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Predictive capacity of histamine release for the diagnosis of drug allergy

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Background: The diagnosis of immediate allergic reactions to drugs is difficult, requiring *in vitro* test development. Basophils are likely to be involved in these reactions, and to evaluate the sensitivity, the specificity, and the predictive values of the histamine-release test, we performed a prospective study in 68 patients tested for suspected drug allergy.

Methods: Positive diagnosis was established by history, skin tests, and, if needed, oral provocation tests. Histamine release in the presence of the drug was assessed on heparinized whole blood by enzyme immunoassay (Immunotech, France), and the cutoff value was set at 5% of total histamine content. Spontaneous and anti-IgE-induced histamine release was also studied in all subjects.

Results: All patients presented to our clinic with reactions ranging from maculopapular exanthema to anaphylactic shock. Thirty-five patients had proven drug allergy; 33 were not allergic to drugs and served as a control group together with 40 other subjects with no history of drug allergy. Net histamine release was positive in 18/35 allergics and 27/73 nonallergics, giving poor sensitivity (51.4%), specificity (63.0%), and positive predictive value (29.3%), but valuable negative predictive value (81.1%).

Conclusions: The usefulness of the *in vitro* histamine-release test for the diagnosis of drug allergy appears to be insufficient.

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Allergic reactions to drugs are responsible for levels of morbidity, mortality, and health-care costs which are underevaluated. These reactions are highly prevalent, as shown by various studies. For example, Classen et al. (1), using a computerized surveillance of adverse drug reactions in the LDS Hospital of Salt Lake City, Utah, USA, found

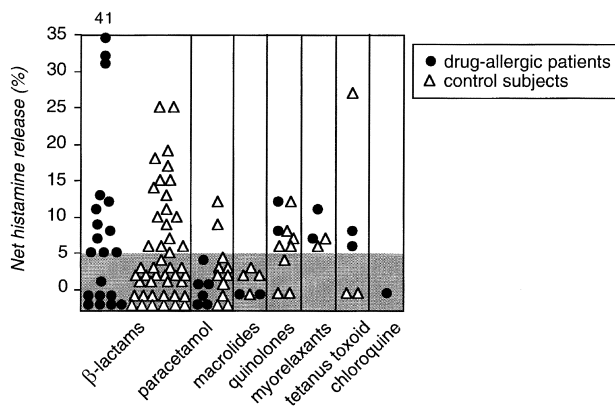


Figure 1. Histamine-release results in 108 subjects, of whom 68 were referred for suspected drug allergy. According to our diagnosis procedure (including provocation tests for most patients; see Material and methods), 35 had proven drug allergy and 33 were not allergic to drugs and were considered as control group, together with 40 other subjects, not provoked, but with no history of drug allergy. Results are expressed as percentage of net histamine release in presence of drug (see Material and methods).

over a period of 18 months that 0.65% of over 36 500 hospitalized patients experienced an allergic drug reaction. In a recent study (2), adverse drug reactions appeared to be the 4th most common cause of death in the USA, with over 106 000 deaths in 1994; 25% of these reactions were of allergic nature. The diagnosis of these reactions is difficult, being based on history and clinical manifestations, and, if possible, on skin tests, biologic tests, and provocation tests as required.

Provocation tests have the best sensitivity and are certainly useful to validate new biologic tests. However, they can be performed only under the most rigorous surveillance conditions due to potentially life-threatening reactions. Biologic tests would be highly desirable to establish the nature of the culprit agent, especially when the patient is receiving several drugs simultaneously. However, there are few available biologic tools, and they have not been fully evaluated. They include serum drug-specific antibodies, histamine release (HR) (3), cysteinyl-leukotriene release (4), histamine and tryptase in body fluids (5), basophil membrane markers (6), and T-cell-based assays (proliferation, activation, clones) (7). HR from total blood in the presence of the drug may be due to both specific IgE and nonspecific mechanisms. In the case of allergy to myorelaxants, the HR correlates well with skin tests and specific IgE (8), but, although there have been numerous case reports in the literature showing some basophil HR, this diagnostic tool has not been fully validated for most other drugs.

To evaluate the sensitivity, the specificity, and the predictive values of the HR test, we studied 68 patients with suspected drug allergy prospectively.

