

# Skin and laboratory tests in amoxicillin- and penicillin-induced morbilliform skin eruption

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## Summary

**Background** Cutaneous amoxicillin- and penicillin-mediated reactions can be classified as immediate and delayed-type reactions. Immediate reactions are thought to involve IgE antibodies and have been studied extensively. In contrast only few data exist about delayed reactions such as morbilliform or maculopapular rash.

**Objective** To assess the predictive value of immediate skin tests, skin-patch tests, specific IgE and lymphocyte transformation tests with regard to the diagnosis of delayed skin eruptions.

**Methods** Skin and *in vitro* tests were performed in 18 subjects. Twelve subjects had penicillin- or amoxicillin-induced morbilliform exanthema and six were controls without hypersensitivity reaction, tested before and after exposure.

**Results** Specific IgE to penicillin and immediate penicillin skin tests were negative in amoxicillin- or penicillin-induced delayed skin eruptions. In contrast, skin-patch testing and LTT were positive in 9/12 or 10/12, respectively, but negative in all six controls.

**Conclusion** These findings substantiate a T-cell-mediated immune pathomechanism in the majority of penicillin-induced delayed skin reaction. Moreover, they underline the necessity to adapt the test procedures to underlying pathomechanisms and support the diagnostic value of skin-patch testing and LTT in delayed cutaneous reactions to penicillins.

**Keywords:** amoxicillin, drug eruptions, drug hypersensitivity, lymphocyte transformation test, maculopapular rash, patch test, penicillin, T-lymphocytes

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## Introduction

The incidence of hypersensitivity reactions to amoxicillin and penicillinG in exposed subjects is estimated to be between 1% and 10% [1,2]. The most common manifestations are cutaneous reactions. These side-effects may involve various types of immunological and non-immunological mechanisms. They can be classified as immediate and delayed reactions [3]. Immediate reactions involve IgE antibodies and have been studied extensively. For these reactions sequential skin tests (prick/scratch followed by intradermal testing), including penicillinG, amoxicillin, penicilloate and penilloate reagents coupled with a polylysine carrier, are a safe and reliable diagnostic tool [4,5].

However, the majority of amoxicillin- and penicillinG-induced skin eruptions are morbilliform or erythematous exanthema. They appear to have increased [6], due probably to the enhanced use of oral aminopenicillins [7,8]. This type of hypersensitivity reaction has been little studied [1,7–13] and the pathogenesis of these reactions has remained controversial: some reactions, in particular the exanthema in association with EBV-infections, have been considered to be not allergic as they do not normally reappear after re-exposure to the drug [14]. On the other hand, clinical features [8] and many experimental data support an allergic, T-cell mediated process in these skin eruptions. Skin infiltration consists of CD4 cells [9], drug-specific T cells can be found in the circulation as revealed by positive lymphocyte transformation tests (LTT) and drug-specific T-cell clones could even be eluted from the affected skin [10]. Thus, patch and LTT seem to be more suitable methods for the

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diagnosis of this type of reaction [11–15]. However, the diagnostic value of patch tests and LTT remains controversial, as these tests have been rarely evaluated in control individuals who tolerated the drug. In this study we examined immediate skin tests, skin-patch tests, specific IgE and LTT in 12 patients with clinically diagnosed penicillinG- or amoxicillin-induced morbilliform exanthema and in six amoxicillin-exposed controls without hypersensitivity reaction.

## Methods

### *Patients and definitions*

The case history documents of all patients ( $n = 94$ ) who were referred between December 1994 and March 1997 to the allergy and immunology outpatient clinic of the university hospital in Bern with supposed hypersensitivity reaction to amoxicillin or penicillinG were analysed by one investigator, who did not know the results of skin or *in vitro* tests. If necessary for the following classification, the history was completed by telephone calls to the patients. After classification, test results were analysed and complemented if necessary and possible.

For this study an amoxicillin- or penicillinG-induced skin rash was defined exclusively by the history fulfilling the following criteria:

- skin manifestations within 2 weeks after application of amoxicillin or penicillinG;
- no involvement of other drugs than amoxicillin or penicillinG; and
- no evidence that the skin reaction may have been elicited by another mechanism, such as viral infection.

