

Original article

Skin test evaluation in nonimmediate allergic reactions to penicillins

Background: Nonimmediate reactions (NIR) to aminopenicillins (AP) are frequent. Although patch testing (PT) and intradermal testing (IT) are used for diagnosis, comparative results have never been adequately performed. We compared PT and IT in subjects with NIR to AP.

Methods: Twenty-one subjects with NIR to AP and positive IT were re-evaluated. Skin tests were performed with amoxicillin (AX) and ampicillin (AM) at different concentrations in petrolatum, 50, 25, and 5% w/w, for PT and in saline, 20, 2, and 0.2 mg/ml for both PT and IT. Skin biopsies from the site of the positive response were studied with haematoxylin–eosin and immunohistochemistry.

Results: In the re-evaluation, one case was IT and PT negative and was excluded; 20 were IT positive and 18 PT positive for both AX and AM. Decreasing concentrations of AP induced a reduction in positivity in both methods when diluted in saline, but not when mixed in petrolatum (PT only). With both PT and IT, immunohistochemical studies showed a perivascular mononuclear infiltrate with CD4 and CD8 memory cells expressing perforin and granzyme B.

Conclusions: Both tests appear valuable for the diagnosis of NIR to AP. However, IT diagnosed more patients than PT. The vehicle (saline or petrolatum) had no influence on the response, although in the former the concentration was critical. The immunohistochemical analysis showed skin infiltrates compatible with a T-cell drug reaction.

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Key words: amoxicillin; ampicillin; intradermal test; nonimmediate reactions; patch test; skin tests; T cells.

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Accepted for publication 26 May 2003

Maculopapular exanthemas, followed by urticaria, are the most common manifestation of cutaneous drug reactions (1–3). Other skin reactions induced by drugs include erythema multiforme, Stevens Johnson syndrome, toxic epidermal necrolysis, fixed drug eruptions, erythroderma (exfoliative dermatitis), hypersensitivity syndrome or drug rash with eosinophilia and systemic syndrome, pseudolymphoma, generalized or localized eczema, baboon syndrome, systemically induced contact dermatitis, flexural cutaneous adverse drug reactions, acute generalized exanthematic pustulosis, purpura, leucocytoclastic vasculitis, lichenoid dermatosis and eczematous photosensitivity reaction (4).

For maculopapular exanthema and urticaria, a number of studies indicate that aminopenicillins (AP) is the most common drug involved (5–7). These cutaneous reactions are reproducible and an immunological mechanism seems to be involved (8, 9). They can be induced after

re-administration of the culprit drug (5, 6), and the application of the drug to the skin may also induce localized erythema and induration, often associated with the formation of vesicles and bullae, with the presence of a lymphocyte infiltrate consisting mainly of CD4 and CD8 cells (6–8, 10–12). Further support for an immunological mechanism is that specific T cells and T-cell clones have been raised from both peripheral blood and affected skin (12–14).

Originally, Levine reported delayed positive intradermal tests (IT), consisting of erythema and variable induration, in patients with skin rashes (15). Since then, a number of studies have reported that patch tests (PT) (6, 10, 16) or IT (5, 6, 17) can be used as a diagnostic procedure for studying nonimmediate reactions (NIR) to drugs. Although several comparisons between PT and IT have been carried out, considerable heterogeneity exists in their performance, including the preparation of the reagents (4–6, 18–20). These differences hinder comparison of the results and are a source of disagreement (4). Our group, for example, mainly uses IT, because this is part of a standardized protocol for evaluating patients claimed to be allergic to betalactams (5, 21).

Abbreviations: AP, aminopenicillins; PT, patch test; IT, intradermal test; NIR, nonimmediate reactions to drugs; AX, amoxicillin; PPL, penicilloyl-poly-L-lysine; MDM, minor determinant mixture; AM, ampicillin.

In this study we compare PT and IT in patients with NIR to AP, using different concentrations of these drugs in saline and petrolatum. Skin biopsies were also obtained for histopathological and immunohistochemical studies. Results indicate that both methods are useful for skin testing, although certain advantages are afforded by IT.

