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Allergology International 71 (2022) 131–136

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original Article

Diagnosis of non-immediate hypersensitivity to amoxicillin in children by skin test and drug provocation tests: A retrospective case-series study

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ARTICLE INFO

Article history:
Received 11 March 2021
Received in revised form
12 June 2021
Accepted 16 June 2021
Available online 9 August 2021

Keywords:
Amoxicillin
Drug hypersensitivity
Drug-induced lymphocyte stimulation test
Drug provocation test
Intradermal test

Abbreviations:
CEX, cephalexin; DLST, drug-induced lymphocyte-stimulation test; DPT, drug provocation test; EM, erythema multiforme; IDT, intradermal test; IQR, interquartile range; MPE, maculopapular exanthema; S.I., stimulation index; SPT, skin prick test

ABSTRACT

Background: Skin rash often occurs upon oral administration of amoxicillin in children, due to non-immediate hypersensitivity. However, information on delayed hypersensitivity to amoxicillin is scarce. Moreover, the appropriate diagnostic method and actual diagnostic rate of delayed hypersensitivity to amoxicillin among Japanese children are unclear. We conducted intradermal tests (IDTs) and drug provocation tests (DPTs) and retrospectively investigated the proportion of children with a definitive diagnosis of non-immediate hypersensitivity to amoxicillin. We then evaluated the characteristics of patients with a positive allergic workup.

Methods: We enrolled children referred for suspected findings of mild or moderate non-immediate hypersensitivity to amoxicillin between August 2018 and March 2020. If the IDT in the delayed phase was negative, DPT with amoxicillin (60–90 mg/kg/day) was performed for 7 days. Non-immediate hypersensitivity to amoxicillin was defined when IDT or DPT was positive. We evaluated the potential of the drug-induced lymphocyte stimulation test (DLST) to reveal hypersensitivity to amoxicillin.

Results: This study enrolled 27 children. Fourteen children (52%) had hypersensitivity to amoxicillin, of whom 12 had positive IDTs and two had positive DPTs. No differences in age, sex, history of allergic disease, days from oral use to symptom onset, type of rash at symptom onset, generalized rash, and DLST results were observed between the hypersensitivity and non-hypersensitivity groups.

Conclusions: Examination should be performed for children with mild or moderate reactions because positive cases have no significant features and half of the suspected cases are negative.

Introduction

The prevalence of immediate and delayed types of penicillin allergy, based on patients' clinical complaints, is approximately 7–10%.[1,2] Penicillin types include penicillin G, aminopenicillin, piperacillin, and others. Aminopenicillin is commonly used in Japan and Spain, unlike in countries such as the United States, and most reported cases of allergies to penicillin are related to aminopenicillin. However, 50–90% of patients with findings suggestive of a delayed hypersensitivity reaction to aminopenicillin did not have true-positive results for allergies.[3–5] Therefore, it is necessary to identify an accurate method for definitive diagnosis of the allergic condition, to avoid over-diagnosing aminopenicillin allergy. However, because only 46.8% of patients with aminopenicillin allergy have cross-reactivity for benzylpenicillin in intradermal tests (IDTs),[6] it is necessary to perform diagnostic methods separately for each drug classified under penicillins. To date, information on delayed hypersensitivity to aminopenicillin remains unclear.

The standard methods for diagnosing delayed hypersensitivity reaction are skin tests (IDT in the delayed phase, patch test) and drug provocation tests (DPTs),[7] but the method for definitive diagnosis has not been determined. IDTs are considered to be associated with lower risk than DPTs. Regarding the diagnostic ability of IDT, the negative-predictive value of IDT for benzylpenicillin has a wide reported range, from 100%[8] to 51%.[5] However, because major and minor determinants of benzylpenicillin are not available for use for IDTs in Japan, it remains unknown whether the negative-predictive value of IDT for benzylpenicillin can be applied for patients with

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Peer review under responsibility of Japanese Society of Allergology.

https://doi.org/10.1016/j.alit.2021.07.009

aminopenicillin allergy. In contrast, DPT can be safely performed for patients with mild-to-moderate delayed penicillin allergy.[1] The administration period in DPTs varies from a single load to a 10-day load. The positive rate in DPTs tends to increase with long-term loading, but the protocol is not standardized. A few reports published from countries worldwide have made a definitive diagnosis by performing DPT in children,[9,10] but no studies have performed DPT for Japanese children. Therefore, the exact diagnostic method and actual diagnostic rate of amoxicillin delayed hypersensitivity to amoxicillin in Japanese children are unknown. The drug-induced lymphocyte stimulation test (DLST) may be useful in diagnosing delayed hypersensitivity to drugs.[11] Although some studies have reported hypersensitivity to benzylpenicillin, which cannot be used to diagnose delayed hypersensitivity to amoxicillin, there are only few reports of DLST for amoxicillin only.