Immediate or accelerated (type I) hypersensitivity skin reactions were defined as:

- skin reactions with wheals and/or angio-oedema; and
- reactions which developed within 1 h after penicillinG or amoxicillin administration

Delayed-type exanthemas were defined as:

- reactions which did not resolve before 72 h; and
- reactions which were described by the patients or treating physician as measles-like or scaly in nature.

Negative controls were seven (four female and three male) volunteers who were tested before and 2 months after exposure with amoxicillin  $2 \times 750$  mg/day for 2 weeks. One healthy volunteer became infected with a viral infection and developed a generalized morbilliform exanthem on day 7 during the amoxicillin exposure and was excluded.

### *Skin tests*

Sequential skin tests (scratch followed by intradermal testing) using commercial available extracts [0.05 mL

benzylpenicilloyl coupled to polylysine (BPO-PLL, 0.175 mg/mL)] and 0.05 mL minor determinants mixture [0.6 mg monosodium-benzylpenicillin/mL, 0.5 mg disodium-benzylpenicilloate/mL coupled to polylysine (MDM-PLL, Allergopharma, Reinbeck 21465, Germany)] were performed on the volar side of the forearms. Results were recorded 15 min after testing and compared to the positive (1 mg/mL histamine diphosphate) and negative control (phosphate buffered saline [PBS]). A test was considered positive when a wheal  $> 5$  mm and a flare developed within 15 min. If the results were negative, so-called scratch/patch skin tests were performed with penicillinG (200 000E/mL PBS), amoxicillin (750 mg/mL PBS) and with PBS as control. With scratch/patch tests, a gentle,  $\approx 1$ -cm-long scratch with a needle at the site of drug application is performed, which removes only the upper layer of the keratinocytes and does not cause bleeding. Patch tests were read 24 h and 48 h later and considered positive when a clear reddening and infiltration could be observed.

### *In vitro tests*

Specific IgE was assessed using a commercially available kit to PenicilloylG and Penicilloyl V (CAP FEIA, Pharmacia AB, Uppsala, Sweden).

Lymphocyte transformation tests (LTT) were performed as described [15]. Briefly, mononuclear cells (PBMC) of the patient were isolated by density gradient centrifugation (Ficoll/Hypaque) and cultured in tubes at a cell density of  $10^6$  cells/tube in 1 mL medium (RPMI-1640 supplemented with 20% autologous plasma or AB serum) with and without penicillinG 0.5 mg/mL and 1 mg/mL, respectively, as triplicate. Amoxicillin was evaluated at a concentration of 0.2 mg/mL and 0.5 mg/mL. These concentrations have been evaluated previously in cytotoxicity tests and found to be not cytotoxic to PBMC. They are about 12 times higher than serum peaks following standard therapy with the corresponding drug. After 5 days the proliferation was determined by [ $^3$ H]thymidine incorporation (counts per minute = c.p.m.). The result was expressed as stimulation index [(c.p.m. of culture with drug):(c.p.m. of culture without drug)]. A stimulation index (SI) of more than 2.5 was interpreted as positive.

## Results

### *Classification*

Twenty-two of 94 patients with supposed penicillin hypersensitivity fulfilled the strict criteria of a very probable amoxicillin- or penicillinG-induced skin reaction. Eight could not be classified clearly as immediate or delayed reaction. Twelve of the remaining 14 subjects had a delayed

reaction with maculopapular exanthem and two an immediate reaction with urticaria and angioedema.

#### Penicillin- and amoxicillin-specific IgE

None of the 12 patients with amoxicillin/penicillinG-induced maculopapular delayed skin reaction had a clear positive specific IgE (11 CAP class 0; 1 CAP class 1). Even in the two patients with immediate and presumably IgE-mediated reaction, circulating IgE was not detected (time interval between exposure and testing in one patient was 5 years, in the other 1 month). In contrast, one individual of the negative control group who had no detectable penicillin-specific IgE before exposure showed a raised BPO-specific IgE (CAP class 3) after exposure.

#### Immediate skin tests

No immediate skin reaction was observed in the 12 patients with amoxicillin/penicillinG-induced maculopapular skin reactions (neither scratch tests with amoxicillin, penicillinG, BPO-PPL and MDM-PLL nor intradermal tests with BPO-PPL and MDM-PLL were positive). Two patients had an unintentional re-exposure to amoxicillin/penicillinG and had developed severe, long-lasting skin eruptions. In both patients an erythematous infiltration appeared 12–36 h after skin testing in the area of the intradermal test with BPO-PPL, which persisted for several days. Such delayed reactions to immediate skin tests are unusual but may occur sporadically [16] and might be a sign of strong sensitization.

