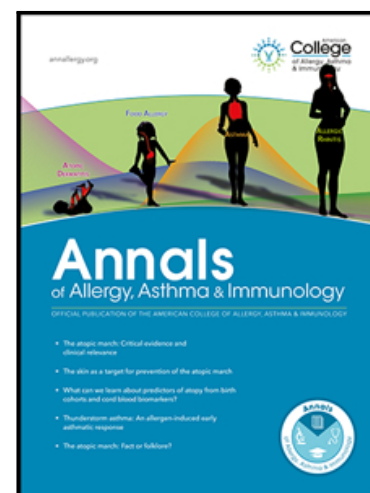


Prospective assessment of diagnostic tests for pediatric penicillin allergy, from clinical history to challenge tests☆☆☆☆



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Title:**Prospective assessment of diagnostic tests for pediatric penicillin allergy, from clinical history to challenge tests.**

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Abbreviations used:

Adverse drug reaction (ADR), amoxicillin (AX), amoxicillin-clavulanic acid (AX-C), penicilloyl polylysine (PPL), drug provocation test (DPT), intradermal test (ID), index reaction (IR), minor determinant mixture (MDM), penicillin (PNC), serum specific IgE (sIgE), skin prick test (SPT), penicillin G (PNC-G), penicillin V (PNC-V), versus (vs).

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Abstract

Background: Diagnostic guidelines for penicillin allergy in children recommend cumbersome protocols based partially on data from adults, which may be suboptimal for pediatric use.

Objective: To assess the accuracy of tools for diagnosis of penicillin allergy in children.

Methods: A prospective multicenter study was conducted in children with reported adverse events related to penicillin, excluding severe reactions. All patients underwent a uniform diagnostic protocol consisting of clinical history, skin tests, serum specific IgE, and, regardless of these results, drug provocation tests (DPT).

Results: 732 children (mean 5.5 years; 51.2% males) completed the allergy work-up, including DPT. Amoxicillin triggered 96.9% of all reactions. None of the patients with an immediate index reaction (IR) developed a reaction on DPT. Penicillin allergy was confirmed in 35 subjects (4.8%): 6(17%) immediate and 29(83%) non-immediate reactions in the DPT. No severe reactions were recorded. The allergist diagnosis based upon the clinical history was not associated with the final outcome at DPT. In 30 of 33(91%) allergic patients, all skin tests and sIgE were negative. A logistic regression model identified the following to be associated with

PNC allergy ($p<0.05$): a family history of drug allergy (OR=3.03; 95% confidence interval (CI): 1.33-6.89), an IR lasting >3 days vs ≤ 24 hours (OR=8.96; 95% CI: 2.01-39.86), and IR while receiving corticosteroids (OR=2.68; 95% CI: 1.30-5.54).

Conclusion: Conventional predictors of allergy to penicillin performed weakly. The authors propose straightforward penicillin provocation testing in controlled, experienced centers for the diagnosis of non-severe penicillin allergy in children.

INTRODUCTION

Beta-lactam antibiotics, especially aminopenicillin (PNC), are the most commonly involved drugs in adverse drug reactions in children¹. Though a remarkable number of children treated with beta-lactams develop maculopapular exanthema or urticaria after exposure^{2,3}, confirmed drug allergy is less frequent than in adults^{4,5}.

In clinical practice, the diagnostic approach taken with these patients is highly inconsistent despite efforts from the scientific community^{6, 7, 8, 9} to create clear-cut algorithms. Usually, skin prick test (SPT), intradermal test (ID), or serum specific IgE (sIgE) to PNC, amoxicillin (AX), and/or its reagents must be negative for a drug provocation test (DPT)—the gold standard for PNC-allergy diagnosis—to be performed^{8, 10, 11}, even in non-immediate reactions¹².

Mainly due to the scarcity of studies carried out in children, the same guidelines used for diagnosis of drug allergy in adults are generally applied to the pediatric population. Contrary to the aforementioned recommendations, recently published evidence suggests the possibility of performing DPT without prior skin/serum testing, though only in children showing non-severe delayed-onset urticarial or maculopapular rashes due to penicillin^{2, 6, 13}.

To our knowledge, no prospective studies with a uniform diagnostic protocol for PNC allergy have been conducted to determine the effectiveness of the most frequently used diagnostic procedures in both immediate and non-immediate index reaction (IR: the drug-related event that is the focus of the current study) compared against the results of DPT. The main objective of this study was to determine the efficacy of available diagnostic tools in PNC allergy relative to the results of DPT. The tools used for comparison were clinical history, skin testing (SPT and ID) and sIgE to PNC and its determinants. The secondary objectives were i) to describe the

characteristics of IR to PNC; ii) to determine the PNC most frequently involved; iii) to assess differences between PNC-allergic and non-allergic patients; iv) to assess differences between immediate and non-immediate reactions; and v) to study tolerance to cefuroxime in case PNC allergy was confirmed. This study may mark a step toward optimizing PNC-allergy diagnosis in the pediatric population.

METHODS

Study design

A task force to study PNC-allergy diagnosis was created in the Children's Allergy Committee of the Spanish Society of Allergy and Clinical Immunology (SEAIC) and a prospective multicenter study to be conducted in Spanish departments of allergy was launched.

Ethics

The study was approved by the ethics committees of each hospital and the Spanish Agency for Drug and Health Products (AEMPS). Written informed consent was signed by the parents or guardians and was obtained from children over 12 years of age.

Participants

Consecutive patients 14 years old or younger presenting to participating allergy departments and who reported an adverse drug reaction (ADR) with benzylpenicillin (PNC-G), phenoxymethylpenicillin (PNC-V), amoxicillin-clavulanic acid (AX-C), or cloxacillin and who had given their written consent were included. Patients were excluded in case of clinical history of severe skin conditions during the IR, presence of skin diseases not allowing skin testing, and/or any condition contraindicating adrenaline use. Anaphylaxis was not an exclusion criterion, except in case of severe reaction (defined as involving hypoxia, hypotension, or neurologic compromise).

Allergy work-up

A uniform protocol was used, including a detailed clinical history, where IRs were classified as immediate if they had developed during the first hour after the intake of the first dose or as non-immediate in all other cases⁶. After gathering the clinical history, the clinical researcher was asked whether the IR was, in their expert opinion, a “clear-positive” case of allergy, a “doubtful-case”, or a “clear-negative” case of allergy, in order to assess the accuracy of the clinical history alone when diagnosing PNC allergy. Afterwards, every patient underwent SPT and ID in accordance with Torres et al¹⁴, sIgE determinations using 2 different thresholds for positiveness (0.35k/L^{10, 11} and also 0.1kU/L, as reported elsewhere²), and DPT with the culprit PNC was performed following the ENDA/EAACI recommendations¹⁵ regardless of the results of the aforementioned tests. If DPT with the culprit PNC was positive, cefuroxime was then tested in a different DPT. Data were recorded on an online platform. For detailed information on the methods, see **Supplementary material file 1**.

