Interval between the first drug administration and the onset of the reaction. RAST: radioallergosorbent test. ANA: anaphylaxis. URT: urticaria. ANG: angioedema. AS: anaphylactic shock. DPT: drug provocation test. ST: skin test. CH: clinical history.

Legend for Figure E1. Correlation of both stimulation indexes in BAT using CD63 and CD203c activation markers. Data are expressed as stimulation index.

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