**Table 1** Skin and *in vitro* tests in patients with amoxicillin/penicillin hypersensitivity

| No.                                                     | Sex | Age | Drug | Scratch | i.d.   | Patch* | CAP  | LTT | Interval |
|---------------------------------------------------------|-----|-----|------|---------|--------|--------|------|-----|----------|
| Delayed skin eruptions                                  |     |     |      |         |        |        |      |     |          |
| 1                                                       | f   | 70¶ | A/P§ | 0       | 0†     | P/A    | 0    | P/A | 2m/15y   |
| 2                                                       | m   | 67¶ | P‡   | 0       | 0†     | A      | 0    | A   | 3m/10y   |
| 3                                                       | m   | 48  | A    | 0       | 0      | 0      | 0    | neg | y        |
| 4                                                       | f   | 47  | A    | 0       | 0      | A      | 0    | A   | y        |
| 5                                                       | f   | 46  | A    | 0       | 0      | P/A    | 0    | neg | 3m       |
| 6                                                       | f   | 46  | P    | 0       | 0      | 0      | 1    | P   | 15y      |
| 7                                                       | f   | 45  | P    | 0       | 0      | P      | 0    | P/A | 1y       |
| 8                                                       | m   | 42  | A    | 0       | 0      | P/A    | 0    | A/P | 10y      |
| 9                                                       | f   | 40  | A    | 0       | 0      | A      | 0    | A/P | 2y       |
| 10                                                      | f   | 35  | A    | 0       | 0      | A      | 0    | A   | 4m       |
| 11                                                      | m   | 30  | A    | 0       | 0      | 0      | n.d. | A/P | 4m       |
| 12                                                      | m   | 25  | A    | 0       | 0      | A      | n.d. | A/P | 6m       |
| Immediate skin eruptions                                |     |     |      |         |        |        |      |     |          |
| 13                                                      | m   | 52  | A    | 0       | PPL    | 0      | 0    | neg | 5y       |
| 14                                                      | f   | 37  | A/P  | PPL/A   | n.d.   | n.d.   | 0    | neg | 1m       |
| Skin eruptions which could not be classified by history |     |     |      |         |        |        |      |     |          |
| 15                                                      | f   | 69  | A    | 0       | 0      | P      | 0    | A   | 5y       |
| 16                                                      | m   | 52  | A    | 0       | 0      | 0      | 0    | A   | 2m       |
| 17                                                      | f   | 44  | A    | 0       | 0      | 0      | 0    | A   | 6m       |
| 18                                                      | m   | 30  | P    | 0       | 0      | 0      | 0    | P   | 2m       |
| 19                                                      | f   | 25  | A    | 0       | 0      | P      | 0    | A   | 2y       |
| 20                                                      | f   | 24  | A    | 0       | 0      | P      | 0    | neg | 10y      |
| 21                                                      | m   | 20  | P    | 0       | 0      | 0      | 0    | P   | 3m       |
| 22                                                      | f   | 20  | A    | P/A     | PPL/MD | 0      | 0    | PA  | 2y       |

§A: amoxicillin; P: penicillin.

LTT: lymphocyte transformation tests with A and P: if stimulation indices were  $\geq 2.5$ , the LTT is considered positive and the reactive drug is indicated. Stimulation indices  $< 2.5$  were considered negative.

\*If positive, the reactive drug is indicated.

†Not immediate, but delayed reaction with onset 12–36 h later.

‡Clinically and in LTT cross-reaction to imipenem.

¶Unintentionally re-exposed with severe generalized skin reaction.

Interval: interval between exposure and test (m = month, y = year).

0: no reaction; n.d.: not done.

