

Determinations of Penicilloyl Specific IgE Antibodies for the Evaluation of Hypersensitivity against Penicillin

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Results from skin tests and radioallergosorbent test (penicilloyl G and penicilloyl V) were compared in 76 patients with suspected penicillin hypersensitivity and in 22 control subjects without such clinical symptoms. The test results were also correlated to the clinical history of adverse reactions and to the levels of total serum IgE. The overall agreement between skin test and RAST results was 89%, in the patient group. Two of the control subjects without symptoms were professionally employed with penicillin production; one of them had circulating IgE-antibodies and the other exhibited positive skin tests. The skin tests were more frequently positive than the RAST test. By means of skin titration some of the skin tests were demonstrated to be false positive. The discrepancy can also be explained by the fact that skin reactions also occur in other situations than IgE-mediated adverse reactions. Circulating IgE-antibodies were not found in any of 23 cases where adverse reactions appeared later than 24 h after the latest penicillin administration.

It is concluded that the measurement of circulating IgE-antibodies is a valuable aid in the diagnosis of penicillin allergy.

Keywords: hypersensitivity; immunoglobulin E; laboratory diagnosis; penicillin; radioallergosorbent test; skin tests.

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Penicillin represents a very potent sensitizing drug capable of causing a wide variety of hypersensitivity symptoms. The evaluation of such manifestations is difficult in many respects.

The diagnosis of penicillin allergy is based mainly on the patients' clinical history and the actual symptoms of hypersensitivity. In cases of multiple drug administration the identification of the drug responsible for the allergic reaction may be difficult. Suspected reactions may actually be manifestations of the patient's illness. Among the available diagnostic tests for the immediate type allergic reactions most

data are available concerning the significance of skin tests. The value of each new method for the diagnosis of penicillin allergy is therefore best evaluated in comparison with skin tests.

In the case of immediate type penicillin allergy skin testing is not free of risk for the patient; it is also time-consuming and requires reliable reagents and experience. Therefore skin testing should be limited to specialized centres. The results of skin tests may not be conclusive and the test cannot be performed during the acute phase of a reaction affecting the skin.

Among several *in vitro* tests that have been

suggested the radioallergosorbent test (RAST) (14) has been reported to give promising results in patients with a history of immediate type penicillin allergy (1, 5, 6, 15). In the present study, skin test and Phadebas RAST results were compared in patients with suspected penicillin hypersensitivity who had been subjected to routine diagnosis. The test results were also correlated with the period of time between penicillin administration and the onset of adverse reaction.

MATERIAL AND METHODS

Patients

Seventy-six patients (aged 10–89 years) with a clinical history of suspected penicillin hypersensitivity were studied. Age and sex distribution is shown in Table 1. Sixty-one patients had been referred to the University Clinic of Dermatology and Venereal Diseases, Innsbruck and 15 had been referred to the II. Med. Dept. of the County Hospital, Graz. Serum samples were collected during the period 1974–1975, except for two samples that were drawn in 1976 and 1977 respectively.

Control individuals

Twenty-two control subjects were also subjected to study. None of them had any symptoms of adverse reactions against penicillin. Three had symptoms of pollen allergy and two were professionally employed in penicillin

production facilities. Nine had been treated with penicillin without developing any symptoms of penicillin hypersensitivity.

Skin tests

Usually the skin tests were performed in connection with the blood sampling for *in vitro* test analysis. Only immediate skin test reactions were recorded for the purpose of this study.

At the Innsbruck clinic the patients and 10 control individuals were skin tested by means of end point titration with prick and intracutaneous methods, with penicillin G and PPL (penicilloyl-polylysine), according to the technique described by Zelger (16). Intracutaneous tests were only performed when the preceding prick tests were negative. Penicillin G was applied in concentrations of 1, 10, 100, 1,000 and 10,000 U/ml and PPL in concentrations of 1×10^{-6} , 1×10^{-5} , 6×10^{-5} , 12×10^{-5} and 50×10^{-5} mol (Cilligen, Sigma Chemical Co., St. Louis, Missouri, U.S.A.).

