

Penicillin Allergy: Value of Including Amoxicillin as a Determinant in Penicillin Skin Testing

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Key Words

Diagnostic tests • Drug allergy • Penicillin allergy • Skin testing • Amoxicillin allergy

Abstract

Background: Allergy to penicillins remains an important issue. Penicillin skin testing (PST) with major and minor determinants has been shown to be a highly valuable tool for identifying IgE-mediated penicillin allergy. The value of additional testing with side-chain-specific moieties from semi-synthetic penicillins such as amoxicillin is not well-established in spite of the widespread use of these medications.

Methods: A retrospective review of all consecutive inpatient PST results from 1995 to 2007 comprising 1,068 patients was performed in our institution on individuals with a self-reported history of beta-lactam allergy to assess the importance of including the amoxicillin determinant in a previously validated PST panel. Descriptive statistics were performed. The PST panel included penicilloyl-polylysine, penicillin G, penicilloate, penilloate and amoxicillin. **Results:** Of 1,068 patients, 243 (23%) had a positive skin test reaction on the PST panel. Testing with amoxicillin was positive in 30.9% of patients, the majority of whom (81%) were also positive to 1 or more standard penicillin reagents. Fourteen of the 243 posi-

tive patients (5.8%) had a positive skin test reaction only to amoxicillin. Additionally, the use of penicilloate and penilloate minor determinants in combination with penicillin G identified a greater percentage of penicillin-allergic individuals compared to using only penicillin G (22.6 vs. 6.6%), demonstrating their importance in the PST panel. **Conclusions:** These data indicate that the inclusion of the amoxicillin determinant appears to identify a small but important group of allergic individuals who may otherwise test negative on a PST panel.

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Introduction

Beta-lactam antibiotics, including penicillins, remain the most common cause of drug-induced hypersensitivity reactions [1]. The incidence of self-reported penicillin allergy is approximately 10% [2–5]; however, over 90% of these individuals are able to tolerate penicillins when evaluated for penicillin allergy [6]. Thus, it appears that there is a significant portion of individuals who are inappropriately labeled as allergic to penicillin. The overdiagnosis of penicillin allergy may be due to many factors, such as declining penicillin IgE antibodies over time [7],

concomitant viral illnesses with symptoms inappropriately attributed to penicillin allergy [8] or misinterpretation of side effects to medication as allergy. The negative consequences of this mislabeling include limited antibiotic options, the unnecessary use of broad-spectrum antibiotics, increased risk of toxicity and increased health-care costs [9, 10]. Thus, the role of penicillin skin testing (PST) is important in evaluating true penicillin allergy in order to optimize the efficacy of medical care [11]. However, there are currently no standardized guidelines regarding which determinants to include in a PST panel. In particular, the value of including semisynthetic penicillins such as amoxicillin is not known.

Historically, while PST panels may vary from institution to institution, the haptens that have generally been used include the major determinant penicilloyl-polylysine and a mixture of minor determinants, including penicilloate, penilloate and penicillin G [6, 12]. Gadde et al. [6] found that 90% of skin test-positive subjects demonstrated sensitivity to penicilloyl-polylysine, whereas 10% displayed sensitivity to the minor determinants alone. Testing sensitivity to the minor determinants is of particular importance given data suggesting that skin test reactivity to these antigens is clinically associated with more severe acute systemic reactions upon subsequent penicillin administration [6, 13]. Inclusion of semisynthetic penicillins such as amoxicillin in penicillin allergy skin testing panels is not widely utilized [14], and data regarding its significance have not been extensively studied in the USA.

In evaluating patients for beta-lactam IgE-mediated allergy, the importance of including testing for hypersensitivity to side-chain-specific moieties has been debated. Given the widespread use of semisynthetic penicillins, there may be a significant number of individuals who react only to the side-chain-related haptens [15–17]. A number of studies on amoxicillin allergy have been performed in Europe. Blanca et al. [15, 16] have described cases of hypersensitivity specifically to amoxicillin with positive skin test and radioallergosorbent test results to amoxicillin and with negative skin test results to the major and minor determinants of penicillin. These results suggest that the acyl side chain may be a significant antigenic determinant apart from the penicillin nucleus [15, 16, 18]. Vega et al. [19] described a group of patients with suspected beta-lactam allergy who demonstrated allergic reactions only to amoxicillin (30.5%), while tolerating penicillin. Of these individuals, 63% were positive for amoxicillin on skin testing and 22% were positive on a radioallergosorbent test [19]. These studies suggest that

amoxicillin may represent an important antigen to include in a PST panel. There is a paucity of studies in the USA regarding amoxicillin allergy, and its incidence may differ from that in other parts of the world.