Material and methods

Patients and diagnosis of drug allergy

Sixty-eight patients were referred to our department with a personal history of a drug reaction consistent with an immunologic mechanism. Forty controls with no history of drug allergy were added. Allergic patients were 8–73 years of age (mean $\pm$ SD, 44 ± 16 years), and control subjects were aged 12–66 (37 ± 14 years).

The diagnosis of drug allergy was carefully evaluated. We always performed skin prick tests with the stock solution, followed by intradermal skin tests with increasing dilutions of the drug. For penicillin allergy, the diagnosis was based on history and skin testing with benzylpenicilloyl-poly-L-lysine, minor determinant mixture, penicillin G, amoxycillin, and cefapirine, as described previously (9, 10). We considered that the patient was allergic to penicillins if skin tests were positive. For rocuronium allergy, it is well accepted that a suggestive clinical history, together with a positive skin test to myorelaxants and negative tests to the other drugs utilized during general anesthesia, establishes the diagnosis (8, 11). Provocation tests were performed when penicillin skin tests were negative (10) and for drugs for which the skin test predictive value is low or unknown (cephalosporins, paracetamol, macrolides, quinolones, and chloroquine). Provocation tests were single-blind, placebo-controlled challenges adapted from Blanca et al. (12). Always performed after informed consent, they started with 0.1–1% of the therapeutic dose of the culprit drug with 5- to 10-fold increments at half-hour intervals until the therapeutic dose was reached. The test was immediately stopped when allergic symptoms occurred, and the reaction was treated.

Histamine release (HR)

HR was measured in peripheral blood basophils before skin testing and provocation, following a procedure previously described (13). Venous blood was drawn into heparinized tubes and diluted 1/3 with PBS containing Ca^{2+} (1 mM) and Mg^{2+} (1 mM) at pH 7.4. The challenge was carried out with 200 μl of diluted whole blood, 70 μl of PBS, and 30 μl of rabbit anti-human IgE (Dako, Versailles, France). Final anti-

Table 1. Sensitivity (Se), specificity (Sp), and positive and negative predictive values (PV) of histamine-release test (only net histamine release of 5% of histamine content was considered positive) in 35 patients with proven drug allergy and 73 controls

	Patients <i>n</i>	Controls <i>n</i>	Se	Sp	Positive PV*	Negative PV*
All drugs						
Total	35	73	51.4%	63.0%	29.3%	81.1%
Onset ≤72 h	31	64	54.8%	62.5%	30.4%	82.4%
Anaphylactic						
shock	10	4	40.0%	50.0%	19.3%	73.6%
Urticaria	23	14	43.5%	69.2%	29.7%	80.4%
β-Lactams						
Total	20	45	60.0%	62.2%	41.6%	77.5%
Onset ≤72 h	16	37	68.7%	62.2%	44.9%	94.7%

* Predictive values were calculated on basis of 23% prevalence of proven drug allergy among patients with compatible history for "all drug" analysis and 31% for "β-lactam" analysis.

IgE concentrations were 0 (spontaneous histamine release), 0.1, 1, and 10 µg/ml, and final (culprit or closest chemically related) drug concentrations were 0.01, 0.1, 1.0, and 10 µg/ml. After a 30-min incubation period at 37°C, cells were centrifuged, and supernatants were collected and frozen at -20°C until histamine measurements were performed. The total histamine content of the blood cells was measured after cell lysis with the freezing and thawing (three times) technique. Histamine was measured by a highly specific and sensitive enzyme immunoassay (Immunotech, Luminy, France) using a monoclonal antibody against acylated histamine (14). The sensitivity of the assay is 0.2 nM. Results were expressed as a percentage of total histamine

content after correction for spontaneous release, and the net HR was calculated by the following formula:

$$\frac{(\text{anti-IgE release or drug release} - \text{spontaneous release})}{(\text{total histamine content} - \text{spontaneous release})}$$

As other authors have suggested (8, 15), we considered 5% the limit of positivity, but we also analyzed other threshold levels, as described below.