Fourteen of the 15 patients investigated in Graz were subjected to scratch tests with penicillin G (200,000 U/ml) and PPL (500 nmol benzyl-penicilloyl = BPO/ml). If scratch tests were negative intracutaneous tests were generally performed with PPL at a concentration of 25 nmol BPO/ml and if negative, by a follow-up test using 250 nmol BPO/ml. Two control subjects were also skin tested, one of them only by the scratch procedure.

Total and specific IgE determinations

The serum samples were stored deep-frozen until transport on dry ice to Pharmacia Diagnostics AB, Uppsala, where RAST analyses were carried out with Phadebas RAST® Penicilloyl G and Phadebas RAST® Penicilloyl V (Pharmacia Diagnostics AB, Uppsala, Sweden). The assays were performed and results evaluated according to the directions for use of the test.

Total serum IgE determinations were

Table 1
Age and sex distribution of investigated patients and control subjects

Age (years)	Patients			Control subjects		
	Female	Male	Total	Female	Male	Total
0–20	1	5	6	2	3	5
21–40	21	15	36	6	2	8
41–60	12	14	26	1	1	2
>60	3	5	8	2	2	4
Unknown				1	2	3
Total	37	39	76	12	10	22

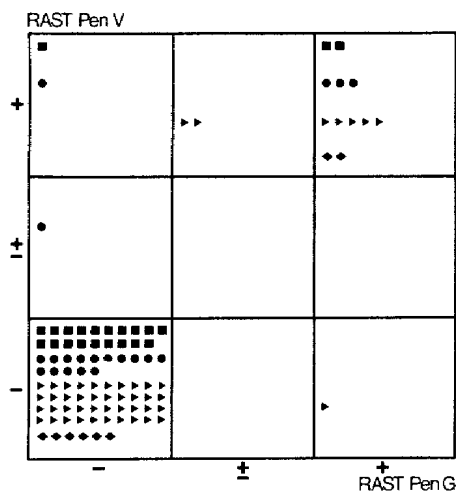


Fig. 1. Comparison between results obtained with Phadebas RAST penicilloyl G and penicilloyl V. $n = 98$. Symbols:

- ◆ = patients with anaphylaxis ($n = 8$)
- ▲ = patients with urticaria without anaphylaxis ($n = 48$)
- = patients with other adverse reactions ($n = 20$)
- = control individuals ($n = 22$)

simultaneously carried out on all control serum samples and on all but two patient serum samples. Phadebas IgE PRIST® (Pharmacia Diagnostics AB, Uppsala, Sweden) was used according to the directions for use.

RESULTS

RAST results with penicilloyl G versus penicilloyl V

Fifteen patients and three control individuals had circulating IgE-antibodies against one or both allergen preparations included in the RAST test. There was good agreement between the two sets of results (Fig. 1). One patient and one control had detectable IgE-antibodies against only penicillol V and one patient was RAST test positive only with regard to penicilloyl G. One patient exhibited a borderline RAST value with regard to penicilloyl V but was negative for penicilloyl G. Two urticaria cases on borderline for penicilloyl G were RAST test positive for penicilloyl V.

RAST versus skin test results

Ninety-eight per cent (52/53) of the skin test negative patients were also RAST test negative with regard to both allergen preparations (Table 2). Sixty-two per cent (13/21) of the skin test positive cases also gave similar results in the RAST test. Of the eight remaining skin test positive patients seven were RAST test negative (four skin test positive, only upon intradermal testing) and one intradermal test positive patient exhibited a borderline RAST result. The overall agreement between skin test and RAST results was 89% (the two borderline cases are not included). In six patients (two anaphylaxis, three urticaria and one serum sickness) immediate skin test reactions appeared but the weal area did not increase with increasing allergen concentration. They were therefore evaluated as non-specific. Circulating antibodies were not detected by the RAST test in any of these six cases.

Anaphylaxis. Out of eight patients, with a history of anaphylactic shock or shock fragments, two (25%) had positive skin reactions upon testing, as well as having IgE-antibodies against penicilloyl G and V

Table 2

Agreement between skin test and RAST results ($n = 75$ patients)

		Skin tests			Total
		-	±	+	
RAST	-	52 (98%)	1	7* (33%)	60
	±			1** (5%)	1
	+	1 (2%)		13 (62%)	14
Total		53 (100%)	1	21 (100%)	75

If borderline cases are excluded:

RAST test agreement with positive skin tests: 65%

RAST test agreement with negative skin tests: 98%

Average agreement between RAST and skin tests: 89% (65/73)

* In four cases skin tests positive only upon intradermal testing.