While the majority of individuals who report an allergy to penicillin are able to tolerate penicillin, the potential for an acute and life-threatening reaction in those with true penicillin allergy mandates appropriate caution and evaluation. The rate of anaphylactic reactions after parenteral administration of penicillin has been found to be approximately 0.2% [20]. Given the limited utility of clinical history as a predictor for beta-lactam allergy [5, 8, 21, 22], PST with major and minor determinants as well as the potential inclusion of amoxicillin is a valuable tool for identifying a true IgE-mediated penicillin allergy. The purpose of this study was to evaluate the importance of the specific determinants used in the PST panel. In particular, the value of including the amoxicillin determinant in the PST panel was assessed.

Patients and Methods

This study was a retrospective review of all consecutive inpatient PST results from tests performed at The University of California – Los Angeles Medical Center from January 1995 to December 2007. We have been performing PST with characterized and validated penicillin determinants since the mid-1970s. Challenge data from our first 15 years of skin testing have been published previously [23, 24]. Amoxicillin at 10^{-2} M (3.65 mg/ml) was added to this panel in January 1995. The amoxicillin concentration of 10^{-2} M was selected as it is the concentration that has been well-established for penicillin as a minor determinant. The cohort consisted of 1,068 patients. Of these, 834 had appropriate positive and negative control responses. PST was performed on those hospitalized individuals suspected of having an allergy to penicillin or a related beta-lactam antibiotic with a documented reaction on medical records or a self-reported history. These patients were referred to the allergy skin testing service based upon the judgment of the primary attending physician. The standardized protocol for PST involved administering a series of skin prick tests followed by intradermal skin tests to penicilloyl-polylysine (major determinant), penicilloate, penilloate, penicillin G and amoxicillin, along with appropriate controls. The respective institutional review board approved the study protocol.

The PST panel used in this study consisted of 7 scratch ('prick') skin tests: histamine at 1 mg/ml (positive control), normal phosphate buffer (negative control), penicilloyl-polylysine (6×10^{-5} M), penicillin G (10^{-2} M), penicilloate (10^{-2} M), penilloate (10^{-2} M) and amoxicillin (10^{-2} M). Patients had the skin on the volar part of their forearm cleaned with alcohol and then 'pricked' with the individual PST determinants. Skin tests were read after 15–20 min. Any determinant giving a wheal response of 4 mm or greater with flare on prick skin testing was not employed on intradermal testing. Intradermal tests involved placing 0.02–0.03

ml of each of the determinants under the skin, which was enough to create a 2 × 2 mm wheal. Intradermal skin tests were read after a 15- to 20-min interval. A skin prick and intradermal result were considered positive if there was a wheal greater than or equal to 4 mm with a surrounding area of erythema. A positive result on the PST panel was defined as having 1 or more positive responses to a skin prick or intradermal determinant. Testing was considered indeterminate if the individual did not have a positive histamine response (i.e. wheal >4 mm with surrounding erythema) and/or had a wheal/flare of 3 mm or more to the negative control. The methods for the preparation of the penicillin determinants have been published previously [25]. When the commercially available material for the major determinant (penicilloyl-polylysine) became unavailable in 2004, we synthesized our own penicilloyl-polylysine (BPL-PLL) according to published methods [26]. The molarity and quality of this benzylpenicilloyl moiety was assessed by comparing it to the previously available commercial materials in the penamaldade assay [27, 28] using HgCl₂ as a substrate. The endotoxin content of BPL-PLL was less than 0.0125 EU/ml endotoxin per 2 mg of BPL-PLL, as assayed by the Pyrogen[®] limulus amoebocyte lysate assay (Lonza Biologics). BPL-PLL was used at a final concentration of 6.15 × 10⁻⁵ M for skin testing.