Statistics

Chi-square test and Fisher exact test were used to compare qualitative variables between allergic and non-allergic children as well as for immediate and non-immediate reactions. Due to the theoretically better performance of diagnostic tests when the IR has occurred in the previous year¹⁶, a subanalysis for this population was carried out. The odds ratio (OR) [95% confidence interval (CI)] was calculated in univariate and multivariate analyses to assess potential factors associated with PNC allergy. It was estimated that 707 patients would provide sufficient power to find statistical differences based on the prevalence given by Caubet² (**Supplementary file 1**). An estimation of the cases of hypothetical misdiagnosis was performed if clinical guidelines had been strictly followed^{6,14}.

RESULTS:

Participants

A total of 67 allergists volunteered to participate, though only 38 (56.7%), from 23 centers, actively participated. Of the 766 patients included, 732 (95.6%) were given a DPT-based final diagnosis (**Figure 1 and Supplementary file 2**) and 34 were lost to follow-up. Thirty-five (4.8%) were finally diagnosed with PNC allergy, 6 (17% of PNC-allergic patients) with immediate reactions, and 29 (83% of PNC-allergic subjects) with non-immediate reactions (**Table 1**). Males comprised 51.2% (n=375) of the population, and mean age at the time of the study was 5.5 years ($\pm$ SD 3.87), and 3.9 at IR ($\pm$ SD 3.1). Family history of drug allergy was associated with a higher risk of PNC allergy (OR=2.56; 95% CI: 1.16–5.65, p=0.016).

Culprit drug

Considering only patients with a final diagnosis of PNC allergy (n=732), AX, either alone (489, 66.8%) or along with clavulanic acid (220, 30.1%), was the most frequent trigger of IR (**Table 1**). The reaction took place on first use by the patient in 31.7% (175) of cases. AX was the only drug that allergic patients had used more often than non-allergic patients prior to the event (25.7% vs 13.8%, p=0.049) (**Table 1**).

Index reaction characteristics

The most frequent cause of antibiotic administration was tonsillitis (268, 36.6%). Immediate IR represented 5.2% (38) of all reactions, and none of these patients was proven to be PNC-allergic after the study. The mean number of doses eliciting the IR was significantly higher ($15 \pm$ SD 6.2) in allergic patients than in non-allergic patients ($10 \pm$ 8.2 SD) (p = 0.016).

The most frequently reported symptom was exanthema (411, 56.1%) and was associated with non-immediate IR (58.4% vs 30% immediate reaction, p<0.0001). Urticaria was the second most common symptom (286, 39.1%), and was associated with immediate reaction (55.5% vs 37.6% non-immediate reaction, p=0.031). The appearance of angioedema increased the risk of

true PNC allergy (OR= 2.44; 95% CI: 1.13–5.24, $p=0.018$). Seven patients reported mild dyspnea (none were allergic) and 6, joint involvement (one of these found to be allergic after the study). None of the reported IRs was severe.

Local reactions occurred more frequently in immediate IRs than non-immediate IRs (34% vs 14.3%, respectively, $p=0.012$), though this factor was not associated with the final diagnosis. Immediate IRs were associated with earlier resolution, as 57.5% immediate vs 21.2% non-immediate IRs resolved within the first 24 hours ($p<0.0001$).

Index reaction treatment

More than half of the patients (457, 62.4%) received some form of treatment, and 416 (56.8%) reported to the emergency room (ER) for the IR (**Table 1**). The following variables were associated with a higher risk of being PNC allergic: receiving medical care in the ER (OR= 2.67; 95% CI: 1.19–5.96, $p=0.013$), receiving antihistamines (OR= 2.30; 95% CI: 1.06–4.98, $p=0.030$), and receiving corticosteroids (OR= 2.77; 95% CI: 1.37–5.59, $p=0.003$). ER attendance during the IR was significantly higher in patients diagnosed with immediate PNC allergy than in those diagnosed with non-immediate PNC allergy. Among PNC-allergic patients, both antihistamines and corticosteroids were used more frequently in non-immediate than in immediate DPTs ($p=0.04$ and $p=0.004$, respectively) (**Table1**). Here, it should be noted that adrenaline was used only once for an IR, in a patient who was later proven not to be PNC-allergic.

Allergy work-up

Allergy work-up was completed in 732 patients (**Figure 1**). The median elapsed time between the IR and the time when the patient presented to the allergy clinic was 95 days (PC25–PC75: 44–490 days). In 492 (67.2%) patients the reaction had occurred in the year prior to the allergy work-up.

Initial Judgment of the attending physician

Thirty-one (4.2%) cases were judged by the clinical researcher as “clear positive”, 518 (70.8%) as “clear-negative”, and 183 (25%) as “doubtful cases” of penicillin allergy. None of the clear-positive cases turned out to be real PNC-allergic patients; 23 cases of those classified as clearly negative and 12 of the so-called doubtful cases were true PNC-allergic subjects. This prediction was clearly influenced by the IR temporality, with significantly more cases of IR suspected to be “clear positive” in patients with immediate IR than in non-immediate IR (26.5% vs 3.4% respectively, $p < 0.0001$).

Skin tests

Only one positive SPT was recorded for PPL, and none for MDM, PNC-G, PNC-V, AX, or AX-C. The ID was found to be positive more frequently among allergic patients than in non-allergic patients ($p < 0.05$) only for PPL in early reading and PNC-G in late reading (**Table 2**). Overall, sensitivity was low (range 0–20%, any positive 9.1%) and specificity high (range 97–100%, any positive 98.3%) (**Supplementary file 3**).

Serum specific IgE

Differences between allergic patients and non-allergic patients were significant at the 0.1 kU/L threshold for PNC-V and AX; additionally, among allergic individuals, sIgE to AX discriminated between subjects with immediate and non-immediate reactions at DPT (**Table 3**). Using the 0.35 kU/L cut-off, no significant differences were found between the subpopulations studied. Sensitivity was low for all determinants and thresholds (2.9–17%), and specificity was high (94.4–99.9%) (**Supplementary file 3**). The subgroup analysis for the performance of sIgE in the population with IR onset during the year prior to the start of the study ($n=492$) also failed to provide better results (**Supplementary file 4**).

Drug provocation tests

The final number of allergic subjects was 35 (4.8%); table 4 shows the characteristics of each patient diagnosed with PNC allergy. DPTs with the culprit antibiotic were carried out on 950 occasions, of which 40 were considered positive.

All 732 patients underwent a first DPT (DPT1), which was positive in 34 subjects. Five of these subjects underwent a second confirmation DPT, producing negative results in 2 of them. The first challenge elicited 6 immediate and 28 non-immediate reactions (**Figure 1**). The symptoms in DPT1 were urticaria (67.7%, 23), generalized erythema (20.5%, 7), generalized pruritus (20.5%, 7), angioedema (11.7%, 4), localized pruritus (8.8%, 3), feeling unwell (5.8%, 2), vomiting (5.8%, 2), and dyspnea, abdominal pain, nausea, and rhinitis in 1 patient each (2.9%). No severe reactions occurred. Rescue medication consisted of oral antihistamines (88.2%, 30) and oral corticosteroids (35.2%, 12), and intramuscular corticosteroids, intramuscular adrenaline, and salbutamol were given on 1 occasion (2.9%) each (patient 6 adrenaline, and patient 31 salbutamol and corticosteroids, Table 4).

The second DPT with the same drug was performed 218 times and was positive in 6 patients, 3 of whom had a previous positive DPT1, while in the other 3 cases the DPT1 had been previously negative (all these patients with IRs occurring more than 1 year prior). In the positive DPT2, 2 were immediate reactions and 4 non-immediate (**Figure 1**). Urticaria was again the most frequent symptom (83.3%, 5), and all reactions were mild.