Therefore, in this study, we performed IDTs and DPTs in Japanese children who had findings suggestive of mild-to-moderate delayed hypersensitivity to amoxicillin, for an accurate definitive diagnosis using a fixed protocol. Furthermore, we retrospectively examined the characteristics and proportion of children with a definitive diagnosis of non-immediate hypersensitivity to amoxicillin and evaluated the diagnostic potential of DLST to reveal delayed hypersensitivity to amoxicillin.

Methods

This retrospective case-series was approved by the Hamamatsu Medical University Ethics Committee (approval number 20-182).

Subjects

The subjects were children aged <18 years who visited the Department of Pediatrics, Hamamatsu Medical University between August 2018 and March 2020 with findings suggestive of delayed hypersensitivity to amoxicillin. The children had the presence of rash after oral administration of amoxicillin or within 3 days after completing oral administration. Patients who met the following criteria were excluded: 1) a period of ≥12 months from the appearance of the rash to the examination, 2) immediate wheal-like rash, 3) findings suggestive of severe hypersensitivity to drugs, 4) not providing consent to undergo the examination, or 5) children with underlying conditions.

Procedure

A skin test (skin prick test [SPT] and IDT) was performed 1–12 months after the appearance of the eruption, and DPT was performed when the IDT in the delayed phase showed negative results (Fig. 1). A skin test was also performed for hypersensitivity to cefalexin (CEX), which is considered to have high cross-reactivity.[12] When the CEX skin test produced negative results and a diagnosis of delayed hypersensitivity to amoxicillin was made, CEX DPT was performed. Patients discontinued oral leukotriene receptor antagonist, systemic antihistamine, systemic steroids, and immunosuppressive drugs 3 days before SPTs, IDTs, and DPTs. All parents were asked to sign written informed consent before their children were tested.

Skin test

We performed SPT (amoxicillin 100 mg/mL, CEX 100 mg/mL (7)) using bifurcated needles (Allergy Laboratories of Ohio, Columbus, OH, USA). We used histamine (10 mg/mL) as the positive control and saline as the negative control for SPTs. The possibility of hypersensitivity was judged after 15 min. Patients tested positive when the average wheal diameter was ≥3 mm.[13] When the test was negative, we performed IDT (amoxicillin 20 mg/mL, 0.02 ml[7]) on the patient's forearm using a 27-G needle. We used saline as the negative control, without positive control, for IDTs. The results were considered positive when the size of the initial wheal increases by 3 mm or greater in diameter after 20 min. In contrast, we determined the delayed phase as positive when doctor found that the average diameter of the infiltrative erythema was ≥5 mm after 48 h.[13] For the skin tests, we used 20% widecillin® granules (Meiji Seika Pharma) and decapsulated contents of cephalexin capsules® (Towa Pharmaceutical), which were oral drugs and contained some additives. We used it after diluting with saline. DPT was performed when only the IDT in the immediate phase was positive, and that in the delayed phase was negative. Because all patients in this study only had a history of non-immediate hypersensitivity, they were administered the first dose of amoxicillin during DPTs at hospital and observed for several hours. Conversely, if the IDT in the delayed phase was positive, then we diagnosed the patient with delayed hypersensitivity to amoxicillin and decided not to perform DPT for amoxicillin (Fig. 1).

DPT

We conducted a DPT in which amoxicillin 60–90 mg/kg/day was administered orally in two or three divided doses, for 7 days, as an open challenge test. For amoxicillin DPT, the initial load (amoxicillin 20–30 mg/kg) was tested at the outpatient department of the hospital, and the patient was followed up for 2 h. If the patient did not develop any immediate hypersensitivity reaction, then the patient was tested for the same amount of amoxicillin at home for 7 days. We judged the condition as positive if symptoms such as rash or erythema appeared within 3 days after the end of the oral administration.[7] For CEX DPT, a load of 40–60 mg/kg/day was tested in three divided doses for 7 days in the same manner as that for amoxicillin DPT.