The information obtained with the University of California – Los Angeles PST results included date of testing, birth date and gender. The skin testing service did not obtain a detailed clinical history of penicillin allergy from all patients, as they were referred by the attending physician based on a suspicious clinical history.

Descriptive statistics were performed on the PST result patterns. Logistic regression analysis was used to calculate odds ratios and 95% confidence intervals for gender differences (male = 0, female = 1). Binomial analysis was used to investigate potential differences in the proportion of positive results on PST panels in females versus males. Multivariate logistic regression including age as a covariate was performed to assess the potential effect of age on positive PST results.

Results

Between January 1995 and December 2007, 1,068 in-patients were referred to the allergy-immunology service for PST due to a history of suspected penicillin allergy. Of the total 243 PST-positive patients, 75 patients (30.9%) reacted to the amoxicillin determinant. Of these, 14 individuals were positive to amoxicillin alone (table 1). Thus, omission of amoxicillin from the test panel would have resulted in failure to identify 5.8% of PST-positive patients.

Testing with penicilloyl-polylysine, penicillin G, penicilloate and penilloate detected 64.6, 45.7, 37.0 and 34.6% of the positive patients, respectively. Omission of penicilloyl-polylysine would have led to a failure to detect 18.5% of PST-positive patients. Omission of penicillin G, penicilloate and penilloate would have led to a failure to detect

Table 1. Positive results to the various penicillin reagents

Agent	Males (n = 89; 37%)	Females (n = 154; 63%)	Total (n = 243; 23%)
Penicilloyl-polylysine	56 (62.9)	101 (65.6)	157 (64.6)
Penicillin G	37 (41.6)	74 (48.0)	111 (45.7)
Penicilloate	32 (36.0)	58 (37.7)	90 (37)
Penilloate	31 (34.8)	53 (34.4)	84 (34.6)
Amoxicillin	28 (31.5)	47 (30.5)	75 (30.9)
Penicilloyl-polylysine only	16 (18.0)	29 (18.8)	45 (18.5)
Penicillin G only	8 (9.0)	8 (5.2)	16 (6.6)
Penicilloate only	3 (3.4)	4 (2.6)	7 (2.9)
Penilloate only	5 (5.6)	11 (7.4)	16 (6.6)
Combination 1	21 (23.6)	34 (22.1)	55 (22.6)
Combination 2	13 (14.6)	26 (16.9)	39 (16)
Amoxicillin only	5 (5.6)	9 (5.8)	14 (5.8)

Results are numbers of patients (percentages in parentheses). Combination 1 = combination of the minor determinants penicillin G, penicilloate and penilloate. Combination 2 = combination of the minor determinants penicilloate and penilloate.

Table 2. PST results by gender

	Females	Males	Total tested	Total evaluable ¹
Positive results	154 (63.4)*	89 (36.6)	243 (23)	243 (29.1)
Negative results	314 (53.1)	277 (46.9)	591 (55)	591 (70.9)
Indeterminate	131 (56)	103 (44)	234 (22)	0
Total	599 (56)*	469 (44)	1,068	834

Results are numbers of patients (percentages in parentheses). * p < 0.0001.

¹ Includes only patients with appropriate positive and negative controls.

6.6, 2.9 and 6.6% of PST-positive patients, respectively. Omission of a combination of 3 minor determinants (penicillin G, penicilloate and penilloate) would have led to a failure to identify 22.6% of PST-positive patients. Omission of a combination of 2 minor determinants (penicilloate and penilloate) would have led to a failure to identify 16% of PST-positive patients (table 1). Of note, out of the total 243 patients who tested positive on our PST panel, 45 were identified on initial prick skin testing and did not require intradermal testing.

In the entire cohort, 243 of 834 patients (29.1%) with appropriate control responses had a positive skin test reaction to 1 or more antigens on the PST panel. Of those

who tested positive, 154 (63.4%) were female and 89 (36.6%) were male (table 2). Female gender was associated with greater odds of positive PST results even when adjusted for age (odds ratio 1.47, 95% confidence interval 1.09–1.98; *p* value = 0.011). There was not a significant interaction between age and gender (*p* = 0.103). Overall, a logistic regression model including gender as the sole predictor variable was not shown to be particularly effective at discriminating between positive and negative PST results.