Cefuroxime tolerance was evaluated with a DPT in 24 out of 35 PNC-allergic patients, and only 1 patient (4.1%) was found to be allergic to this drug (Patient 26, Table 4).

Potential diagnostic failures

If penicillin-allergy diagnosis had been performed only on the basis of a clinical history and positivity either to any of the serum determinants (using the 0.35 kU/L threshold) or to any of the skin tests, only 9.1% of the allergic patients and 2.2% of non-allergic patients would have received an accurate diagnosis. Overall, applying the above-mentioned criteria, 6.2% of the

studied cohort would have been misdiagnosed (detailed information for potential diagnostic failures for each test appears in **Table 5**).

Multivariate analysis of parameters associated to PNC allergy

In the logistic regression model, the following variables were shown to be associated with PNC allergy: family history of drug allergy (adjusted OR= 3.03; 95% CI: 1.33–6.89, $p=0.008$), IR duration >3 days compared to an IR duration of <24 hours (adjusted OR= 8.96; 95% CI: 2.01–39.86, $p=0.004$), and having received corticosteroids for the IR (adjusted OR= 2.68; 95% CI: 1.30–5.54, $p=0.007$).

DISCUSSION:

In the present prospective multicenter study, PNC-allergy diagnosis based on positive DPT was established in 35 (4.8%) out of 732 patients, providing further support to the mounting evidence that penicillins are very unlikely causes of rashes in pediatric population. At the time of DPT, 6 were immediate and 29 were non-immediate reactions (0.8% and 4% of the total studied population, respectively). As previously described¹⁷⁻¹⁹, amoxicillin (96.9%) was the most frequent penicillin involved in the IR, mirroring its real-life frequency of use in Spain²⁹.

The first step toward diagnosis in drug allergy is a precise clinical history, including a detailed description of the IR. Many authors have identified an increased likelihood of beta-lactam hypersensitivity in immediate IRs^{9,14}. In the present study, none of the IRs reported as immediate occurred in real allergic patients, and 6 out of the 35 allergic individuals with non-immediate IR displayed an immediate reaction on DPT. In addition, none of the 30 patients with a “clear-positive” clinical assessment by the attending allergist prior to the allergy work-up were PNC allergic while 23 considered “clearly negative” were shown to be allergic. This information suggests significant limitations in the reliability of chronology or the “experience” of the attending physician, unless the reaction is observed first-hand by the physician, as previously suggested²¹, challenging the robustness of referred temporality and clinical judgment as the main criterion to be applied in the allergy work-up in each case.

As found in other series^{9,12}, most patients (95%) had maculopapular exanthema or urticaria during the IR, and in 95% of the cases, the IR was non-immediate. Only 38 (5%) of our patients had immediate IR, and all were revealed to be negative on DPT, providing indirect evidence that immediate reactions could also be caused by infections². None of the reported IRs was considered indicative of anaphylaxis by the attending physician despite the presence of 4 events of bronchial and skin symptoms in patients who eventually proved to be non-allergic following DPT. In another noteworthy result, the only case requiring adrenaline in the IR was a non-allergic patient. Data obtained from patient medical histories, such as ER attendance, use of antihistamines during the IR, >3 days of IR duration, having received corticosteroids, and

parental history of drug allergy were associated with PNC allergy diagnosis in the univariate analysis. Only IR duration of >3 days vs <24 hours, the use of corticosteroids for the IR, and, as recently reported also elsewhere²², parental history of drug allergy, were confirmed in the multivariate analysis.

DPT is usually carried out only if preliminary tests are negative. In 3 different large studies with DPT performed only in case of negative skin tests and sIgE, PNC diagnosis was established in 16%⁹, 8%¹¹ and 7.4%¹² of the studied population, respectively. On the other hand, in studies using DPT in every patient, the prevalence of PNC allergy was lower, varying from 3.4% in a population of 119 children developing mild non-immediate reactions (13), to 6% among 818 children with both immediate and non-immediate reactions²², or 5% according to our own data. These data suggest the need to run DPT in every patient to decrease potential overdiagnosis caused by more conservative algorithms.

The differences between these 2 diagnostic approaches to PNC allergy can be attributed to the fact that protocols designed for adults, calling for DPT only if preliminary tests are negative^{7,8,14,23-25}, are applied in children, while other protocols are specifically designed for use in the pediatric population. Although many well-designed studies support the diagnostic accuracy of the aforementioned tests in adults^{7,8,14,23-25}, to date very few studies carried out in children have compared the results of other diagnostic techniques to DPT, the gold standard.

Evidence from different pediatric studies suggests that ID tests perform poorly in this population. In the study by Caubet et al.², 64% of patients (7 out of 11) with PNC non-immediate reactions and positive ID had a negative DPT. Taking a different approach, in their retrospective study of 783 suspected cases of PNC allergy in children, Zambonino et al.¹² found that ID was positive in only 6.5% of 62 PNC-allergic children. Similarly, we also found a high rate of false-positive results (80%) when any ID was positive; on the other hand, just 9.1% (1 immediate and 2 non-immediate DPT) of our PNC-allergic patients were correctly detected by a

positive ID. Only PPL and PN-G ID tests in early and late readings, respectively, were more frequently positive among allergic subjects ($p < 0.05$) than in non-allergic subjects.

Serum sIgE has been specially recommended for children under 6 years of age¹⁴, though, like ID, the published evidence of the accuracy of these tests is not encouraging either. Of 88 subjects studied for PNC allergy², only 2 had anti-PNC sIgE titers over 0.10 kU/L and below 0.35 kU/L, and neither was allergic according to the final DPT. In our study only 1 (2.9%) and 7 (20%) of the 35 PNC-allergic patients had specific IgE > 0.35 or > 0.1 kU/L to any determinant, respectively. For the same thresholds, 7 (1%) and 49 (7%) of the non-allergic population also displayed positive sIgE. It is remarkable that only the 0.1 kU/L threshold value for amoxicillin differentiated between immediate and non-immediate DPT reactions, and for PNC-V and amoxicillin IgE discriminated allergic and non-allergic patients ($p < 0.05$). In an attempt to assess the performance of sIgE in the best of the scenarios, and considering that sIgE to PNC tends to decline over time²⁶, we carried out a subanalysis of a cohort in which the IR occurred no later than 1 year previously, though the results were not better than those obtained for the whole group.

The low number of immediate IR cases in our study ($n = 38$, 5%) might hamper the robustness of our findings regarding the relevance of sIgE. This modest representation is mostly due to the stringent definition of immediate reaction appearing in the pediatric section of Mirakian et al.⁶: reactions occurring within one hour after the first drug administration. By using this pediatric-focused definition, we reinforce one of the objectives of this manuscript, namely, to argue for specific diagnostic protocols in children. In contrast, for adults it is generally accepted that an immediate reaction is that which occurs within 1 hour after the last drug intake^{6,8}. In addition to being more age-appropriate, the pediatric-specific definition used here provides further advantages: when a reaction occurs after several doses, it is difficult to pinpoint whether the reaction is immediate after the last dose or if the trigger was a previous dose that had taken time to be clinically relevant, thus indicating a non-immediate reaction. Moreover, the chronology of events is less reliable if more than one dose was administered owing to recall bias, but also

because parents might not witness the reaction. Had we used the more permissive definition (i.e. immediate reactions considered to be those with onset within one hour after any dose), our number of immediate IRs would have risen to 96 subjects (13.1%, data not shown). Despite this increase in IRs, the sIgE performance would have remained low, as the only allergic patient in this group (subject 1, table 4) had negative values.