Drug-induced lymphocyte stimulation test

We performed two DLST assays: one in the acute phase, i.e., when the rash first appeared (within 20 days) and the other in the non-acute phase, i.e., 30–180 days after the rash appeared. We excluded the results of children who used systemic steroids at the time of sampling. DLST in the non-acute phase was performed at the same timing as that when the skin test was performed. The measurement was performed by SRL Inc. (Tokyo, Japan). The drug was then added to the lymphocytes separated from the patient's plasma. The lymphocytes were cultured for 72 h, and ^{3}H-thymidine was added. After culturing the lymphocytes for 16–18 h, we measured radioactivity resulting from the uptake of ^{3}H-thymidine by the cells during DNA synthesis, in counts per minutes (cpm). The stimulation index (S.I.) was calculated as the ratio of proliferation (cpm) with the drug/proliferation (cpm) without the drug. The S.I. cutoff values for DLST assessed by SRL Inc. are 1.8 in Japan, compared to 2.5–3.0 in other countries.[11] In this study, we performed DPT as an intradermal examination, regardless of the DLST result.

Outcomes

Definition of non-immediate hypersensitivity to amoxicillin

A patient was diagnosed with delayed hypersensitivity to amoxicillin (hypersensitivity group) if the IDT in the delayed phase was positive or if the DPT was positive. If the DPT result was negative, patients were considered as being non-hypersensitive to the antibiotic.