In our cohort, 551/834 patients (66%) had a documented clinical history of a reaction to penicillin in their medical records. Of these patients with a documented clinical history, 145/551 (26.3%) had clinical histories suggestive of an IgE-mediated reaction (including anaphylaxis, pruritic rash, urticaria, angioedema and respiratory symptoms). Of the 243 patients with positive PST results, 57 (23%) reported a clinical history suggestive of an IgE-mediated reaction. Eighty-eight of the 591 patients (15%) with negative PST results reported a clinical history suggestive of an IgE-mediated reaction. Given the incomplete documentation of the clinical history of our cohort, it is difficult to make any definitive conclusions on the predictive ability of clinical history in penicillin allergy. Of note, several previous studies failed to show utility of the clinical history as a predictor for penicillin allergy [5, 8, 21, 22].

The overall mean age ($\pm$ SD) of the study population was 58 ± 18 years. In the patients with positive PST, the mean age was 54 ± 18 years. In those with negative PST, the mean age was 56 ± 18 years. In those with indeterminate PST, the mean age was 62 ± 18 years. There were no statistically significant differences in the ages based on the PST result.

Discussion

In our study, the overall rate of positive PST was 29.1% of the evaluable patients. This is a somewhat higher positive rate than has been previously reported in the literature (7–20%) [6, 21]. It may be due to the fact that our population was strictly inpatient in a tertiary care hospital, and thus represented patients with medical concerns of higher acuity who may have had more exposure to antibiotics in the past. Additionally, in-depth evaluation of these patients by inpatient house staff and hospitalists may have resulted in a selected test population with a higher pretest probability for true penicillin allergy. Differences in the overall rate of positive PST may also be due

to the varying number of determinants included in the skin testing panel at each institution. The inclusion of the amoxicillin determinant at our institution was shown to increase the overall positive skin test rate by a statistically significant amount.

The amoxicillin determinant was found to identify 5.8% of patients who otherwise had negative PST results. Of the 243 patients with positive results on the PST panel, 30.9% reacted to the amoxicillin determinant. This finding contrasts with those of Vega et al. [19], who found that 63% of their cohort were positive to amoxicillin on PST. This large discrepancy may be due to differences in prescribing patterns, as the study performed by Vega et al. [19] was conducted in Spain, where there is a higher rate of prescriptions (up to 45%) given for amoxicillin [16]. Sastre et al. [29] reported that 21% of their patients were allergic to amoxicillin but demonstrated good tolerance to penicillin. Our finding suggests that amoxicillin may be a useful determinant to include in the PST panel but may not be as essential as other studies have found. The inclusion of amoxicillin in a PST panel raises the question of whether to administer penicillin or a semi-synthetic penicillin such as piperacillin to an individual who tests positive only to amoxicillin. While it may appear to be reasonable to administer penicillin or a semi-synthetic penicillin in this setting, further studies will need to be performed to assess safety before one could make a firm recommendation. The current practice at our institution is to recommend that patients with a positive skin test result on the PST panel avoid penicillin, semisynthetic penicillins, cephalosporins and beta-lactams, if possible, due to the variable risk of cross-reactivity with these drugs as well as medicolegal concerns. This is usually feasible given the wide choice of alternative antibiotics now available. In patients with a documented positive skin test, desensitization or graded challenge was performed only if the identified antibiotic was deemed absolutely medically necessary. We have had no serious adverse outcomes following desensitization or graded challenge.

Sixteen percent of the patients in our study responded only to the minor determinants penicilloate and/or penilloate, which is consistent with previous findings reported by Macy et al. [25] and supports the importance of including these minor determinants in the PST panel. The addition of the minor determinants penicilloate and penilloate with penicillin G clearly identifies a greater percentage of individuals compared to using only penicillin G alone (22.6 vs. 6.6%), showing the value of including all 3 minor determinants in the PST panel.