Methods

Patients and controls

A positive group was composed of patients with NIR to penicillins and positive IT to AP selected from our database. Some of these patients have been included in previous reports (5, 22, 23). Non-immediate reactions were considered to be those appearing more than 1 h after drug administration and manifested mainly by exanthema and urticaria, generally during the 24–48 h after drug intake (19), and include both accelerated and delayed reactions under the Levine classification (15). All cases were re-evaluated, with a new clinical history to assess the presence of a new reaction since the first evaluation, a PT and IT to evaluate skin-test positivity, and *in vitro* determination of immunoglobulin E (IgE) antibodies to benzylpenicillin and amoxicillin (AX) by Pharmacia CAP System RAST FEIA (Pharmacia, Uppsala, Sweden), as described (24), to exclude a type I immediate reaction.

A negative control group consisted of 30 age and sex-matched subjects with negative skin tests to penicillins and good tolerance to different AP, established by controlled administration, using previously described protocols (21).

The study was approved by the institutional review board and informed consent for all the diagnostic procedures was obtained from all patients and controls.

Intradermal skin testing

This was performed as described (5) using major and minor determinants of benzylpenicillin, penicilloyl-poly-L-lysine (PPL) and minor determinant mixture (MDM), (both from Allergopharma, Merck, Darmstadt, Germany) at concentrations of 5×10^{-5} and 2×10^{-2} mM respectively, AX (Beecham, Toledo, Spain) and ampicillin (AM) (Antibiotic SA, León, Spain) both at 20 mg/ml. These were the maximum concentrations used (5, 22). A 0.02 ml of each hapten was injected into the volar forearm and the erythema and induration area were evaluated as described (5, 19). Readings were made at 20 min, and at 24, 48 and 96 h. Once a positive response was confirmed, three 10-fold decreasing dilutions in 0.9% NaCl were used to estimate the optimal and the minimum concentrations inducing a positive response. Negative controls were performed with saline in each individual tested and all proved negative.

Patch testing

This was performed as described (4) with some modifications, using the same haptens as for IT, with two different procedures: (a) by embedding the patch disk (leukotest[®]; Beiersdorf, Hamburg, Germany) in the same hapten solution prepared for IT, and (b) by mixing the drug in petrolatum at 50% w/w concentrations.

In all cases the occlusion time was 48 h and readings were made as described (25) 20 min after removal of the strips and 24, 48 and 96 h later. Faint erythema was considered a doubtful reaction; erythema, infiltration, and discrete papules – a weak positive reaction (+); erythema, infiltration, papules and vesicles – a strong

positive reaction (++); intense erythema, infiltration, and coalescing vesicles – an extremely positive reaction (+++). Negative controls were performed with saline and petrolatum in each individual tested and all proved negative.

Once a positive response was confirmed, decreasing dilutions of AX and AM were used to estimate the optimal and the minimum concentrations inducing a response. In the first method three 10-fold dilutions in 0.9% NaCl were used and in the second, 50, 25 and 5% w/w in petrolatum.

In order to compare the powder taken directly from the vial with the powder obtained from pills both formulations were also mixed in petrolatum at 50, 25 and 5% w/w concentrations.

Immunohistochemical studies

In order to compare positive results elicited by PT and IT, 4 mm punch (Stiefel Laboratories, Madrid, Spain) skin biopsies were obtained after both tests. Tissue segments were fixed and embedded in paraffin. Microtome sections (4 μ m) were processed for haematoxylin-eosin and immunohistochemical staining with the following monoclonal antibodies: CD4, granzyme B (Novocastra Laboratories, Newcastle upon Tyne, UK), CD8, CD45RO and perforin (Dako, Ely, Cambridgeshire, UK), using the automatized immunostaining system Techmate 500 plus (Dako) with the Envision Plus visualization system.

Results

A group of 21 patients with NIR who had been IT positive were finally recruited for the study, 16 historical patients (5, 22, 23) and five new. In the re-evaluation for the present study, the detailed questionnaire showed that none had