Statistical analysis

The Kruskal–Wallis and Mann–Whitney U tests were used for statistical comparisons between the groups. Sensitivity (Se), specificity (Sp), and ROC (receiver operating character-

Table 2. Ten subjects with drug-induced anaphylactic shock underwent histamine-release test with culprit or closest chemically related drug (only net histamine release of ≥5% of histamine content was considered positive)

Patients	Sex/age (years)	Drug	Time interval ¹	Total histamine ²	Histamine release Spontaneous	Drug ³
BM	F/64	Cefatrizine	2	61	4	0 (10 µg/ml)
NV	M/62	Cefatrizine	2	59	16	25 (1 µg/ml)
SN	M/47	Amoxycillin	12	32	3	13 (0.1 µg/ml)
CA	F/28	Amoxycillin-clavulanate	22	34	28	0 (10 µg/ml)
WA	M/49	Paracetamol	2.5	140	<1	4 (10 µg/ml)
DC	F/39	Paracetamol	2	89	3	0 (10 µg/ml)
FM	F/47	Paracetamol	2.5	63	2	0 (10 µg/ml)
LM	F/8	Paracetamol	3.5	112	2	1 (10 µg/ml)
MA	F/36	Rocuronium	120	79	1	8 (10 µg/ml)
VB	M/49	Rocuronium	12	103	0	12 (10 µg/ml)

¹ Period of time in months from shock to blood sample. ² Total histamine is expressed in ng/ml and equals maximum histamine release caused by freeze/thawing (see Material and methods). ³ Net histamine release in percentage of histamine content after correction for spontaneous release; drug concentrations are in (parenthesis).

istics) curves were calculated according to McNeil et al. (16). The predictive values (PV) were calculated according to the Bayes theorem (17), with the following formula:

$$\text{Positive PV} = P \times \text{Se} / P \times \text{Se} + (1 - P) \times (1 - \text{Sp})$$

$$\text{Negative PV} = (1 - P) \times \text{Sp} / (1 - \text{Se}) \times P + \text{Sp} \times (1 - P)$$

To calculate the above predictive values, we utilized three different prevalence rates (P) of drug allergies, according to our personal data and data taken from the literature. Over 380 patients with a history of reactions consistent with an immunologic mechanism were referred last year to our department for diagnosis of drug allergy and provoked with the culprit drug: 23% were allergic and 77% were not. Thus, 23% was the P prevalence rate we chose when all drugs were considered. Among these 380 patients, 115 were treated with β -lactam antibiotics, but only 36 (31%) were truly allergic to them, according to the procedure described above. Thus, 31% was the P prevalence rate we chose when only β -lactam antibiotics were considered. Moreover, since Bigby et al. (18) documented over 104 cutaneous allergic reactions per 1000 β -lactam administrations to hospitalized patients, giving a prevalence of around 10%, we also chose 10% as a P prevalence rate when only β -lactam antibiotics were considered.

Results

Drug allergies

Among the 68 patients referred to our clinic, 35 had proven drug allergy (A) and 33 were not allergic to drugs (C1). We added 40 controls (22 aeroallergen-allergic patients and 18 laboratory staff with no history of drug allergy [C2]) for better assessment of the specificity of the HR test. Positive diagnosis was made for paracetamol ($n=6$), macrolides ($n=2$), β -lactams ($n=20$), muscle relaxants ($n=2$), quinolones ($n=2$), tetanus toxoid ($n=2$), and chloroquine ($n=1$). Clinical manifestations ranged widely from maculopapular exanthema, urticaria ($n=37$), and angioedema ($n=17$) to anaphylactic shock ($n=14$). Most reactions were of rapid onset (21/35 appearing less than 6 h and 31/35 less than 72 h after drug intake).

Histamine release (HR)

The total HR upon freeze/thawing did not differ among the three groups (median [25–75th percentiles]): 61 ng/ml (43–89) for A, 50 ng/ml (31–90) for C1, and 55 ng/ml

(37–79) for C2. When 5% of net histamine content release in the presence of the drug was chosen as positive limit, 18/35 A, 12/33 C1, and 15/40 C2 had positive net HR (Fig. 1) with the following global statistical values (Table 1): sensitivity, 51.4%; specificity, 63.0%, positive predictive value, 29.3%; and negative predictive value, 81.1%. Net HR results for patients experiencing an anaphylactic shock and patients given β -lactams are detailed in Tables 2 and 3, respectively. The specificity of HR for the diagnosis of β -lactam allergy was 62.2%. The sensitivity of HR was higher when β -lactams were analyzed alone (60.0%), but lower when anaphylactic shocks were analyzed alone (40%). However, when only urticaria was taken into account, the specificity of HR was better (69.2%); when only β -lactam reactions occurring within 72 h after drug intake (reactions thought to be IgE-dependent) were taken into account, the sensitivity was slightly better (68.7%) (Table 1). The positive predictive values were very low, always under 50%. A better positive predictive value was obtained when β -lactams were analyzed alone and a prevalence of β -lactam allergy of 31% was chosen (Table 1). However, the positive predictive value dropped from 41.6% to 15% when a prevalence of β -lactam allergy of 10% was chosen. The negative predictive value was high (81.1%), but not better, when onsets of less than 72 h, β -lactam allergies, anaphylactic shocks, or urticaria was analyzed separately. The negative predictive value was higher for β -lactam reactions with an onset of less than 72 h (94.7%) (Table 1).