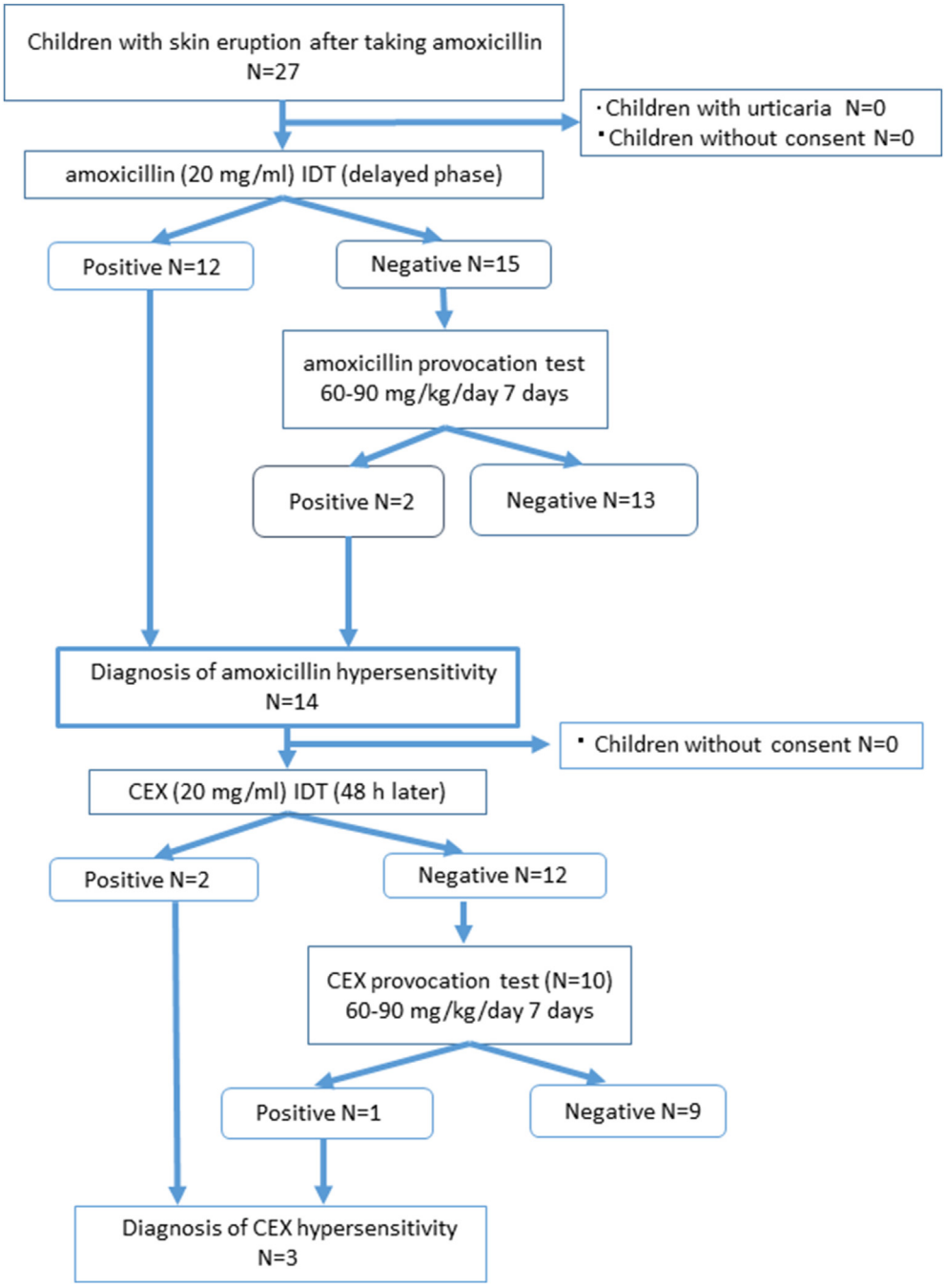

Fig. 1. Flowchart of the study participant selection process. We checked the intradermal test (IDT) results after 48 h. When the IDT or drug provocation test (DPT) for amoxicillin was positive, we performed IDT and DPT for cefalexin (CEX) to examine cross-reactivity.

Other outcomes

We classified the types of skin eruptions into disseminated erythema multiforme (EM), maculopapular exanthema (MPE), and others. When a rash was present on the trunk and other parts, it was classified as "general."

Statistical analysis

Differences between the hypersensitivity and non-hypersensitivity groups were analyzed using Fisher's exact test. Wilcoxon's rank-sum test was used to compare continuous variables. We set the significant difference value as $p < 0.05$. Statistical analysis was performed using JMP® 14.0.0 (SAS Institute, Cary, NC, USA).

Results

Twenty-seven children were enrolled as research subjects. The characteristics of the patients in the two groups are shown in Table 1. The median patient age (interquartile range [IQR]) was 73 (range 18–87) months, and 56% had a history of allergic diseases. The reason for taking amoxicillin was streptococcal infections, identified as per a positive rapid streptococcal test, in 81% of cases. The most common type of rash was EM. There were no patients with blood test results showing EBV infection or mycoplasma

Table 1
Baseline characteristics.

N = 27	
Age (months), median (IQR)	73 (18–87)
Sex (male), N (%)	11 (41)
History of allergic disease, N (%)	15 (56)
Interval time from the first dose of initial reaction (day), median (range)	8 (2–11)
Symptoms, N (%)	
MPE (maculopapular exanthema)	8 (30)
EM (erythema multiforme)	15 (56)
Others	4 (15)
EBNA antibody-positive conversion, N (%)	0 (0)
More than four -fold increase in *Micoplasma neumoniae* antibody titers[†], N (%)	0 (0)

IQR, interquartile range; SD, standard deviation; EBNA, Epstein–Barr viral nuclear antigen.
[†] Particle agglutination test.

infection. A flowchart of participant selection, examination, and diagnosis is shown in Fig. 1. None of the children met the exclusion criteria. All 27 children had negative results for amoxicillin SPT, and 13 of 27 children had positive results for amoxicillin IDT in the delayed phase. The negative predictive value for IDT was 87%. We performed DPT for the 15 children who had negative amoxicillin IDT results, and two had positive results for DPT. Therefore, 14 of 27 (52%) children were diagnosed with delayed hypersensitivity to amoxicillin.

Details of the 14 patients with hypersensitivity to amoxicillin are shown in Table 2. An initial rash was recorded in 12 patients: eight patients with EM and five with MPE. In the two patients who tested positive for amoxicillin DPT, the type of rash was the same as that observed initially, but the number of days from the start of oral administration to the onset of symptoms was different from the initial occurrence (the number of days to development of the initial rash vs the number of days to development of the DPT rash were 8 vs 1 day in Case 1, and 5 vs 7 days in Case 2). The induced symptoms were mild in both patients, and itching was successfully treated with oral antihistamine only.