**Table 2.** Skin and *in vitro* tests in controls

| No.                                                                           | Sex | Age | Before exposure |      |       |      |      | After exposure |      |       |     |     |
|-------------------------------------------------------------------------------|-----|-----|-----------------|------|-------|------|------|----------------|------|-------|-----|-----|
|                                                                               |     |     | Scratch         | i.d. | Patch | CAP  | LTT  | Scratch        | i.d. | Patch | CAP | LTT |
| Controls without rash                                                         |     |     |                 |      |       |      |      |                |      |       |     |     |
| 1'                                                                            | m   | 49  | 0               | 0    | 0     | 0    | neg  | 0              | 0    | 0     | 3   | neg |
| 2'                                                                            | f   | 40  | n.d.            | n.d. | n.d.  | n.d. | n.d. | 0              | 0    | 0     | 0   | neg |
| 3'                                                                            | f   | 36  | 0               | 0    | 0     | 0    | neg  | 0              | 0    | 0     | 0   | neg |
| 4'                                                                            | m   | 36  | 0               | 0    | 0     | 0    | neg  | 0              | 0    | 0     | 0   | neg |
| 5'                                                                            | f   | 35  | 0               | 0    | 0     | 0    | neg  | 0              | 0    | 0     | 0   | neg |
| 6'                                                                            | f   | 30  | n.d.            | n.d. | n.d.  | n.d. | n.d. | 0              | 0    | 0     | 0   | neg |
| Control with viral infection and a morbilliform rash on day 7 during exposure |     |     |                 |      |       |      |      |                |      |       |     |     |
| 7'                                                                            | m   | 40  | 0               | 0    | 0     | 0    | neg  | 0              | 0    | 0     | 0   | neg |

§ A: amoxicillin; P: penicillin.

LTT: lymphocyte transformation tests with A and P: if stimulation indices were  $\geq 2.5$ , the LTT is considered positive and the reactive drug is indicated. Stimulation indices  $< 2.5$  were considered negative.

¶ Positive reaction to a combination of amoxicillin (75%) and clavulanic acid (25%) with an SI of 2.5 (0.2 mg) and 2.8 (0.5 mg/mL).

0: no reaction; n.d.: not done.

Both patients with clinically clear immediate reactions showed a strong immediate positive test, one in the scratch tests to BPO-PPL and amoxicillin, the other in the intradermal tests to BPO-PPL. In the control group no immediate skin reaction was observed before ( $n = 4$ ) and after exposure ( $n = 6$ ).

#### Scratch/patch skin tests

In nine of the 12 patients with amoxicillin/penicillinG-induced maculopapular skin reactions the scratch/patch

testing was positive, whereas no reaction occurred in the two patients with clinically immediate reactions (Table 1). In the control group no skin reaction was observed before or after exposure. The person with a generalized morbilliform exanthem after a viral infection with concomitant amoxicillin treatment remained negative in skin tests (Table 2). From these data a negative predictive value of 67% and a positive predictive value of 100% for the diagnosis of penicillin-antibiotic-induced maculopapular delayed skin reaction can be calculated.

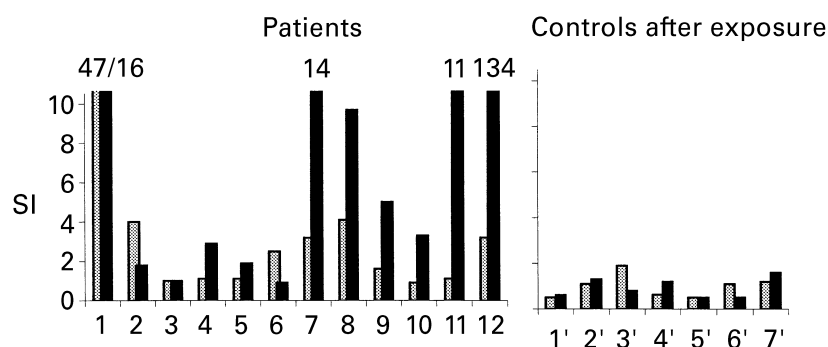


**Fig. 1.** Lymphocyte transformation tests in patients with delayed skin eruptions and in exposed controls.

Shaded bars: stimulation indices with penicillinG at a concentration of 0.5 mg/mL.

SI-values  $> 10$  are indicated above the bars.

Solid bars: stimulation indices with amoxicillin at a concentration of 0.5 mg/mL.

SI-values  $> 10$  are indicated above the bars.

Values for penicillin at a concentration of 1 mg/mL and amoxicillin at 0.2 mg/mL, respectively, were in the same range and are not indicated. Four of the controls were also evaluated before exposure, with negative values ( $SI < 2.5$ ).