ROC curves were not informative because there was no net HR at all in 33/108 of the subjects. However, when 10% of net histamine content release in the presence of the drug was chosen as positive limit, 8/35 A, 6/33 C1, and 9/40 C2 had positive net HR with the following global statistical values: sensitivity, 22.8%; specificity, 79.5%; positive predictive value, 24.9%; and negative predictive value, 77.5%.

Discussion

Thus, in the present study, we showed that the positive value of the *in vitro* HR test for the diagnosis of drug-induced hypersensitivity (including anaphylaxis) is poor, confirming what was generally recognized, but not demonstrated. However, the high negative predictive value may be used to rule out allergic drug reactions, especially in some patient subgroups. When only β -lactam reactions of rapid onset (less than 72 h after drug intake) were taken into

Table 3. Net histamine-release (HR) results in 20 subjects with proven β -lactam allergy. HR results are expressed as percentage of histamine content released in presence of drug

Patients	Sex/age (years)	Drug	Symptoms ¹	Chronology	Time interval ²	Skin tests ³	Drug challenge	HR (%)
<i>Positive skin tests and positive HR</i>								
DJ	M/73	Penicillin G	urt.	24 < $\leq$ 72 h	240	Md; PG, Ax	ND	5
SN	M/47	Amoxycillin	shock	$\leq$ 1 h	13	Md, PG, Ax	ND	13
PT	F/59	Amoxycillin	urt.	8 < $\leq$ 24 h	6	Md, PG	ND	8
BA	F/57	Penicillin G	urt.	$\leq$ 1 h	96	PL	ND	31
MY	F/63	Ampicillin	fever	> 72 h	204	PL, Ax	ND	5
PC	F/31	Amoxycillin-clavulanate	Qcke	24 < $\leq$ 72 h	24	PL, MD, PG, Ax	ND	9
FO	F/48	Amoxycillin	urt.	$\leq$ 24 h	120	Ax	ND	11
NV	M/62	Cefatrizine	shock, urt.	$\leq$ 1 h	2	Ax	ND	25
AA	F/51	Amoxycillin	urt.	$\leq$ 1 h	60	Ax	ND	7
<i>Positive skin tests and negative HR</i>								
RJ	F/43	Amoxycillin	Qcke	$\leq$ 6 h	216	PL, PG, Ax	ND	0
CH	F/33	Amoxycillin-clavulanate	urt., br.	4 days	10	PL, MD, PG, Ax	ND	0
GE	M/68	Penicillin G	urt.	> 72 h	24	PG, Ax	ND	0
MN	F/50	Amoxycillin	urt.	8 days	3	Ax	ND	0
FI	F/33	Amoxycillin-clavulanate	Qcke, urt.	$\leq$ 1 h	6	Ax	ND	1
<i>Negative skin tests and positive HR</i>								
VS	F/26	Amoxycillin	urt.	8 h	3	–	urt.	41
BL	M/71	Ceftriaxone	urt.	$\leq$ 1 h	1	–	urt.	5
KJ	M/65	Penicillin G	urt.	$\leq$ 8 h	480	–	urt.	12
<i>Negative skin tests and negative HR</i>								
CA	F/28	Amoxycillin-clavulanate	shock, Qcke, br.	$\leq$ 3 h	22	–	shock	0
BM	F/64	Cefatrizine	shock, Qcke, urt.	$\leq$ 3 h	2	–	shock	0
DC	F/25	Amoxycillin	urt.	24 < $\leq$ 72 h	2	–	urt.	0

¹ Allergic symptoms: urt., urticaria; br., bronchospasm; Qcke, Quincke's edema. ² Period of time in months from allergic reaction to blood sample. ³ Only positive skin tests are listed: PL, penicillin poly-L-lysine; Md, mixture of minor determinants; PG, penicillin G; Ax, amoxycillin; ND, not done.

account, the specificity, the sensitivity, and the negative predictive value were improved.

