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Non-specific nasal provocation test with histamine. Analysis of the dose-response curve*

G. Zambetti[1], M. Moresi [2], R. Romeo[1], M. Luce[1], F. Filiaci[1]

[1] Department of Otolaryngology, Policlinico Umberto I, University of Rome La Sapienza, Italy
[2] Institute of Food Technology, University of Tuscia, Viterbo, Italy

SUMMARY

Non-Specific Nasal Hyper-reactivity (NSNH) is described as a clinical condition characterized by the presence of rhinitic symptoms that are a consequence of non-specific stimulations. Because of its effects on vascular, epithelial, and glandular receptors, NSNP Test (NSNPT) with histamine allows the study of NSNH.

The aims of this study are 1. to analyze the behavior of NSNH both in non-allergic chronic vasomotory patients and in healthy control subjects 2. to correlate total nasal resistance(TNR) to each dosage of histamine to derive the dose/response curves and 3. to study these curves to analyze and possibly define different stages according to the intensities of response of NSNH.

We have studied 26 subjects affected by non-allergic vasomotor rhinitis and 10 healthy control subjects. We sprayed a NSNPT with histamine-phosphate (0.2-0.3-0.4-0.5-0.6-0.8mg) in different sessions to avoid accumulation phenomena. Five minutes before and five minutes after each challenge, TNR was determined by active anterior rhinomanometry . TNR was correlated to the doses of histamine by an empirical equation.

The most important results of this study are as follows: a) the variation of TNR follows a model of exponential curve, b) it is possible to classify NSNH, as a function of the regression b coefficient belonging to the empirical equation used, in reactivity classes, c) from one reactivity class to another, post-stimulation TNRs double; 0.5mg of histamine of the NSNPT is the optimal dose, d) there is an overlap between the responses of some normal subjects and rhinopathic patients that will be the subject of a further study.

Finally, our data suggest that, in a future perspective, it is possible to use the NSNPT with histamine for diagnostic, prognostic and therapeutic control purposes.

Key words: dose-response curve, nasal hyper-reactivity, non-specific nasal provocation test with histamine, total nasal resistance

INTRODUCTION

Nasal provocation tests may be specific or non-specific (NSNPT); the latter make use of physical or chemical stimuli. NSNPTs are widely used to study the physiopathological mechanisms of rhinopathy and their response to pharmacological treatment. Even though bronchial challenge tests have become better standardized, there is a remarkable interest for NSNPTs because nasal cavities are more accessible than bronchi, allowing easier manoeuvres and internal controls. Furthermore, at a nasal level it is possible to distinguish the direct and indirect effects of structural stimulation. Responses to the NSNPT can be easily measured in its components, and the change of the nasal resistance can be measured by rhinomanometry (RRM) (Pipkorn, 1988).

NSNPTs are used to study non-allergic vasomotor rhinitis, which is clinically characterized by nasal symptoms caused by exposure to a series of non-specific physical and chemical stimuli. This hyper-reaction manifests itself through sneezes, rhinorrhea and nasal obstruction caused by an increased response of the sensorial nerves, of the nervous reflexes and of the end organs (Andersson et al., 1989; Filiaci and Zambetti, 1988; Svensson et al., 1989; Pipkorn et al., 1986; McDonald, 1987; McDonald, 1994). The NSNPT with histamine determines an immediate response in terms of itching, sneezing, hyper-secretion and obstruction (Secher et al., 1982). Such effects are due to the stimulation of H_1 histamine receptors of the sensorial nerves, of H_1 and H_2 histamine receptors of the capacity vessels and of the H_1 histamine receptors of the post-capillary venules,

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that lead to a gradual and repeatable plasmatic exudation (Svensson et al., 1989; Shirasaki et al., 1992; Gawin et al., 1992). Except for nasal obstruction, nasal hyper-secretion and sneezes caused by NSNPT with histamine show a considerable tachyphylaxis which make the analysis of the responses more difficult (Secher et al., 1982). On the whole, nasal stimulation with histamine is the test that best stimulates a rhinitic response, without causing eosinophilia or increasing responsiveness (Gronborg et al., 1986; Walden et al., 1991), but usually the doses used are too high and cause a greater plasmatic exudation than the one caused by an allergenic exposure (Brofeldt et al., 1986). Contrary to what happens at a bronchus level, at a nasal level there is an overlap between normal subjects and patients with rhinitis in terms of hyper-reactivity caused by NSNPT with histamine, but usually the stimulation dose for normal subjects is five times lower than the one for those presenting with rhinitis symptoms (Plavec et al., 1994). Furthermore, increasing the doses of stimulation both in normal subjects and rhinopatics (VanWijk et al., 1989; Van De Heyning et al., 1989) nasal resistance improves. NSNPTs with histamine and with methacholine makes it possible to define the respective impact of nasal hyper-reactivity on the alteration of sensorial nerves, of nervous reflexes and of the alteration of the glands in this process (Van Wijk and Dieges, 1994).