A high specificity and a low sensitivity for ID and sIgE was expected in light of results described in adults^{10,27}. In the present study, overall specificity was very high for both ID and sIgE (98.3% and 99%), whereas sensitivity was extremely low (9.1% and 2.9% for ID and sIgE, respectively), which is lower than that seen in the non-pediatric population, where it ranges from 42% to 78% for skin tests and 38% to 54% for specific IgE²⁷. These discouraging results, despite the relatively short interval between IR and allergy work-up in most of the patients, make it difficult to recommend that such tests be performed in every pediatric patient, especially if cost-efficiency is taken into account.

Approximately 2280 ID (with standardized extracts) and 2200 sIgE tests were performed to correctly diagnose 3 and 1 allergic patients with the ID and sIgE (>0.35 kU/L), respectively. By way of estimation, and according to the reagents' prices in one of the participating centers (Hospital Niño Jesús, Madrid), the cost of all ID and sIgE tests performed throughout this study is 74,100 and 11,800 euros, respectively, not including the expense of hospital personnel and indirect costs. Another drawback of both skin and venipuncture is the great emotional stress these procedures cause for children, with around 20% of children showing an aversion to them². In spite of the recent recommendations of the Penicillin Allergy in Antibiotic Resistance Workgroup of the AAAAI²⁸, the low test sensitivity, along with the aforementioned factors, triggered several new publications advocating DPT without prior testing in mild non-immediate reactions^{2, 6, 13}.

Drug provocation tests were crucial to correctly categorize a significant number of subjects in our study and avoided the misclassification of 15 non-allergic subjects who showed positive

results on ID or sIgE (0.35 kU/L) and 30 allergic patients who did not display any positivity in the aforementioned tests. As previously reported^{2, 5, 12, 29}, most reactions revealed by DPT were mild (85% only skin involvement) and were easily managed with antihistamines and/or corticosteroids, providing further evidence of the safety of DPT in this scenario. Adrenaline was used according to the researcher criteria in only one out of 40 positive DPTs, i.e., in a patient who suffered only skin involvement.

Although DPT remains the gold standard, it is also hampered by false-positive and false-negative results. It is known that a second DPT should be conducted in subjects in whom the IR occurred a long time previously¹⁴. In our study, 164 subjects with an initial negative DPT and an IR more than 1 year prior underwent a second DPT, with a positive result in 3 cases. On the other hand, 5 subjects with a first positive/doubtful DPT underwent a second DPT, and in 2 of them the second test was negative, suggesting that even under controlled exposure children may develop non-specific skin reactions.

To provide a safe alternative to beta-lactam in PNC-allergic subjects⁸, a challenge with cefuroxime was proposed; in 23 of 24 tested subjects the drug was tolerated, with the single reactive patient showing no more than mild skin symptoms. In our population, the prevalence of cefuroxime hypersensitivity among amoxicillin-allergic children (4.1%) was lower than in other series (28%)⁹.

Over the past few years it has been established that skin testing, or even direct challenges, are the primary tools to be used to determine tolerance to penicillins in low-risk individuals, defined as those who develop non-urticarial rashes, gastrointestinal upset, or headaches^{22,30}. However, there is currently a lack of worldwide consensus among clinicians regarding the diagnosis and management of beta-lactam allergy in childhood³¹. The strength of the present study is that, while severe reactions were not included, every patient underwent DPT regardless of the characteristics of the IR and the results of previous allergy tests. In addition, the reactions elicited in positive DPT were all of mild intensity.

In light of the data provided by our large prospective cohort studied conducted following a uniform protocol, and after identifying several key facts such as the poor association between IR temporality and DPT outcome, the low efficiency of ID and sIgE determinations and the good safety profile of DPT, the authors propose that drug provocation tests performed in specialized centers be used as the first diagnostic tool in children with mild to moderate penicillin IRs when anaphylaxis and severe skin syndromes have been previously ruled out. This study represents a significant contribution toward optimization of a protocol for penicillin-allergy diagnosis in the pediatric population.

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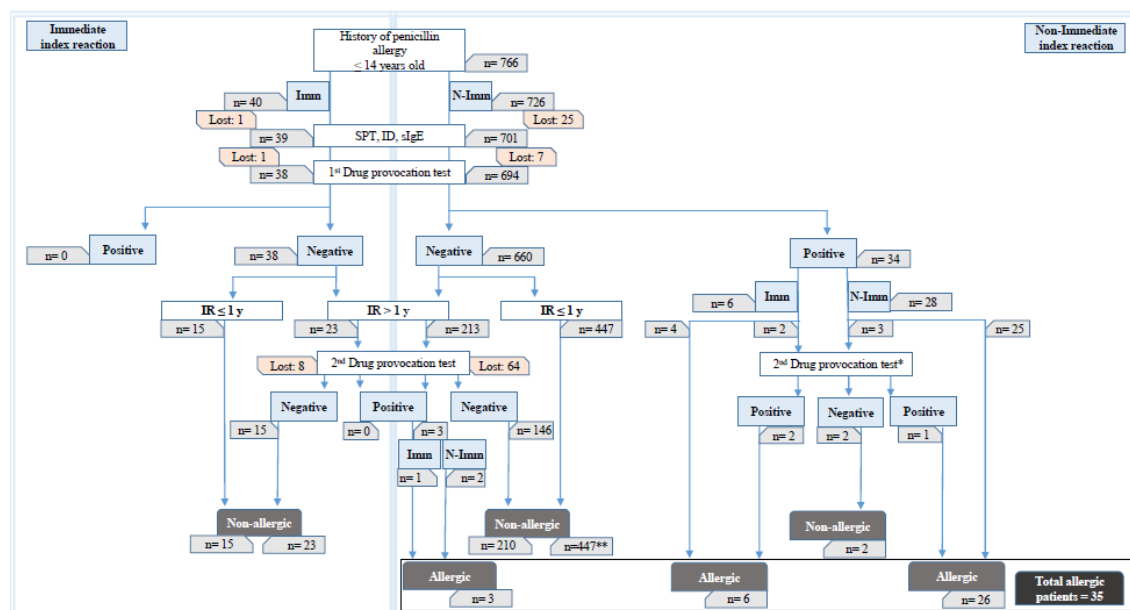


Figure 1. Patient flow chart of penicillin allergy work-up.

Reactions are initially classified according to the temporality reported by the patient in the index reactions (blue double-lined rectangles), and second, in terms of the temporality observed in the drug provocation test. According to the protocol, a second drug provocation test was mandatory if the index reaction occurred more than one year before and the first drug provocation test was negative. There were some protocol deviations, and these were as follows:

* 5 out of 34 patients with a positive first drug provocation test underwent a second drug provocation test at the request of the investigator. In 2 subjects this test was negative, and the patients were finally classified as non-allergic.