Characteristics of patients in the hypersensitivity and non-hypersensitivity groups

There was no significant difference in patients' background characteristics between the hypersensitivity and non-hypersensitivity groups. There were no differences in the interval (days) from the first dose to symptom onset ($p = 0.11$), type of rash at the time of onset ($p = 0.46$), spread of symptoms ($p = 0.43$), or the presence or absence of fever ($p = 0.79$) and itching ($p = 0.19$) between the groups (Table 3).

We also examined complications of hypersensitivity to CEX in children in the amoxicillin hypersensitivity group. We noted that two of 14 (14%) patients with hypersensitivity to amoxicillin tested positive for CEX IDT. Among the 12 patients who tested negative for CEX IDT, 10 underwent CEX DPT and one tested positive (thus, IDT had a negative-predictive value of 90%). Therefore, three of 14 (21%) patients with hypersensitivity to amoxicillin also had hypersensitivity to CEX (Fig. 1, Table 2).

DLST

There was no significant difference in the S.I. of DLST between the hypersensitivity and non-hypersensitivity groups in both the acute and non-acute phases (Table 3). In the amoxicillin hypersensitivity group, the median DLST S.I. (median [IQR]) was 1.3 (1.03–1.93) ($n = 8$) in the acute phase and 1.31 (1.13–2.41) ($n = 10$) in the non-acute phase. Only three (38%) of the eight patients who had a DLST value exceeding the Japanese cutoff value of 1.8 at least once were included in the amoxicillin hypersensitivity group. In addition, the two patients (Cases 1 and 2) who had a positive amoxicillin DPT had a DLST S.I. of 1.7 and 2.0, respectively, in the acute phase, and 4.4 and 1.75, respectively, in the non-acute phase; thus, their S.I. value was $\geq$1.8 only once. Over time, changes in the acute and non-acute DLST S.I. values in the hypersensitivity group decreased in two patients and increased in five patients, and the median change rate (IQR) was 1.07 (0.86–1.42). In summary, there was no fixed tendency associated with DLST timing (Fig. 2).

Discussion

In this study, half of the children with a suspicious history had a diagnosis of delayed hypersensitivity to amoxicillin, based on IDT or DPT. Because there was no significant difference in patients' background characteristics between the hypersensitivity and non-hypersensitivity groups, it is necessary to examine patients by IDT or DPT for diagnosis. There were no severe adverse events in patients who underwent both IDT and DPT, as has previously been reported.[5,7]

Method for diagnosis of delayed drug hypersensitivity

The diagnostic ability of skin tests is an issue that warrants further research. The negative predictive value of IDT for amoxicillin

Table 2
Characteristics of children diagnosed with delayed hypersensitivity to amoxicillin and allergy workup results.

Case no.	Age (months)	Interval days of the initial reaction (days)	Initial symptom	Amoxicillin						CEX		
				SPT	IDT (immediate)	IDT (48 h)	DPT	Interval days of DPT (days)	Symptoms of DPT	IDT (48 h)	DPT	Symptoms of DPT
1	61	8	EM	−	−	−	+	1	EM	−	−	
2	18	5	MPE	−	+	−	+	7	MPE	−	+	MPE
3	53	2	EM	−	−	+	Not done			−	−	
4	39	9	EM	−	−	+	Not done			−	−	
5	96	9	EM	−	−	+	Not done			−	−	
6	43	3	EM (partial)	−	−	+	Not done			−	−	
7	88	8	EM (partial)	−	−	+	Not done			−	−	
8	86	10	EM (partial)	−	−	+	Not done			−	−	
9	81	8	MPE	−	−	+	Not done			−	−	
10	83	11	MPE (partial)	−	−	+	Not done			−	−	
11	80	8	EM	−	−	+	Not done			−	Not done	
12	67	8	MPE	−	−	+	Not done			−	Not done	
13	111	8	MPE (partial)	−	−	+	Not done			+	Not done	
14	62	8	Unknown	−	−	+	Not done			+	Not done	

CEX, cefalexin; SPT, skin prick test; IDT, intradermal test; DPT, drug provocation test; EM, Erythema multiforme; MPE, maculopapular exanthema.