Table 1. Clinical characteristics of the patients included in the study

ID	Age	Sex	Drug	Reaction	Interval	
					Drug reaction	Reaction study
1	30	M	AM	DESQ. EXA.	48 h	30 years
2	47	F	AX	DESQ. EXA.	6 h	7 years
3	72	F	AX	MPE	48 h	20 years
4	64	M	AM	DESQ. EXA.	168 h	15 years
5	69	F	PENI	URTICARIA	8 h	6 years
6	67	M	AX	DESQ. EXA.	48 h	0.08 years
7	58	F	AX	URTICARIA	21 h	5 years
8	49	F	PENI	URTICARIA	12 h	10 years
9	57	F	AX	MPE	8 h	0.08 years
10	43	F	AM	URTICARIA	4 h	15 years
11	41	F	AM	URTICARIA	24 h	2 years
12	43	M	AX	URTICARIA	48 h	3 years
13	42	F	AX	URT/ANGIO	32 h	0.08 years
14	37	M	AX	DESQ. EXA.	144 h	0.5 years
15	50	F	AX	URTICARIA	24 h	0.5 years
16	60	F	AM	URTICARIA	48 h	3 years
17	40	F	AM	URTICARIA	4 h	3 years
18	52	F	PENI	URT/ANGIO	48 h	20 years
19	62	M	PENI	MPE	12 h	10 year
20	53	F	AX	DESQ. EXA.	24 h	10 years

DESQ. EXA., desquamative exanthema; MPE, maculopapular erythema; URT/ANGIO, urticaria and angioedema.

had any further reactions because, but one of the historical cases proved to be both IT and PT negative, and so was excluded from the study. The clinical characteristics of the remaining 20 patients are given in Table 1. The mean age was 52 years (range: 30–72). Six of the nine patients with maculopapular exanthema presented mild desquamation

Table 2. Results of intradermal and patch testing and the optimal times in the study patients. Tests were undertaken with amoxicillin (AX) and ampicillin (AM) at the following doses: intradermal and patch testing diluted in saline at 20 mg/ml and patch testing in petrolatum at 50% w/w

	Intradermal tests		Patch tests			
			Saline		Petrolatum	
	AX	AM	AX	AM	AX	AM
1	8 × 9 (48 h)	10 × 9 (48 h)	+ 48 h	+ 48 h	+ 48 h	+ 48 h
2*	10 × 16 (24 h)	9 × 16 (24 h)	+++ 48 h	+++ 48 h	+++ 48 h	+++ 48 h
3	7 × 6 (48 h)	8 × 7 (48 h)	+ 48 h	+ 48 h	+ 48 h	+ 48 h
4	10 × 12 (48 h)	9 × 7 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
5	8 × 7 (48 h)	10 × 12 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
6	7 × 6 (48 h)	8 × 9 (48 h)	+ 48 h	+ 48 h	+ 48 h	+ 48 h
7	10 × 12 (48 h)	10 × 14 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
8	8 × 9 (48 h)	7 × 6 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
9	10 × 15 (48 h)	10 × 16 (48 h)	+++ 48 h	+++ 48 h	+++ 48 h	+++ 48 h
10	7 × 7 (24 h)	8 × 9 (24 h)	—	—	—	—
11	7 × 6 (48 h)	10 × 12 (48 h)	+ 48 h	+ 48 h	+ 48 h	+ 48 h
12	8 × 12 (96 h)	10 × 12 (96 h)	++ 240 h	++ 240 h	++ 240 h	++ 240 h
13	8 × 10 (48 h)	12 × 14 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
14	7 × 8 (48 h)	8 × 9 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
15	9 × 13 (96 h)	8 × 14 (96 h)	++ 96 h	++ 96 h	++ 96 h	++ 96 h
16	9 × 10 (48 h)	9 × 14 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
17	7 × 8 (24 h)	9 × 9 (24 h)	—	—	—	—
18	14 × 15 (48 h)	10 × 13 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h
19	9 × 10 (48 h)	9 × 11 (48 h)	++ 96 h	++ 96 h	++ 96 h	++ 96 h
20	11 × 15 (48 h)	10 × 13 (48 h)	++ 48 h	++ 48 h	++ 48 h	++ 48 h

* This patient developed a systemic reaction after intradermal testing.

that appeared 48–92 h after the reaction and resolved completely within 1 week. The mean time interval between drug intake and appearance of the symptoms was 39 h (range: 4–168) and the time elapsed between the occurrence of the initial reaction and this re-evaluation was 8 years (range: 1 month to 30 years). In three patients (5, 9), reactions had occurred on more than one occasion. Case 5 developed two reactions, 15 and 6 years before this study, case 9 developed two reactions 20 years apart, and case 20 had three episodes 18, 17 and 10 years prior to this study. CAP test was negative for both benzylpenicillin and AX in all 21 patients.