** 49 of out 447 cases with an index reaction that developed during the previous year and a negative first drug provocation test underwent a second provocation test at the request of the investigator. All such secondary tests were negative.

Imm: immediate reaction, IR: Index reaction, N-Imm: non-immediate; n: number of subjects.

SPT: skin prick test; ID: intradermal test

Table 1. Characteristics according to the final classification of the population into groups (for per-protocol population).

Final diagnosis according to drug provocation test results					
Characteristic	Immediate reaction (n=6)	Non-immediate reaction (n=29)	Total allergic (n=35)	Non-allergic (n=697)	Total (n=732 patients, PP)
Age at study time, y	6.3 (+/-3.3)	5.3 (+/-3.7)	5.4 (+/-3.7)	5.5 (+/-3.8)	5.5 (+/-3.8)

(+/-SD)

Male, n patients (%)	4 (66.7%)	11 (37.9%)	15 (42.9%)	360 (51.6%)	375 (51.2%)
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Female, n patients (%)	2 (33.3%)	18 (62.1%)	20 (53.1%)	337 (48.4%)	357 (48.8%)
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Baseline disease, n patients (%)

None	4 (67.7)	22 (75.8)	26 (74.3)	452 (64.8)	478 (64.5)
Atopic dermatitis	1 (16.7)	3 (10.3)	4 (11.4)	123 (17.6)	127 (17.3)
Respiratory allergy	2 (33.3)	3 (10.3)	5 (14.3)	79 (11.3)	84 (11.5)
Food allergy	0	2 (6.9)	2 (5.7)	41 (5.9)	43 (5.9)
Drug allergy	0	0	0	5 (0.7)	5 (0.7)

Family history of allergy, n patients (%)

None	1 (16.7)	15 (51.7)	16 (45.7)	330 (47.3)	350 (47.8)
Respiratory allergy	3 (50)	8 (27.6)	11 (31.4)	228 (32.7)	239 (32.7)
Drug allergy*†	3 (50)*	6 (20.7)*	9 (25.7)†	83 (11.9)†	92 (12.6)
Atopic dermatitis	0	1 (3.4)	1 (2.9)	40 (5.7)	41 (5.6)
Food allergy	0	2 (6.9)	2 (5.7)	37 (5.3)	39 (5.3)

Disease causing penicillin administration in the patient, n patients (%)

Tonsillitis	1 (16.7)	11 (37.9)	12 (34.3)	256 (36.7)	268 (36.6)
Otitis	2 (33.3)	7 (24.1)	9 (25.7)	177 (25.4)	186 (25.4)
Others/UN	3 (50)	6 (20.6)	9 (25.7)	104 (14.9)	113 (15.4)
Upper respiratory tract infection	0	3 (10.3)	3 (8.6)	109 (15.6)	112 (15.3)
Pneumonia	0	2 (6.9)	2 (5.7)	32 (4.6)	34 (4.6)
Pyrexia of unknown origin	0	0	0	19 (2.7)	19 (2.6)

Trigger drug, n patients (%)

Amoxicillin	3 (50)	18 (62.1)	21 (60)	468 (67.1)	489 (66.8)
Amoxicillin-clavulanic acid	2 (33.3)	11 (37.9)	13 (37.1)	207 (29.7)	220 (30.1)
Penicillin V	0	0	0	19 (2.7)	19 (2.6)
Penicillin G	1 (16.7)	0	1 (2.9)	3 (0.4)	4 (0.5)

Route of administration, n patients (%)

Oral	5 (83.3)	29 (100)	34 (97.1)	685 (98.3)	719 (98.2)
Intramuscular	1 (16.7)	0	1 (2.9)	4 (0.6)	5 (0.7)
Intravenous	0	0	0	8 (1.1)	8 (1.1)
Previous tolerance to trigger drug, n patients (%)					
Previously tolerated	3 (50)	19 (65.5)	22 (62.9)	365 (52.4)	387 (52.9)
First time of use	1 (33.3)	2 (6.9)	3 (8.5)	172 (32.7)	175 (31.7)
Previous reaction	1 (16.7)	8 (27.6)	9 (25.7)	104 (14.9)	113 (15.4)
Symptoms in index reaction, n patients (%)					
Exanthema	1 (16.7)	14 (48.3)	15 (42.9)	396 (56.8)	411 (56.1)
Urticaria	4 (66.7)	15 (51.7)	19 (54.3)	267 (38.3)	286 (39.1)
Angioedema* †	1 (16.7)*	9 (31)*	10 (28.6)†	98 (14.1)†	108 (14.8)
Temporality of IR, n patients (%)					
Non-immediate IR	6 (100)	29 (100)	35 (100)	659 (94.5)	694 (94.8)
Immediate IR	0	0	0	38 (5.5)	38 (5.2)
Mean doses until IR*†	8 (±7.2 SD)*	16 (±5.4 SD)*	15 (±6.5 SD) †	10 (±8.2 SD) †	10 (±8.2 SD)
IR occurring after the first dose	1 (16.7)	0	1 (2.9)	99 (14.2)	100 (13.6)
IR occurring after the 2nd or more doses†	5 (83.3)	29 (100)	34 (97.1)†	598 (85.8)†	632 (86.3)
Time until onset of symptoms after the last dose in IR, n patients (%)					
<1 hour	1 (16.7)	0	1 (2.9)	95 (13.6)	96 (13.1)
1 to 8 hours	3 (50)	20 (69)	23 (65.7)	404 (58)	427 (58.3)
>8 hours	1 (16.7)	5 (17.2)	6 (17.1)	89 (12.8)	95 (13)
UN	1 (16.7)	4 (13.8)	5 (14.3)	109 (15.6)	114 (15.6)
Distribution of IR, n patients (%)					
Generalized symptoms	4 (66.7)	26 (89.7)	30 (85.7)	589 (84.5)	619 (84.6)
Local symptoms	2 (33.3)	3 (10.3)	5 (14.3)	108 (15.5)	113 (15.4)
IR duration, n patients (%)					
<24 hours†	2 (33.3)	0	2 (5.7)†	167 (24)†	169 (23.1)
24 hours-3 days	3 (50)	12 (41.4)	15 (42.9)	349 (50.1)	364 (49.7)

>3 days*†	1 (16.7)*	17 (58.6)*	18 (51.4)†	181 (25.9)†	199 (27.2)
IR treatment, n patients (%)					
None*†	3 (50)*	4 (13.8)*	7 (20)†	268 (38.5)†	275 (37.6)
ER visit†	4 (66.7)	23 (79.3)	27 (77.1)†	389 (55.8)†	416 (56.8)
Antihistamines*†	3 (50)*	23 (79.3)*	26 (74.3)†	388 (55.7)†	414 (56.6)
Corticosteroids*†	1 (16.7)*	13 (44.8)*	14 (40)†	135 (19.4)†	149 (20.4)
Adrenaline	0	0	0	1 (0.1)	1 (0.1)

Footnote: There were no episodes of anaphylaxis in the index reaction. Percentages in each column were calculated using the total number of subjects in each defined group in columns and in parentheses. Significant statistical differences ($p < 0.05$) were calculated comparing immediate vs non-immediate PNC-allergic populations, and were marked with *, and for PNC allergic vs non-PNC-allergic population, which were marked with †. IR: index reaction. UN: unknown. ND: not determined; PP: per protocol, SD: standard deviation; y: years.