Our study demonstrates that there is a 1.47-fold greater odds of a positive PST result in women compared to men in those with a reported history of penicillin allergy in an inpatient hospital setting. The age of the patient did not significantly affect these greater odds for a positive skin test in women. Park et al. [30] showed a significantly higher odds ratio of 3.6 for female gender as a risk factor for a positive PST than that found in our study (1.47). In contrast, Sullivan et al. [31] showed no effect of gender on PST results. Discrepancies in the findings between our studies may be due to differences in the patient populations. While our study was composed entirely of an inpatient population, the individuals studied by Park et al. [30] were drawn exclusively from an outpatient setting. Sixty-two percent of the population examined by Sullivan et al. [31] was based in an outpatient setting.

The use of PST in a patient with suspected penicillin or beta-lactam allergy provides valuable information,

given that the majority of individuals with suspected penicillin allergy have a negative result and are able to tolerate penicillins. The return of the commercially manufactured major determinant product, penicilloyl-polylysine, which was removed from the market in 2004, offers clinicians the opportunity to perform standardized PST [32]. The availability of the major determinant in conjunction with access to a protocol for preparing the minor determinants (penicilloate and penilloate) published by Macy et al. [25], as well as the ability to test with amoxicillin, allows clinicians to be better equipped to evaluate patients with reported penicillin allergy. Data from 12 years of PST at our institution confirm the importance of including 3 minor determinants in addition to the major determinant in the PST panel. The inclusion of the amoxicillin determinant appears to identify a small but important group of individuals who may otherwise test negative on PST.

References

- Gruchalla RS: Drug allergy. *J Allergy Clin Immunol* 2003;111(2 suppl):S548–S559.
- Neugut AI, Ghatak AT, Miller RL: Anaphylaxis in the United States: an investigation into its epidemiology. *Arch Intern Med* 2001; 161:15–21.
- Kerr JR: Penicillin allergy: a study of incidence as reported by patients. *Br J Clin Pract* 1994;48:5–7.
- Kagy L, Blaiss MS: Anaphylaxis in children. *Pediatr Ann* 1998;27:727–734.
- Solensky R, Earl HS, Gruchalla RS: Penicillin allergy: prevalence of vague history in skin test-positive patients. *Ann Allergy Asthma Immunol* 2000;85:195–199.
- Gadde J, Spence M, Wheeler B, Adkinson NF Jr: Clinical experience with penicillin skin testing in a large inner-city STD clinic. *JAMA* 1993;270:2456–2463.
- Blanca M, Torres MJ, García JJ, Romano A, Mayorga C, de Ramon E, Vega JM, Miranda A, Juárez C: Natural evolution of skin test sensitivity in patients allergic to beta-lactam antibiotics. *J Allergy Clin Immunol* 1999; 103:918–924.
- Stember RH: Prevalence of skin test reactivity in patients with convincing, vague, and unacceptable histories of penicillin allergy. *Allergy Asthma Proc* 2005;26:59–64.
- Arroliga ME, Radojicic C, Gordon SM, Popovich MJ, Bashour CA, Melton AL, Arroliga AC: A prospective observational study of the effect of penicillin skin testing on antibiotic use in the intensive care unit. *Infect Control Hosp Epidemiol* 2003;24:347–350.
- Lee CE, Zembower TR, Fotis MA, Postelnick MJ, Greenberger PA, Peterson LR, Noskin GA: The incidence of antimicrobial allergies in hospitalized patients: implications regarding prescribing patterns and emerging bacterial resistance. *Arch Intern Med* 2000; 160:2819–2822.
- Solensky R, Earl HS, Gruchalla RS: Clinical approach to penicillin-allergic patients: a survey. *Ann Allergy Asthma Immunol* 2000; 84:329–333.
- Levine BB, Redmond AP: Minor haptenic determinant-specific reagins of penicillin hypersensitivity in man. *Int Arch Allergy Appl Immunol* 1969;35:445–455.
- Gorevic PD, Levine BB: Desensitization of anaphylactic hypersensitivity specific for the penicilloate minor determinant of penicillin and carbenicillin. *J Allergy Clin Immunol* 1981;68:267–272.
- Abuaf N, Rostane H, Rajoely B, Gaouar H, Autegarden JE, Leynadier F, Girot R: Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy* 2008;38: 921–928.
- Blanca M, Perez E, Garcia J, Miranda A, Fernandez J, Vega JM, Terrados S, Avila M, Martin A, Suau R: Anaphylaxis to amoxicillin but good tolerance for benzyl penicillin. In vivo and in vitro studies of specific IgE antibodies. *Allergy* 1988;43:508–510.
- Blanca M, Vega JM, Garcia J, Carmona MJ, Terrados S, Avila MJ, Miranda A, Juárez C: Allergy to penicillin with good tolerance to other penicillins; study of the incidence in subjects allergic to beta-lactams. *Clin Exp Allergy* 1990;20:475–481.
- Silviu-Dan F, McPhillips S, Warrington RJ: The frequency of skin test reactions to side-chain penicillin determinants. *J Allergy Clin Immunol* 1993;91:694–701.
- de Haan P, de Jonge AJ, Verbrugge T, Boersma DM: Three epitope-specific monoclonal antibodies against the hapten penicillin. *Int Arch Allergy Appl Immunol* 1985;76:42–46.
- Vega JM, Blanca M, García JJ, Carmona MJ, Miranda A, Pérez-Estrada M, Fernández S, Acebes JM, Terrados S: Immediate allergic reactions to amoxicillin. *Allergy* 1994;49: 317–322.
- International Rheumatic Fever Study Group: Allergic reactions to long-term benzathine penicillin prophylaxis for rheumatic fever. *Lancet* 1991;337:1308–1310.
- Salkind AR, Cuddy PG, Foxworth JW: The rational clinical examination. Is this patient allergic to penicillin? An evidence-based analysis of the likelihood of penicillin allergy. *JAMA* 2001;285:2498–2505.
- Park MA, Li JT: Diagnosis and management of penicillin allergy. *Mayo Clin Proc* 2005; 80:405–410.
- Saxon A, Beall GN, Rohr AS, Adelman DC: Immediate hypersensitivity reactions to beta-lactam antibiotics. *Ann Intern Med* 1987;107:204–215.