Results of IT and both methods of PT, all performed at the maximum dose, are shown in Table 2. All patients remained negative to PPL and MDM in all the three test methods. After IT, the three cases with an optimal response at 24 h were still positive at 48 h and the two with optimal responses at 96 h were also positive at the 48-h reading. The positive response in all cases was an indurated erythema between 7 × 6 mm and 10 × 16 mm, accompanied in three cases by small vesicles and in one case by a bulla. No immediate or nonimmediate systemic symptoms appeared after skin testing except for one case who developed a mild exanthema on the legs 24 h after IT, corresponding to a patient who had originally had mild desquamative exanthema. As can be seen in the columns for the two methods of PT in Table 2, there was a close relationship between methods. In five cases there was a weak positive reaction (+), in 10 a strong positive reaction (++) and in two an extremely positive reaction (+++). The maximum concentrations for both IT and PT were assayed in the negative control group described in the methods section, with negative results in all cases.

In order to assess the optimal concentration and the minimum concentration eliciting a positive response in

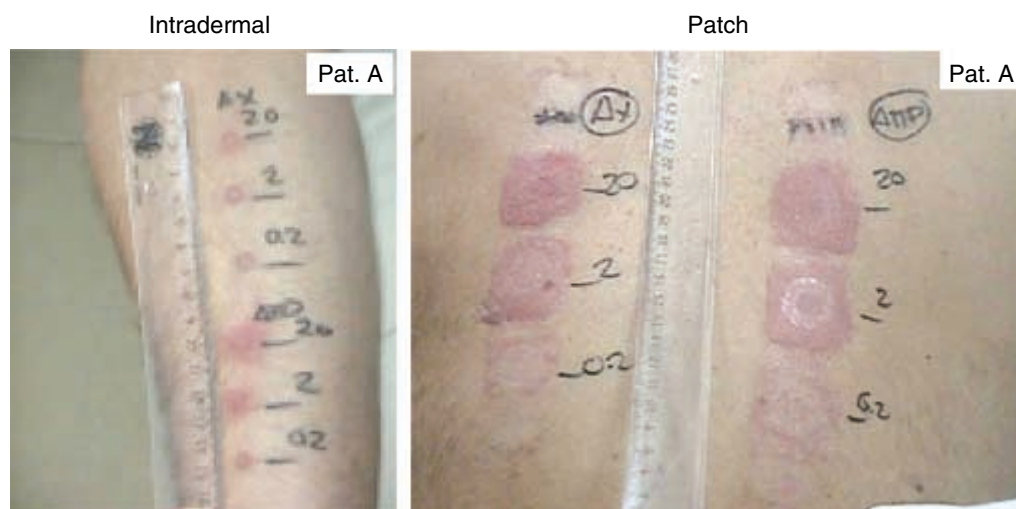


Figure 1. Representative intradermal testing (IT) (left) and patch testing (PT) (right) in one of the patients studied, performed with three different concentrations of amoxicillin and ampicillin, diluted in saline. This patient was positive in both IT and PT.