Table 2. Positive intradermal test results to different determinants according to the final diagnosis readings at 15 minutes (early reading) and 24 hours (late reading).

	Immediate n=6	Non- immediate n=29	Allergic n=35	Non-allergic n=697
PPL				
15 min (n=701) †	1 (16.7%)	0	1 (2.9%)†	1 (0.1%)†
24 hours (n= 359)	0	0	0	0
MDM				
15 min (n=702)	0	0	0	0
24 hours (n= 359)	0	0	0	0
Amoxicillin				
15 min (n= 724)	0	0	0	1 (0.1%)
24 hours (n= 355)	0	0	0	0
Penicillin G				
15 min (n= 235)	0	0	0	3 (1.2%)
24 hours (n= 120) †	0	2 (6.9%)	2 (5.7%)†	4 (0.6%)†
Clavulanic acid				
15 min (n= 132)	0	0	0	0
24 hours (n=30)	0	0	0	1 (0.1%)

Percentages refer to the total of patients with positive test in each subgroup. † indicates a significant difference ($p<0.05$) between the percentage of positive or negative tests detected in the comparison between allergic patients vs not allergic patients. There were no significant differences between immediate and non-immediate reactions in DPT for these determinations.

Table 3. Results of sIgE to different determinants according to the diagnosis

	Immediate (n=6)	Non-immediate (n=29)	Allergic (n=35)	Non-allergic (n=697)
Penicillin G				
0.1 kU/L	1 (16.7%)	2 (6.9%)	3 (8.6%)	26 (3.7%)
0.35 kU/L	1 (16.7%)	0	1 (2.9%)	1 (0.1%)
Penicillin V				
0.1 kU/L †	2 (33.3%)	4 (13.8%)	6 (17.1%)†	35 (5%)†
0.35 kU/L	1 (16.7%)	0	1 (2.9%)	4 (0.6%)
Amoxicillin				
0.1 kU/L *†	3 (50%)*	3 (10.3%)*	6 (17.1%)†	39 (5.6%)†
0.35 kU/L	1 (16.7%)	0	1 (2.9%)	2 (0.3%)

For each test, two different positive thresholds were evaluated.

The asterisk (*) denotes a significant difference ($p<0.05$) between the percentage of positive and negative tests detected when comparing immediate vs non-immediate reactions and † ($p<0.05$) indicates differences between allergic vs non-allergic subjects.

Table 4. Characteristics of PNC-allergic cases.

Pat no.	Gender, Age (y)	Drug	IR symptoms	*Skin tests	**Specific IgE (kU/L)	DPT1 onset and symptoms	DPT2 onset and symptoms	Cefuroxime tolerance
1	F, 1.4	AX	Exanthema, nausea & vomiting	-	-	Imm: urticaria & pruritus	NP	NP
2	M, 2.2	AX	Urticaria	-	-	Imm: urticaria	NP	Yes
3	M, 3.0	AX-C	Local urticaria	-	-	-	Imm: urticaria	NP
4	M, 6.0	AX-C	Vomiting	-	PNC -V 0.1; AX 0.1	Imm: abdominal pain, vomiting & nausea	NP	Yes
5	F, 9.1	PNC-G	Urticaria & angioedema	SPT for PPL; ID: early reading PPL	PNC -G 8.7; PNC-V 8.2; AX 2.6	Imm: urticaria	Imm: urticaria, palmoplantar pruritus & generalized pruritus	Yes
6†	M, 9.3	AX	Urticaria	-	AX 0.2	Imm: urticaria, pruritus & erythema	NP	Yes
7	M, 0.1	AX	Exanthema	-	-	Non-imm: urticaria, local pruritus & rhinitis	NP	NP
8	M, 1.0	AX-C	Urticaria & exanthema	-	-	Non-imm: erythema, pruritus & urticaria	NP	NP
9	M, 1.2	AX	Urticaria & angioedema	-	PNC -V 0.1; AX 0.34	Non-imm: urticaria	NP	NP
10	M, 1.4	AX	Exanthema	-	-	Non-imm: erythema	NP	Yes
11	M, 1.7	AX	Urticaria	-	-	Non-imm: urticaria	NP	Yes
12	F, 1.9	AX	Exanthema	-	-	Non-imm: erythema & pruritus	NP	Yes
13	M, 2.0	AX	Exanthema	-	-	Non-imm: urticaria	Non-imm: urticaria	Yes
14	F, 2.0	AX-C	Urticaria & angioedema	ID: Late reading PNC-G	-	Non-imm: angioedema & urticaria	NP	Yes

15	F, 2.0	AX-C	Urticaria & angioedema	-	-	Non-imm: urticaria, hands & feet; angioedema	NP	Yes
16	M, 2.6	AX	Exanthema	-	-	Non-imm: erythema	NP	Yes
17	F, 2.7	AX	Exanthema	-	PNC-G 0.2; PNC-V 0.2; AX 0.2	Non-imm: urticaria & local pruritus	NP	NP
18	F, 3.0	AX	Exanthema	-	-	Non-imm: erythema & pruritus	NP	Yes
19	F, 3.2	AX	Urticaria	-	-	Non-imm: urticaria	NP	NP
20	F, 3.4	AX-C	Urticaria & angioedema	-	-	Non-imm: urticaria	NP	NP
21	F, 3.9	AX	Exanthema & angioedema	-	-	Non-imm: urticaria & angioedema	NP	Yes
22	F, 4.3	AX	Urticaria	-	-	Non-imm: urticaria, pruritus & erythema	NP	Yes
23	M, 4.3	AX	Urticaria & angioedema	-	-	Non-imm: urticaria	NP	Yes
24	F, 4.4	AX	Exanthema	-	-	Non-imm: urticaria	NP	Yes
25	F, 5.4	AX	Local exanthema	-	-	Non-imm: local urticaria	NP	Yes
26	F, 6.0	AX-C	Urticaria & angioedema	-	-	Non-imm: urticaria & angioedema	Non-imm: urticaria & angioedema	No
27	M, 6.0	AX-C	Local exanthema	-	PNC-V 0.22	-	Non-imm: urticaria & angioedema	NP
28	F, 6.6	AX	Urticaria	-	-	Non-imm: urticaria	NP	Yes
29	F, 6.7	AX-C	Exanthema	-	-	Non-imm: local pruritus	NP	Yes
30	F, 8.0	AX-C	Local erythema, elbows and knees; raised temperature	ID: Late reading PNC-G		Non-imm: feeling unwell and elbows and knees; raised temperature & erythema	NP	Yes

31	M, 8.5	AX	Urticaria & angioedema	-	PNC-G 0.1; PNC-V 0.1; AX 0.1	Non-imm: urticaria, angioedema, disnea & generalized pruritus	NP	NP
32	M, 9.0	AX	Exanthema	-	-	-	Non-imm: erythema & pruritus	NP
33	F, 9.1	AX-C	Exanthema	-	-	Non-imm: erythema	NP	Yes
34	F, 12.0	AX-C	Urticaria	-	-	Non-imm: urticaria	NP	Yes
35	F, 12.4	AX-C	Urticaria & angioedema	-	-	Non-imm: urticaria	NP	Yes

All reactions finally classified as allergic cases were originally non-immediate index reactions.