** Skin tests positive only upon intradermal testing.

conjugates in their serum (Table 3 and Fig. 1). The six test negative patients all experienced their adverse reactions more than 2 years prior to the blood sampling for RAST analysis.

Urticaria. Eight of the 48 (16.7%) patients with urticarial reactions, possibly caused by penicillin treatment, had circulating IgE antibodies against one or both conjugates used (Table 3 and Fig. 1). All of them also exhibited positive skin test reactions. In six of the cases the adverse reaction was experienced in the year prior to blood sampling, and the remaining two patients had had their reactions 2 and 6 years respectively prior to blood sampling. Weal and flare reactions upon skin testing also occurred in six patients in whom circulating IgE-antibodies could not be detected with the RAST test. Three of them were skin test positive only upon intradermal testing.

Other adverse reactions. Four (20%) of the patients exhibiting reactions other than anaphylaxis or urticaria had circulating IgE-antibodies as detected by the RAST test (Table 3 and Fig. 1). Three of them were also skin test positive and had had their adverse reactions in the year prior to blood sampling. In the fourth case 6 years had passed since the penicillin injection, which had been followed by exanthema. In one RAST test negative case (serum sickness) the intracutaneous tests were positive with both penicillin G and PPL.

Table 3
Distribution of patients and control individuals included in the study according to negative and positive skin test and RAST results

Reaction upon drug treatment	Skin tests				RAST			
	-	±	+	Total	-	±	+	Total
Anaphylaxis	6	2	8	6	2	8		
Urticaria	33	14	47	40	8	48		
Serum sickness	2	3	5	3	2	5		
Other reaction	12	1	2	15	12	1	2	15
Controls	11	1	12	19	3	22		
Total	87				98			

Test results versus time of onset of reactions related to penicillin administration

In seven RAST test positive cases the time between the latest penicillin administration and the onset of the adverse reaction was known. In each of these seven patients the reaction appeared within 1 day (Table 4).

Of the eight patients where skin test results were borderline or positive, but no circulating IgE-antibodies were detected by the RAST test, seven had their first adverse symptoms 1-12 days after completed antibiotic treatment. In the eighth patient in this category the urticaria appeared 30 min after administration of the first tablet (Table 4).

Of the patients where both skin test and RAST results were negative, information on the onset of reaction related to administration of antibiotic drugs was available in 20 of the cases. In 16 of them symptoms appeared 1-10 days after the last treatment, in three between 1.5 and 4 h after and only in one case within minutes after the drug administration (Table 4).

Total serum IgE

Of 26 patients with total serum IgE levels < 100 U/ml 7.7% were found to have specific IgE-antibodies against penicilloyl conjugates.

Table 4
Test results compared with onset of reaction related to the latest penicillin administration (*n* = 35 patients)

	Period of time between latest penicillin administration and onset of symptoms of adverse reaction		
	< 1 hour	1 hour - < 1 day	1 - 12 days
RAST test positive	4	2	
Skin test positive			
RAST test positive	1		
Skin test negative			
RAST test negative	1		7
Skin test positive			
RAST test negative	1	3	16
Skin test negative			

The corresponding figures for patients with IgE levels of 100–500 and > 500 U/ml were 27.6% and 22.2% respectively.

Control results

IgE-antibodies were detected with the penicilloyl V conjugates in the serum from three control individuals. Two of them also had circulating IgE-antibodies against the penicilloyl G conjugates. They all had negative skin tests. The two individuals who had both RAST tests positive had, however, markedly elevated total IgE-levels and had been treated with penicillin. One of them was a 74-year-old woman and the other one a chronic alcoholic. Neither of them could remember any adverse reactions upon treatment with penicillin in the past and no allergic reaction was observed during recent hospitalization during which penicillin treatment was given and the blood sample was drawn.

The third control individual in whom IgE-antibodies were detected had not received penicillin treatment recently. He was, however, professionally occupied in facilities where penicillin was prepared and was suffering from asthma bronchiale. Another control individual who worked in facilities for penicillin preparation was symptom-free, had not been treated with penicillin and had no detectable IgE-antibodies against the two antigen preparations applied in the RAST test. However, the intracutaneous tests were both positive.