Table 3
Characteristics of patients in the hypersensitivity and non-hypersensitivity groups.

Characteristics	Hypersensitivity group (N = 14)	Non-hypersensitivity group (N = 13)	p value
Age (months), median (IQR)	74 (18–85)	73 (22–88)	0.56
Sex (male), N (%)	6 (43)	5 (38)	0.60
History of allergic disease, N (%)	7 (50)	8 (62)	0.36
History of atopic dermatitis, N (%)	4 (29)	4 (31)	0.49
History of other drug allergy, N (%)	0 (0)	0 (0)	
Family history of drug allergy, N (%)	2/14 (14)	1/12 (8)	1.0
Interval between the first dose to the initial reaction (day), median (range)	8 (2–11)	8 (5–11)	0.11
Spread of symptoms, N (%)			0.43
General	8 (57)	6 (46)	
Partial	5 (36)	7 (54)	
Unknown	1 (7)	0 (0)	
Symptoms, N (%)			0.46
Maculopapular exanthema	5 (57)	3 (23)	
Erythema multiforme	8 (57)	7 (54)	
Others	1 (7)	3 (23)	
Complications, N (%)			
Fever	5 (36)	4 (31)	0.79
Pruritus, n/N (%)	11/12 (92)	7/10 (70)	0.19
CEX IDT positive, N (%)	2 (14)	0 (0)	0.17
DLST (S.I. value), median (IQR)			
Acute phase (0–30 days)	1.30 (1.03–1.93) (N = 8)	1.20 (1.15–1.40) (N = 11)	0.68
Non-acute phase (31–180 days)	1.31 (1.13–2.41) (N = 10)	1.33 (1.14–2.14) (N = 13)	0.85

CEX, cefalexin; IDT, intradermal test; DLST, drug-induced lymphocyte-stimulation test; IQR, interquartile range; SD, standard deviation; SI, stimulation index.
Fisher's exact test was used for categorical variables, and the Wilcoxon rank-sum test for continuous variables.

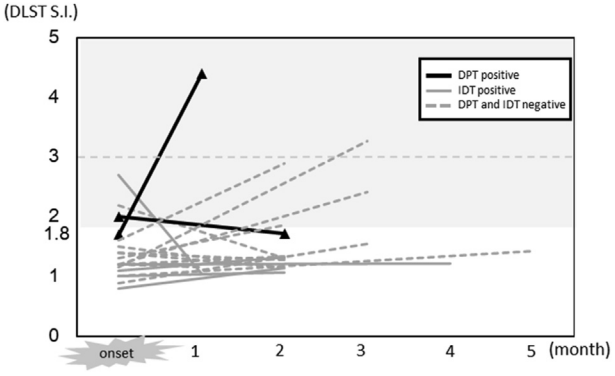

Fig. 2. Changes in drug-induced lymphocyte stimulation test (DLST) results (n = 18). The result is considered positive when the value of the stimulation index (S.I.) is greater than 1.8 in Japan, although it is judged to be positive when it is 2.5–3.0 or higher in other countries. Among the eight participants who had an S.I. value of 1.8 or higher in the acute or non-acute phase DLSTs, five had a negative result in the drug provocation test (DPT). When a DLST cutoff value of 3.0 was used, only two patients had a positive result in the DLST, one who was positive and one who was negative for the DPT. DPT-positive (n = 2), intradermal test (IDT)-positive (n = 5), and DPT-and-IDT-negative (n = 11).

in previous studies was reported to be 87–98%,[4,14–16] which was consistent with the result of the present study. Barni *et al.* conducted IDT and DPT for 352 patients with delayed-type allergy to amoxicillin and reported that the sensitivity of IDT was 8%.[17] In our study, DPT was not performed for IDT-positive patients; hence, the possibility of false-positive IDT results cannot be ruled out. Recently, there have been reports of suspected mild-to-moderate drug allergy cases among pediatric patients undergoing DPT without skin tests, with a positive rate of 0–3%.[1,9,18] Since the countries, drugs, and subjects differ among studies, it is not easy to make comparisons, and the diagnostic rate of 52% obtained in the present study may be too high. Because our hospital is a high-tier medical institution, it is possible that the diagnostic rate was high because the study population included patients with a higher likelihood of allergies, but the possibility of false-positive results for IDT also remains. This is one of the issues warranting analysis in future. Skin tests for non-immediate drug hypersensitivity include patch tests in addition to the IDT delayed phase; however, we did not perform patch tests. It may be necessary to consider how to use it together in the future.