Obvious explanations of low sensitivity are the possibility that the reactive compounds may not be the drug itself but one of its metabolites (11), and/or the injectable soluble form of the drug may not be available (ready to be tested on blood basophils) and/or the mechanism is not IgE-dependent. This may be particularly true for paracetamol, macrolides, and cephalosporins, of which the metabolites are not available for testing or even all known. Thus, among the six out of 10 patients with cases of anaphylactic shock and negative HR, four were due to paracetamol, one to cefatrizine (of which the injectable soluble form is not available), and one to amoxycillin-clavulanate (patient with a high spontaneous HR). However, for penicillins, where major and minor determinants, together with injectable forms of penicillin G,

amoxycillin, and ampicillin are readily available and account for most B-cell epitopes (14 out of 20 had positive skin tests in this study), the HR test does not appear to be powerful either, giving a sensitivity of 60% and a positive predictive value of 15.0–41.6%. Moreover, for the reactions reported to be IgE-dependent (i.e., β -lactam reactions occurring within 72 h after drug intake) (19), where the HR test is expected to be positive, the sensitivity was only slightly better (68.7%), suggesting either a true low sensitivity of the assay or the involvement of non-IgE mechanisms. The low specificity of the HR test may be related to the fact that, as for specific serum IgE, positive results demonstrate sensitization to the drug but do not confirm that it caused the allergic reaction.

However, there may exist subgroups of drug-allergic patients for which HR may be of interest. For example, in

the case of myorelaxant allergy, HR has been shown to correlate well with skin tests and specific IgE (8), but we did not have a sufficient number of such patients to confirm this report. Our two myorelaxant-allergic patients had positive HR although five out of eight controls also had positive results. In the paper of Mata et al. (8), the sensitivity and specificity of the HR test were 65% (26/40) and 100% (0/44), respectively. In the paper of Vervloet et al. (20), the sensitivity was lower (eight out of 25 patients), but the specificity was not studied. HR could be of interest as additive value, complementing β -lactam skin tests, for example. Thus, in our experience, 30% of true β -lactam-allergic patients have negative skin tests (6/20) (Table 3). They represent almost 10% of the 115 patients referred last year to our clinic with a history of β -lactam allergy but negative skin tests. Current protocols hold it safe to allow patients with nonimmediate history of penicillin allergy and negative penicillin skin tests to take penicillins when needed without previous provocation test (21). The six patients in the present study had personal histories of variable onsets, both immediate ($n=1$) and accelerated ($n=5$). In this regard, although HR could have been of interest to avoid the provocation test, only 3/6 of these patients had positive HR (Table 3). However, for firm conclusions in this regard, we would need more patients.

Allergies to medically prescribed drugs play significant roles in iatrogenic drug-related illness and are responsible for levels of morbidity, mortality (2), and health-care costs which are presently underevaluated. Their symptoms are

wide ranging, from mild urticaria to potentially fatal anaphylactic shock. The underlying mechanisms are varied and include all four types of the immunologic reactions described by Gell and Coombs (22), leading to some difficulty in diagnosis. There are also numerous other types of nonallergic reactions which mimic symptoms of allergy, and which have polymorphic origins. Finally, there are many differential diagnoses including infectious agent-induced exanthema and food allergy, so that, in our experience, only 23% of the patients referred to our department for suspected drug-allergy are truly allergic. Therefore, it would be highly desirable to have discriminating biologic tests available; firstly, to establish or rule out drug-allergy, and, secondly, to specify the culprit drug in patients receiving several drugs simultaneously. However, the biologic diagnostic tools at our disposal are few and have not yet been fully evaluated. Moreover, a formal diagnosis of allergy is required in order to institute proper preventive measures and treatment. In this paper, we demonstrated that the HR test cannot be, at the present stage, a routine biologic test for the diagnosis of drug allergies. Moreover, the analysis of HR from total blood in the presence of a drug is not a routine biologic assay; it requires certain skills and is expensive. However, it certainly is relevant to the research to demonstrate an IgE-dependent mechanism in patients with a suggestive clinical history.

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