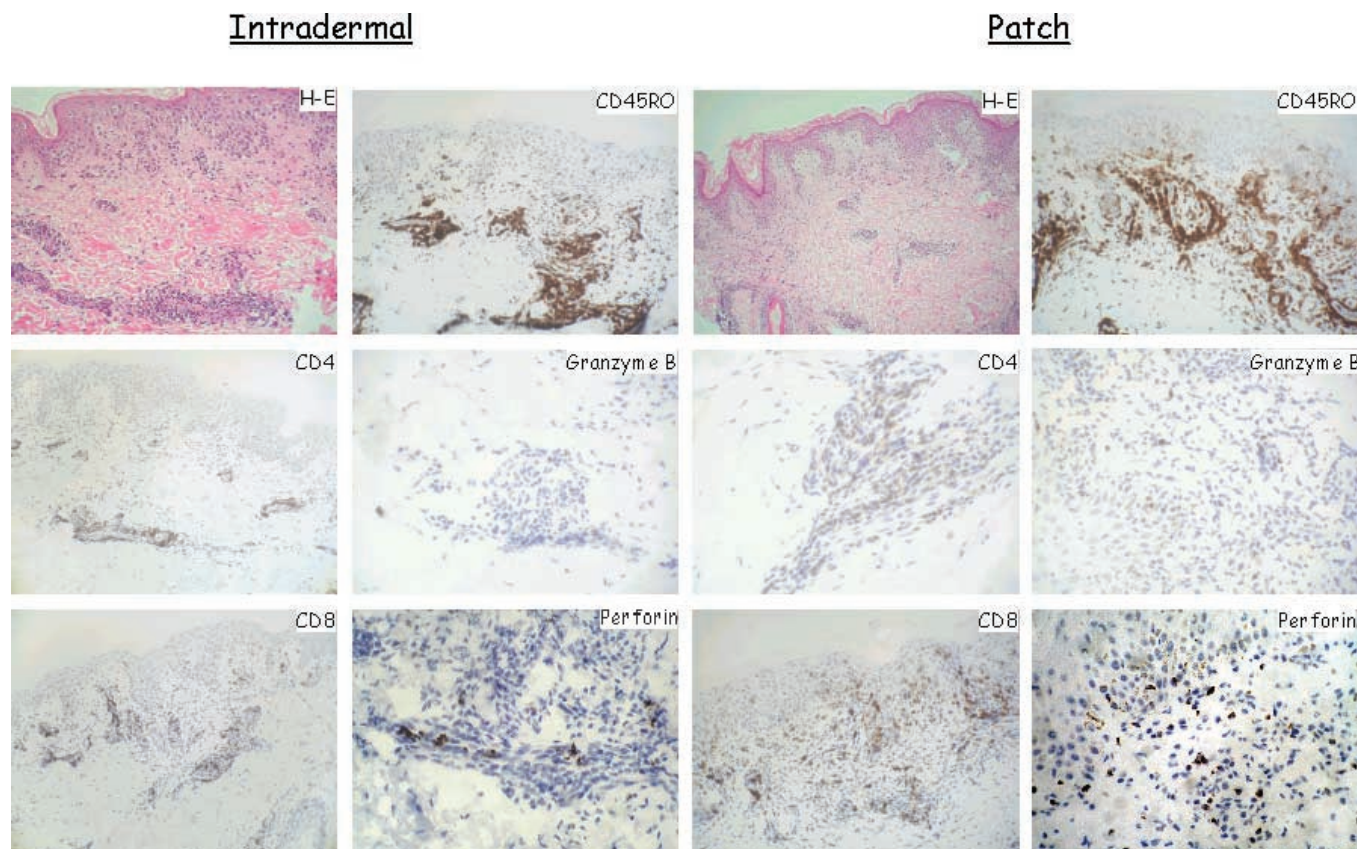


Figure 2. Skin biopsy specimens in intradermal (left) and patch biopsies (right) taken from the amoxicillin positive results in patients in Fig. 1 and processed for haematoxylin–eosin, 20×; immunohistochemical stains: lymphocyte CD4 (Novocastra Laboratories, Newcastle upon Tyne, UK), CD8 and CD45RO (Dako, Ely, Cambridgeshire, UK) 20×; cytotoxic markers granzyme B (Novocastra), 20×; perforin (Dako), 40×.

each method, a group of six patients, positive with all three methods, were further tested. After IT with 20, 2 and 0.2 mg/ml of AX and AM, we saw that at 2 mg/ml only three remained positive and at 0.2 only one remained positive. When we evaluated PT performed with liquid dilutions at the same concentrations, we found that at 2 mg/ml only two patients remained positive and none at 0.2 mg/ml (Fig. 1). The PT performed in petrolatum, at 50, 25 and 5% w/w, gave a positive response in all cases at all three concentrations. No differences were seen between PT performed with powder taken directly from the vial and the powder obtained from pills.

Representative haematoxylin–eosin staining of biopsies from positive IT and PT are shown in Fig. 2. In the IT there was a mononuclear cell infiltrate with a perivascular and upper dermal distribution. In the PT, oedema was observed together with a mononuclear cell infiltrate in the dermoepidermal junction, and also a moderate perivascular infiltrate. The immunohistochemical study is also shown in Fig. 2. Most of the infiltrating cells were lymphocytes. The CD4 cells were mainly located perivascularly while the CD8 cells were mostly located in the

dermis, with the distribution described in the haematoxylin–eosin staining. The memory cell marker CD45RO was expressed in almost all visible infiltrating cells in IT and PT. The cytotoxic markers granzyme B and perforin were moderately expressed (< 33%) in the dermis in both IT and PT.