None of the remaining control individuals had detectable levels of circulating IgE-antibodies. Two of the pollen allergic controls had significantly elevated total IgE-levels.

DISCUSSION

In patients with penicillin allergy of type I (reaginic type) the performance of skin tests using high concentrations of penicillin may cause anaphylactic reactions. In rare cases, lethal reactions have occurred (2). The risk may be reduced to a minimum by skin test titrations with penicillin reagents (4, 9, 16).

The skin titration with penicillin also allows

for the distinction between specific and non-specific test reactions. Using stepwise increasing penicillin concentrations, the specific test reactions become stronger while non-specific test reactions do not change with antigen concentration. However, there is a possibility of misinterpretation if only the highest penicillin concentration causes a positive (erythematous-urticarial) reaction in the intracutaneous test. It is likely that this was the case in three of the six urticaria patients where the skin test was evaluated as positive but the RAST results were negative.

Another cause for discrepancies between skin test and RAST results could be the difference in the duration of the presence of specific antibodies in serum and skin. According to Zelger (17) the repetition of the skin tests after 3 months to 6 years, using penicillin G in 28 patients resulted in no increase in any patient. Five patients showed identical titers and 23 patients presented lower or no titers. A decrease in circulating specific IgE-antibody levels also has been reported by Kraft et al. (5).

Further, discordant results in some cases are related to the fact that the RAST results demonstrate only type I allergies whereas skin titrations indicate also type IV (delayed hypersensitivity) allergies. Patients with contact allergy to penicillin present an eczematous reaction at the sites of the intracutaneous tests to the highest penicillin concentrations after 24–48 h. In the present study there were three patients with such delayed reactions and histories of contact allergy. These three patients had negative RAST results and no immediate skin test reaction.

Although skin test results are positive more frequently than the RAST results, it is likely, that the RAST method sufficiently identifies the immediate type reactions mediated by IgE-antibodies, which are of the greatest clinical importance. Although only few patients with a history of anaphylactic reactions against penicillin were included in this study there was a 100% accordance between skin test and RAST results.

The finding of more frequent positive skin tests than positive RAST results may be explained by the fact that some patients who have had past allergic reactions to penicillin do not show an adverse reaction to a second challenge despite positive skin test (3). The discrepancy could also be due to reaginic antibodies other than IgE which are not detected by the penicilloyl specific IgE Phadebas RAST used in this study. The possible role of IgG4-antibodies for discordance between skin test and RAST results in various allergies is discussed by Vijay et al. (12).

Systematic prospective studies of penicillin administration to patients with positive skin tests do not seem to exist on a large scale for obvious reasons. Levine & Zolov (8) report on eight penicillin allergic patients with positive skin tests against the major determinant and negative results with the minor determinants, five of whom showed accelerated urticarial reactions to penicillin therapy. In three further penicillin allergic patients with positive skin tests only to the minor determinant mixture, two presented accelerated and one an immediate urticarial reaction.

Although it seems that not all of the above patients have reacted despite a history of penicillin allergy and a positive skin test result, the administration of penicillin to patients under such conditions can be a very high risk for the patient. A positive result obtained in a properly performed skin test should mean a contraindication for the administration of penicillin probably for the lifetime of the patient. On the other hand, a true negative result under the same optimal conditions does not absolutely exclude a possible hypersensitivity reaction, especially in cases where the first allergic reaction had happened many years ago.

The detection of specific IgE-antibodies correlates with the time interval between the latest penicillin administration and the onset of the symptoms of hypersensitivity reactions. It is evident from Table 4 that the RAST result is positive in five out of seven cases where the

reaction occurred within 1 hour after the latest penicillin administration, whilst none of the 23 patients, with symptoms appearing more than 24 h after the latest administration, had circulating IgE-antibodies.

From our results (Fig. 1) it is also evident that there is better yield from the RAST method when both conjugates are tested than when only one hapten is tested. Single cases show positive reactions with only penicilloyl G or penicilloyl V. This might be an indication that better information could be obtained from this *in vitro* test when several antigenic determinations of penicillin derivatives are introduced into the system. It is known that in penicillin allergy many different haptenic determinants may be involved. The majority of penicillin allergic reactions is due to the penicilloyl group (major determinant), but also other metabolites of penicillin (minor determinants) may cause serious reactions (7, 10, 11, 13).