Method and safety of DPT

We believe that a 7-day DPT for IDT-negative children is safe. Two of the 15 patients who had negative results for the IDT were actually positive, but both patients had only a mild rash, were symptomatic, and received only oral antihistamines as a treatment for itching.

Regarding the DPT method, there have been reports of once load, 1-day load,[10] several-day load,[4] 7-day load,[19] etc.; all of these loads could be tested without the occurrence of major adverse events. Furthermore, long-term DPT may have a higher positive rate.[10,20] It remains unknown how many days of loading is best, as long loading periods may also increase the risk of sensitization. In this study, we believe that the 7-day load we chose for this study was an appropriate number of days, because the number of days until the appearance of eruption in DPT did not match the history in our two cases. We believe that the 7-day load we chose for this study was an appropriate number of days, because the number of days until the appearance of eruption in DPT did not match the history in our two cases.

Diagnostic ability of DLST

In our study, DLST was not found to be useful for diagnosing non-immediate hypersensitivity to amoxicillin, and we consider that DLST alone should not be used to diagnose delayed hypersensitivity to amoxicillin. The EAACI position paper states that

DLST should be used as an adjunct to determine whether to perform high-risk tests, such as the DPT.[7] In addition, when drug-induced hypersensitivity syndrome is suspected, the timing of DLST should be considered to avoid the acute phase.[11] For mild-to-moderate drug hypersensitivity reactions, such as MPE and EM, the appropriate timing for performing DLST has not yet been clarified. In this study, DLST was examined at two timings, namely during the acute phase, i.e., within 20 days of onset, and the non-acute phase, i.e., between 30 and 180 days, but neither result was useful for diagnosis. DLST was examined only for two children who had positive DPTs; thus, it was not possible to set an appropriate cutoff value.

Cross-reactivity with CEX

In one review, the complication rate of hypersensitivity to cephalosporin, which has an R chain with high homology to that of amoxicillin, was reported to be 16.4%.[21] In this study, three children (21%) in the amoxicillin hypersensitivity group were diagnosed with hypersensitivity to CEX.

Limitations

In this study, we cannot rule out the possibility of false-positive IDT results because we judged that IDT in the delayed phase was positive, without performing DPT in most cases, as described above. Since raw materials for skin tests are not available in Japan, oral drugs containing other additives were used for IDT with the consent of parents in our study. Therefore, false positives that responded to additives instead of amoxicillin cannot be denied. Conversely, there may have been false-negative IDT results because IDT reagents, such as major determinants, are not available in Japan. It is possible that this was also the reason for the two IDT-negative/DPT-positive cases in this study. In addition, DPT is conducted for patients in a healthy state, but even if the DPT is negative, symptoms may appear under the influence of cofactors, such as fever and infectious diseases, so that false-negative DPT results may be obtained. A subsequent questionnaire survey was conducted among adults who had findings suggestive of delayed hypersensitivity to amoxicillin, but were DPT negative, and it revealed that the negative-predictive value of DPT was 94.9%.[22] The strength of our study is that we were able to demonstrate that half of the suspected patients did not have hypersensitivity to amoxicillin, by performing DPT in addition to IDT, and that these tests were safe. Since IDT may have been false positive, it is necessary to perform DPT regardless of the results of IDT and examine the accuracy of the IDT in future.

Conclusion

We conducted IDT and DPT for pediatric patients who developed eruptions after the oral administration of amoxicillin, and 52% of children were diagnosed with delayed hypersensitivity to amoxicillin. The absence of differences in background characteristics of patients in the hypersensitivity and non-hypersensitivity groups, emphasizes the necessity to examine patients by IDT or DPT for diagnosis, and to assess cases of moderate or mild symptoms.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

YK wrote the manuscript; All authors collected clinical data. ON, and RY gave technical support and conceptual advice. All authors read and approved the final manuscript.

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