Discussion

The results of this study indicate that in subjects with NIR to AP, both PT and IT can give positive results when tested at different concentrations and with different methods. No other systematic studies comparing different techniques in the same patients have been undertaken. The low prevalence of NIR makes it difficult to gather enough cases to undertake such studies, with periods of at least several years required in order to have a sufficient number (5, 6, 18). Our historical database, which includes a large number of cases, enabled us to carry out this study. Some of these patients have already been included in other studies (5, 6, 22) but some were new cases who

had been referred to Carlos Haya Hospital, Elche Hospital or La Paz Hospital.

It is generally accepted that skin test sensitivity remains in patients after NIR (5, 6). However, in our study one of the 16 patients became negative and, indeed, a higher percentage of negativity has been reported (16). Nevertheless, the long interval between the initial study and the repetition of the skin tests supports previous findings indicating that NIR to AP remain for a long time (5, 6).

As in other studies (5, 11, 19, 22), no case was positive to PPL or MDM. Regarding PPL, this negativity may be related to the nature of the carrier, as poly-L-lysine is a nonimmunogenic carrier, as seen by Levine in delayed reactions to penicillins (15), and for MDM, the response was negative because the group was selective to AP. Furthermore, the concentration is critical in delayed positive skin responses and previous experience indicates that a positive response is seen at concentrations higher than those contained in commercially available MDM (5).

Not all IT positive were PT positive, demonstrating the greater sensitivity of IT in diagnosing NIR to AP. This supports the results of others who have suggested advantages of IT compared with PT (10, 18). Furthermore, IT enables both immediate and NIR to be evaluated at the same time, which is especially important in cases of uncertainty regarding the timing of the original reaction.

Although generalized systemic reactions have been reported (26), in our study just one patient, who had originally had a desquamative exanthema, developed a mild exanthematic rash on the legs after IT. Nevertheless, the possibility of the existence of a systemic reaction after skin testing should be borne in mind, and the previous symptoms taken into account.

The fact that there were no positive results in our negative control group indicates that there were no false positive cases, although a local irritative response at the concentrations used cannot be ruled out in other cases. The possibility of a subject being enrolled as a negative control but who is really true positive to penicillins obviously exists, although we have not experienced this situation to date (5, 21, 22).

Variable concentrations have been used for skin testing, with IT concentrations ranging from 375 to 1 mg/ml (5, 17–19), and with PT, ranges in petrolatum have varied from 20 to 1% (17, 18) and in saline from 75 to 5% (7, 12, 19). In our study, for IT and PT in saline,

we used 20, 2, and 0.2 mg/ml and for PT in petrolatum 50, 25, and 5%. We were therefore able to cover a wide range of concentrations. For IT 20 mg/ml were more sensitive than 2 or 0.2 mg/ml, with only one of the six patients remaining positive at the lowest concentration. As all our cases responded at 20 mg/ml, we cannot rule out the possibility of other patients only responding at higher concentrations. As stated above, no false positive response was detected at this concentration in the negative control group. For PT, the concentration is much more important in saline than in petrolatum, with 20 mg/ml producing the optimal response in our patients.

As our study group was initially selected as IT positive and comparison was made with PT, questions remain as to what would have happened if the subjects had been selected on the basis of being PT positive to AP. Current data indicate that persons who are PT positive are invariably IT positive, and in fact some PT negative can even be IT positive (6, 10, 18).

Pathological and immunohistochemical studies obtained at the optimal response after PT and IT confirmed previous reports that an immunological response is involved in NIR (8, 9, 11–14, 23). No histological differences were observed between PT and IT, indicating that, independent of sensitivity, the same immunological phenomenon is induced although different techniques are used to apply the hapten to the skin.

In summary, we conclude that both IT and PT are valuable instruments for the diagnosis of NIR to AP, and a positive response remains over time. However, the sensitivity of IT seems to be superior to that of PT for the diagnosis of NIR to AP, IT is easy to perform, and the patient can report exactly when a positive response appears. We therefore suggest that IT should be the method of choice when evaluating patients for NIR, and that a single reading at 48 h would appear optimal. However, should PT be preferred, then PT in saline is a good alternative and easy to prepare, although care should be taken with the concentration used.

Acknowledgments

This study was partly supported by grants from the Spanish Ministry of Health (00/0838 and 01/0014).

We thank Ian Johnstone for his help with the English language version.

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