Three persons out of 22 controls had circulating IgE-antibodies against the determinants studied. They had no history of penicillin allergy. Two had previously received penicillin treatment without adverse reaction. The third was professionally employed in penicillin production and suffered from bronchial asthma. The skin tests in these cases were also negative. However, in the two individuals showing positive RAST to both penicilloyl G and V markedly elevated total IgE-levels, >2000 and 890 U/ml respectively, were observed. It is suggested that total IgE-levels should also be measured in such cases of positive penicillin RAST results with no evidence of allergic reactions against penicillin.

The RAST test is frequently positive at the time of the allergic reaction when the performance of the skin test is not possible (6). This is very valuable for early diagnosis, especially in severe infections when several drugs have been administered and when continued administration of penicillin is indicated. However, if no circulating IgE-antibodies are detected at the time of the drug

reaction the test should be repeated within the next few weeks to avoid a false negative diagnosis (5).

The data in our study confirm the clinical significance of the RAST technology as a valuable addition to the presently available diagnostic means in penicillin allergy.

REFERENCES

- Adkinson, N. F., Jr.: Recent developments in the use of RAST for determining hypersensitivity to penicillin. In R. Evans III (ed.): *Advances in diagnosis of allergy: RAST*, p. 125-136. Symposia Specialists, Miami, 1975.
- Dogliotti, M.: An instance of fatal reaction to the penicillin scratch-test. *Dermatologica* 136, 489-496, 1968.
- Girard, J. P. & Cuevas, M.: Analyse clinique et immunologique de 1047 réactions allergiques à la pénicilline. *Schweiz. Med. Wochenschr.* 105, 953-956, 1975.
- Hellenbroich, H. & Lückerrath, I.: Hautteste bei Penicillin-Allergie. *Hautarzt* 22, 18-24, 1971.
- Kraft, D., Roth, A., Mischer, P., Pichler, H. & Ebner, H.: Specific and total serum IgE measurements in the diagnosis of penicillin allergy. A long term follow-up study. *Clin. Allergy* 7, 21-28, 1977.
- Kraft, D. & Wide, L.: Clinical patterns and results of radioallergosorbent test (RAST) and skin test in penicillin allergy. *Br. J. Dermatol.* 94, 593-601, 1976.
- Levine, B. B.: Immunochemical mechanisms of drug allergy. *Ann. Rev. Med.* 17, 23-38, 1966.
- Levine, B. B. & Zolov, D. M.: Prediction of penicillin allergy by immunological tests. *J. Allergy Clin. Immunol.* 43, 231-244, 1966.
- Luton, E. F.: Methicillin tolerance after penicillin G anaphylaxis. *J. Am. Med. Assoc.* 190, 151-152, 1964.
- Parker, C. W., Shapiro, J., Kern, M. & Eisen, H. N.: Hypersensitivity to penicillenic acid derivatives in human beings with penicillin allergy. *J. Exp. Med.* 115, 821-838, 1962.
- Stewart, G. T.: Allergy to penicillin and related antibiotics: antigenic and immunochemical mechanism. *Ann. Rev. Pharmacol. Toxicol.* 13, 309-324, 1973.
- Vijay, H. M., Perelmutter, L. & Bernstein, I. L.: Possible role of IgG4 in discordant correlations between intracutaneous skin tests and RAST. *Int. Arch. Allergy Appl. Immunol.* 56, 517-522, 1978.
- de Weck, A. L.: Penicillin allergy: its detection by an improved haemagglutination technique. *Nature (Lond.)* 202, 975-977, 1964.
- Wide, L., Bennich, H. & Johansson, S. G. O.: Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet* ii, 1105-1107, 1967.
- Wide, L. & Juhlin, L.: Detection of penicillin allergy of the immediate type by radioimmunoassay of reagins (IgE) to penicilloyl conjugates. *Clin. Allergy* 1, 171-177, 1971.
- Zelger, J.: Hauttitration mit Penicillin. *Wien. Klin. Wochenschr.* 85, 790-793, 1973.
- Zelger, J.: Hauttitration bei Penicillin-Allergie. *Hautarzt, Suppl.* 1, 83-85, 1976.

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