- 24 Sogn DD, Evans R 3rd, Shepherd GM, Casale TB, Condemni J, Greenberger PA, Kohler PF, Saxon A, Summers RJ, VanArsdel PP Jr, et al: Results of the National Institute of Allergy and Infectious Diseases Collaborative Clinical Trial to test the predictive value of skin testing with major and minor penicillin derivatives in hospitalized adults. *Arch Intern Med* 1992;152:1025–1032.
- 25 Macy E, Richter PK, Falkoff R, Zeiger R: Skin testing with penicilloate and penilloate prepared by an improved method: amoxicillin oral challenge in patients with negative skin test responses to penicillin reagents. *J Allergy Clin Immunol* 1997;100:586–591.
- 26 Vemuri P, Harris KE, Suh LA, Grammer LC: Preparation of benzylpenicilloyl-polylysine: a preliminary study. *Allergy Asthma Proc* 2004;25:165–168.
- 27 Schwartz MA, Delduce AJ: Kinetic analysis of penicilloic acid and penicilloamides in combination: application to products of reaction of penicillin with tromethamine (tris) and poly-lysine. *J Pharm Sci* 1969;58:1137–1139.
- 28 Schneider RH: Penamaldete assay: improved methodology for the quantitation of penicilloic acids, penicilloyl amides and penicilloyl esters. *Arzneimittelforschung* 1984;34:1247–1249.
- 29 Sastre J, Quijano LD, Novalbos A, Hernandez G, Cuesta J, de las Heras M, Lluch M, Fernandez M: Clinical cross-reactivity between amoxicillin and cephadroxil in patients allergic to amoxicillin and with good tolerance of penicillin. *Allergy* 1996;51:383–386.
- 30 Park MA, Matesic D, Markus PJ, Li JT: Female sex as a risk factor for penicillin allergy. *Ann Allergy Asthma Immunol* 2007;99:54–58.
- 31 Sullivan TJ, Wedner HJ, Shatz GS, Yecies LD, Parker C: Skin testing to detect penicillin allergy. *J Allergy Clin Immunol* 1981;68:171–180.
- 32 Schafe JA, Mateo N, Parlier GL, Rotschafer JC: Penicillin allergy skin testing: what do we do now? *Pharmacotherapy* 2007;27:542–545.