First 6 lines (white and clear-blue lines) are the cases diagnosed with immediate allergy, and lines from 7 to 35 (white and dark-blue lines) are the non-immediate allergic subjects.

* The text shows the type of test, the determinant that was positive, and reading time when the test was positive.

** The text shows the determinant that was positive and the test value.

-. negative result, AX: amoxicillin, AX-C: amoxicillin-clavulanic acid, DPT: drug provocation test, F: female, M: male, y: years old.

†Patient receiving adrenaline in the DPT1

Table 5. Skin and sIgE accurate diagnoses and misdiagnoses.

Diagnosis based upon positive test*	Allergic patients n=35	Non-allergic patients n=697	Diagnosis based upon negative tests*	Allergic patients n=35	Not allergic patients n=697	Total potential diagnostic failures n= 732
	Positive/Tests performed (correct diagnosis)	Positive/Tests performed (misdiagnosis)		Negative/Tests performed (misdiagnosis)	Negative/Tests performed (correct diagnosis)	Failures/Tests performed
Any ID positive (any test + any reading)	3/33 (9.1 %)	12/693 (1.7%)	All ID negative (any reading, any test)	30/33 (90.9%)	683/693 (98.6%)	42/726 (5.8%)
Any sIgE positive (0.1 kU/L threshold)	7/35 (20%)	49/697 (7%)	All sIgE negative (0.1 kU/L threshold)	28/35 (80%)	648/697 (93%)	77/732 (10.6%)

Any sIgE positive (0.35 kU/L threshold)	1/35 (2.9%)	7/697 (1%)	All sIgE negative (0.35 kU/L threshold)	34/35 (97.1%)	690/697 (99%)	41/732 (5.6%)
Any ID positive OR any sIgE positive (0.1 kU/L threshold)	9/33 (27.3%)	57/693 (8.2%)	All ID negative AND all sIgE negative (0.1 kU/L threshold)	24/33 (72.7%)	636/693 (91.8%)	81/726 (11.2%)
Any ID positive OR any sIgE positive (0.35 kU/L threshold)	3/33 (9.1%)	15/693 (2.2%)	All ID negative AND all sIgE negative (0.35 kU/L threshold)	30/33 (90.9%)	678/693 (97.8%)	45/726 (6.2%)

*Percentage with regard to the population indicated in each defined group in each column. As some tests were not performed in all patients, percentages are given according to the number of total tested patients for each subgroup and not for the total number of allergic patients or non-allergic patients.

Supplementary material. File 1

Ethics

The study was approved by the Independent Institutional Ethics Committee of Hospital Universitario Niño Jesús of Madrid (internal code R-SEA-PEN-2013-01) and the ethics committees of each participating hospital as well as by the Spanish Agency for Drug and Health Products (AEMPS). The work was classified as “post-authorization study with designs other than prospective follow-up” (EPA-OD) by the Spanish Agency for Drug and Health Products (AEMPS) in April 2013.

Written informed consent was signed by the patients’ parents or guardians, and was obtained from patients over 11 years of age. The authors guarantee the accuracy of the data and analysis, as well as compliance with the established protocol.

Clinical History

The interval between the last reported ADR and the allergy study was four weeks minimum. The clinical history collected information regarding age, sex, personal and family history of atopy, previous exposure to PNC, drug involved in the reaction (PNC-G, PNC-V, AX, AX-C, or cloxaciline), clinical characteristics of the IR, treatment, and outcome of the reaction.

Patients

Severe skin conditions considered as contraindications were: hypersensitivity syndromes (DRESS/DHIS), bullous diseases, Stevens-Johnson syndrome, toxic epidermal necrolysis, and serum sickness.

Skin tests

An SPT wheal 3 mm larger than the negative saline control was considered positive. In ID, using 0.02-0.05 ml of the drug solution, the wheal area was initially marked, and 20 min after testing, an increase in mean diameter >3 mm surrounded by erythema was considered positive. An ID reading was taken after 48 h in the case of non-immediate reactions. Physiological saline was tested as a negative control. Systemic antihistamines were discontinued at least 1 week before performing in vivo tests. The reagents used for SPT and ID were as follows: benzylpenicilloyl polylysine (PPL) (5×10^{-5} mM), minor determinant mixture (MDM) (2×10^{-2} mM), AX (20 mg/ml for SPT and ID), clavulanic acid (20 mg/mL for SPT, 5 mg/ml for ID), PCN-G (250,000 U for SPT and 10,000 U for ID) (Diater, Madrid, Spain). Tests with cefuroxime (25 mg/ml for SPT and 10 mg/ml for ID) (Ramon Sala SL, Barcelona, Spain) were conducted only in case of positive PNC-DPT.

Serum specific IgE

Serum specific IgE determination was performed using the CAP-FEIA system (Thermo Fisher Scientific, Waltham, MA, USA), using PNC-G, PNC-V, and AX. The results were analyzed for two positive cut-offs (≥ 0.1 kU/L and ≥ 0.35 kU/L).

Drug provocation tests

All patients were subjected to provocation with the culprit drug regardless of the skin test or sIgE results. In cases where the investigator considered that the patient had developed a severe IR contraindicating DPT, an external group of three experts determined whether the exposure test was appropriate or not. The recommended interval between skin testing and DPT was from 2 days to 1 month. DPT was performed under close medical supervision in a hospital setting. Record of vital signs (TA, heart rate, O₂ saturation and peak flow) was done before the DPT was started and in case of a positive DPT. DPT was performed with the culprit drug in a simple-blind procedure following the ENDA/EAACI recommendations (15). Gradually increasing doses (1/8, ¼, and 5/8) were administered at 20-minute intervals until the age- and weight-appropriate, therapeutically recommended cumulative dose was reached. The cumulative dose of oral AX and AX-C for children below 40 kg was 30 mg/kg, and 750 mg for children over that weight. For PNC-G, children below 27 kg were given 600,000 UI, and subjects exceeding 27 kg were given 1,200,000 UI. For oral PNC-V, patients below 27 kg received 250 mg, and patients over 27 kg, 500 mg. Lastly, 50 mg/kg of cloxacilin was administered for children below 40 kg and 500 mg if they were over 40 kg.

If no symptoms appeared during the hospital phase of the DPT and the patient had a non-immediate IR, the challenge was continued at home with doses given every 12 hours to match the number of days of treatment triggering the IR.

If the patients developed cutaneous, digestive, and/or respiratory symptoms or alterations in vital signs, the procedure was discontinued and the symptoms were assessed and treated. Every patient was kept under surveillance for at least 2 hours (4 for non-immediate reactions) after the last dose was administrated or, in children who failed DPT, 2 hours (4 for non-immediate reaction) after symptoms had totally subsided.

Either to confirm a positive but mild/doubtful reaction in the first provocation (according to the investigator's judgment) or because the IR had occurred more than one year previously (14), a second DPT was performed 4 to 8 weeks later.

If the DPT was positive, SPT and DPT with cefuroxime were performed.

Data record

Data were recorded on an online platform (APENIN website) for further analysis. The recording sheet contained the following information: i) coding patient and center, ii) personal details (coded to ensure anonymity), iii) personal and family allergic background, iv) data of IR, v) data of study drug, vi) doctor prediction on the probabilities of the reaction being true allergy, graded as “clear negative”, “doubtful case” or “clear positive”, vii) in vivo tests, viii) in vitro tests, and ix) DPT results.