### *Lymphocyte transformation test (LTT)*

The LTT was positive for penicillin or amoxicillin in 10 of 12 patients with amoxicillin/penicillinG-induced maculopapular skin reaction and in neither of the two patients with immediate reaction. In the control group no positive LTT developed and even the subject with positive serum IgE remained negative (Fig. 1). In our small study sample, the negative predictive value for the diagnosis of penicillin-antibiotic-induced maculopapular delayed skin reaction was 75%, the positive predictive value 100%. The person with exanthem after concomitant viral infection and amoxicillin exposure had a slightly elevated LTT against the combination of amoxicillin (75%) and clavulanic acid (25%) after exposure (Table 2).

### Discussion

Non-immediate skin reactions to penicillin are a common clinical problem but only few data about the diagnostic methods for this type of drug-induced skin reaction have been generated. Since no established test procedures are available, provocation with the supposed causative drug is thought to be the 'gold standard'. However, the lack of reactivity in a provocation test does not rule out the existence of drug-specific sensitization. If the precursor frequency of drug specific T-cells is low and/or the TCR has a low affinity for the hapten, clinical symptoms may only reappear upon re-exposure when additional, but hitherto ill-defined factors, are present (i.e. generalized viral infections [17]). Indeed, one volunteer caught a viral infection during treatment with amoxicillin and developed a generalized morbilliform exanthem on day 7. He remained negative in skin tests but showed a small reactivity in the LTT against the combination of amoxicillin and clavulanic acid. An oral provocation with 1 g amoxicillin did not elicit a reaction. Moreover, the value of provocation tests in delayed skin reactions is controversial, as the delayed drug reactions may not appear before several days of a new exposure (own observations in patients with history of unintentional re-exposure) and/or require more than one provocation dose. In addition, although delayed skin reactions are usually believed to be harmless, even they may be hazardous [18] as two of our patients with unintentional re-exposure developed a long-lasting and severe reaction. Therefore, and because we were mainly interested in detecting a sensitization to  $\beta$ -lactam antibiotics, we chose the history as 'gold standard', which had to be extremely suggestive (cf. Methods).

Only 12 of 94 patients consulting our outpatient clinic because of suspected penicillin allergy fulfilled our rigorous criteria for penicillin allergy. We also included six volun-

teers as a control group, who were tested before and after taking amoxicillin for 10 days without developing side reactions. In spite of the low number of patients we obtained clear results which allow the following conclusions. In all 12 patients with delayed skin reactions to amoxicillin/penicillinG, no evidence of IgE mediated sensitization was found; the serology was negative, skin tests with BPO-PLL and MDM-PLL were negative, and scratch tests with the incriminated penicillin itself were also negative. In contrast, patch tests and LTT, which detect T-cell reactions, were positive in 9/12 and 10/12, respectively. The clinical specificity of these tests in our small sample size was excellent, as none of the amoxicillin-exposed asymptomatic controls were positive in the LTT or skin tests. Thus, the findings substantiate a specific immune reaction with involvement of drug-specific T cells in the majority of amoxicillin/penicillinG-induced delayed skin eruptions. Such a pathomechanism is underlined further by previous studies showing histological findings of skin biopsies with T-cell infiltration in [7,8] and the cultivation of drug-specific T-cell clones [10,19].

In all patients with amoxicillin-induced morbilliform rash, which had a positive test result, the patch reaction, and LTT were directed also/or only to amoxicillin, which underlines the value of tests with the culprit drug and the role of the amino group in the (D-) alpha position in delayed-type allergic reaction to aminopenicillins [20].

Our data indicate that the choice of tests to pinpoint the incriminated drug is highly relevant and that the pathomechanism underlying the clinical symptoms must be considered: in non-immediate reactions skin and laboratory tests for T cell sensitization may be positive in the absence of an IgE-mediated sensitization. Immediate skin tests are a fairly safe and reliable diagnostic tool for immediate reactions involving IgE antipenicillin antibodies, but do not detect sensitization causing delayed skin eruptions such as morbilliform exanthema. The examination of the latter reactions should include, as well as immediate tests to rule out an IgE-mediated component, skin-patch tests and/or LTT. Unfortunately, the latter are poorly standardized and not commercially available. The relevance of a positive test (patch or LTT) for the development of clinical symptoms is difficult to predict in the individual case. One should, however, be aware that re-exposure can also be hazardous in delayed amoxicillin/penicillinG reactions [18,21]. If alternative medications are available, a positive test may be a sufficient indicator to avoid further penicillinG or amoxicillin treatment.

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