Statistics

Sensitivity, specificity, positive and negative predictive values, and the rate of accuracy for these diagnostic tests were calculated. To calculate the sample size needed to estimate the incidence of cases of ADR with penicillin per 100,000 inhabitants, the prevalence given by Caubet of 6.8% cases of penicillin allergy in pediatric patients was used, despite the difference in geographical area, due to the similarities in the population to be included and the diagnostic approach. To ensure sufficient accuracy ($\pm 2\%$), the final maximum prevalence was established at a maximum of 8%, with a total calculation of $n=707$ subjects.

Supplementary file 2. Characteristics according to the temporality of the Index Reaction (IR) of the population (for per-protocol population).

Characteristic	Immediate IR (n=38)	Non-Immediate IR (n=694)	Total (n=732)
Age	6.8 (+/-3.5)	5.5 (+/-3.8)	5.5 (+/-3.8)
Male, n patients (%)	17 (44.7%)	358 (51.6%)	375 (51.2%)
Baseline disease, n patients (%)			
None	24 (63.2%)	454 (65.4%)	478 (65.3%)
Atopic dermatitis	6 (15.8%)	121 (17.4%)	127 (17.3%)
Respiratory allergy	7 (18.4%)	77 (11.1%)	84 (11.4%)
Food allergy	1 (2.6%)	42 (6.1%)	43 (5.8%)
Drug allergy	0 (0%)	5 (0.7%)	5 (0.7%)
Family history of allergy, n patients (%)			
None	19 (50%)	331 (47.7%)	350 (47.8%)
Respiratory allergy	10 (26.3%)	229 (33%)	239 (32.5%)
Drug allergy	6 (15.8%)	86 (12.4%)	92 (12.5%)
Atopic dermatitis	5 (13.2%)	36 (5.2%)	41 (5.6%)
Food allergy	3 (7.9%)	36 (5.2%)	39 (5.3%)
Disease causing penicillin administration in the patient, n patients (%)			
Tonsillitis	14 (36.8%)	254 (36.6%)	268 (36.6%)
Otitis	6 (15.8%)	180 (25.9%)	186 (25.4%)
Others/UN	7 (18.4%)	106 (15.3%)	113 (15.5%)
Upper respiratory tract infection	5 (13.2%)	107 (15.4%)	112 (15.3%)
Pneumonia	4 (10.5%)	30 (4.3%)	34 (4.6%)

Pyrexia of unknown origin	2 (5.3%)	17 (2.4%)	19 (2.6%)
Trigger drug, n patients (%)			
Amoxicillin	19 (50%)	470 (67.7%)	489 (66.8%)
Amoxicillin-clavulanic acid	17 (44.7%)	203 (29.3%)	220 (30.1%)
Penicillin V	1 (2.65%)	18 (2.6%)	19 (2.6%)
Penicillin G	1 (2.65%)	3 (0.4%)	4 (0.5%)
Route of administration, n patients (%)			
Oral	32 (84.2%)*	687 (99%)*	719 (98.2%)
Intramuscular	0	5 (0.7%)	5 (0.7%)
Intravenous	6 (15.8%)*	2 (0.3%)*	8 (1.1%)
Previous tolerance to trigger drug, n patients (%)			
Previously tolerated	19 (50%)	368 (53%)	387 (52.9%)
First time of use	13 (34.2%)	475 (31.6%)	175 (31.7%)
Previous reaction	6 (15.8%)	107 (15.4%)	113 (15.4%)
Distribution of IR, n patients (%)			
Generalized symptoms	26 (68.4%)	593 (85.4%)	619 (84.6%)
Local symptoms	12 (31.6%)	101 (14.6%)	113 (15.4%)
IR duration, n patients (%)			
<24 hours*	23 (60.5%)	146 (21%)	169 (23.1%)
24 hours-3 days	12 (31.6%)	352 (50.7%)	364 (49.7%)
>3 days*	3 (7.9%)	196 (28.2%)	199 (27.2%)
IR treatment, n patients (%)			
None	13 (34.2%)	262 (37.8%)	275 (37.6%)
ER visit	21 (55.3%)	395 (56.9%)	416 (56.8%)
Antihistamines	22 (57.9%)	392 (56.5%)	414 (56.6%)
Corticosteroids	8 (21.1%)	141 (20.3%)	149 (20.4%)
Adrenaline	1 (2.6%)	0	1 (0.1%)

*p<0.0001

Supplementary file 3. Accuracy of diagnostic tests

	Sensiti- vity (%)	Specifi- city (%)	Positive predictive value (%)	Negative predictive value (%)	False positives (%)	False negatives (%)
Intradermal tests						
MDM	0.0	100.0	Null	0.95	Null	4.6
PPL	3.1	99.9	50.0	0.96	50.0	4.4
Amoxicillin	0.0	99.9	0.0	0.95	100.0	4.6
Penicillin G	12.5	97.0	22.2	0.94	77.8	5.8
Clavulanic acid	20.0	99.2	50.0	0.97	50.0	3.1
Any positive	9.1	98.3	20.0	95.8	80.0	4.2
Serum sIgE						
Penicillin G (0.1 kU/L)	8.6	96.3	10.3	95.4	89.7	4.6
Penicillin V (0.1 kU/L)	17.1	95.0	14.6	95.8	85.4	4.2
Amoxicillin (0.1 kU/L)	17.1	94.4	13.3	95.8	86.7	4.2
Any positive for (0.1 kU/l)	20	93	12.5	95.9	87.5	4.1
Penicillin G (0.35 kU/L)	2.9	99.9	50.0	95.3	50.0	4.7
Penicillin V (0.35 kU/L)	2.9	99.4	20.0	95.3	80.0	4.7
Amoxicillin (0.35 kU/L)	2.9	99.7	33.3	95.3	66.7	4.7
Any positive for (0.35 kU/l)	2.9	99	12.5	95.3	87.5	4.7

Null values could not be estimated because there were no positive tests to perform the calculations. For the intradermal tests, any positivity (either at 15 minutes or 24 hours) was used to consider the test as positive or negative.

Supplementary file 4. Results of sIgE to different determinants according to the final diagnosis in subjects in whom the study was performed during the year following the IR (n=492).

	Immediate n=4	Non-immediate n=25	Allergic n=29	Non-allergic n=463
Penicilloyl G				
0.1 kU/L	1 (25%)	2 (8%)	3 (10.3%)	14 (3%)
0.35 kU/L	1 (25%)	0	1 (3.4%)	1 (0.2%)
Penicilloyl V				
0.1k U/L †	2 (50%)	3 (12%)	5 (17.2%) †	22 (4.8%) †
0.35 kU/L	1 (25%)	0	1 (3.4%)	4 (0.9%)
Amoxicillin				
0.1 kU/L *†	3 (75%)*	3 (12%)*	6 (20%) †	22 (4.8%) †
0.35 kU/L	1 (25%)	0	1 (3.4%)	0

For each test, two different positive thresholds were evaluated.

The asterisk (*) denotes a significant difference ($p<0.05$) detected between the percentage of positive or negative tests in the comparison of immediate vs non-immediate reactions, and † ($p<0.05$) indicates differences between allergic and non-allergic subjects.