The aims of this study are: 1. to analyze the dose/response curve of the NSNPT with histamine in normal subjects and rhinopathic patients in order to verify differences in behavior, 2. to describe the response curve with a mathematical model, relating empirically total nasal resistance (TNR) for each dose of stimulation with the reactivity to histamine, 3. to verify if TNR is a valid parameter to monitor reactivity to histamine, which represents the non-specific nasal hyper-reactivity (NSNH).

MATERIAL AND METHODS

Thirty-six subjects (12 women and 24 men, mean age 42±3.5 years) were recruited for this study. They were divided into two groups: 26 patients affected by perennial non-allergic rhinopathy (PNAR) and 10 control healthy subjects. All subjects underwent anamnesis, ENT-examination, skin-tests, and RAST to exclude any sort of allergic pathogenesis. The radiograph of the sinuses of the nose, carried out as routine procedure, excluded any sinuses involvement, both of clinical and radiological importance. Therefore, those patients can be considered as being affected by first degree rhinitis according to Wayoff (Wayoff, 1983).

The patients had a clinical history of perennial non-allergic rhinitis dating back from 2 to 10 years. All 36 subjects belonging to both groups received a non-specific nasal challenge test with histamine (Luce et al., 1991). Nasal stimulation with histamine phosphate was performed in different sessions (every day, and at the same hour) to avoid accumulation phenomena. Each subject received a solution in the nasal cavity containing 1, 1.5, 2, 2.5, 3, and 4 mg of histamine per ml with a pre-dosed spray in a volume of 0.1 ml so to gradually give a dose of histamine of 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 mg. The Total Nasal Resistance (TNR) was determined with an active anterior rhinomanometry (RRM) to each subject 5 minutes before and 5 minutes after each stimulation, after blowing the nose to free it from any sort of secretion and with a 1 to 3 minutes pause after the provocation to give way to the maximum number of sneezes present at that moment. The TNR was calculated as [rightR x leftR / (rightR + leftR)] (Clement, 1984).

All the TNR values before stimulation were within the normal range. The response to NSNPT with histamine was considered positive when the TNR after stimulation increased by at least 100% vis : vis basic values. This choice was made considering that a 100% increase in TNR cannot be a variation caused by a normal nasal cycle; other studies have illustrated important increases already starting from 75-100% (Clement et al., 1983; Plavec et al., 1994; Randerath et al., 1998), though without a proper standardization. Histamine stimulation adequately simulates the response to an allergenic stimulation (if doses are not excessively high), for which many studies have shown an important correlation between a TNR 100% minimum increase and other diagnostic tests. The TNR values were compared to histamine doses by using the following exponential equation:

$$\text{Ln TNR}=a+bH \qquad \text{(equation 1)}$$

where “a” and “b” are generic regression coefficients, TNR is the response to the total nasal resistance and “H” is the independent variable (the quantity of histamine inhaled). All regression coefficients were evaluated with the method of the minimum squares (Box and Draper, 1987), with their standard error and the total correlation coefficients (R^2).

The statistical correlation between the two groups was performed with the “Student t test”, comparing the difference among the mean values of the subjects.

RESULTS

The normal control subjects and the vasomotor rhinopatics were devided into groups to evaluate and compare their nasal response, as shown in Table 1: group A, healthy control subjects; group B, normo-reactive subjects of group A; group C, hyper-reactive subjects of group A; group D, rhinopatics; group E, hyper-reactive patients of group D; group F, normo-reactive patients of group D; group G, normo-reactive subjects of groups A and D; group H, hyper-reactive subjects of groups A and hyper-reactive patients of group D. As shown in Table 1 groups B, F, and G responded to the NSNPT normally, since the groups are made up by normo-reactive subjects present both in the controls and in the vasomotor rhinopatics. The incidence of normo-reactivity in rhinopatic patients is 32.5%, while the incidence of hyper-reactivity in control subjects is 30% (group C). Groups D, C, E, and H show a positive response to the NSNPT, since they represent subjects nasal hyper-reactivity. TNRs increase as the doses of inhaled histamine by the hyper-reactive subjects increase starting from 0.2 mg, while in group C, such an increase occurs starting from a dose of 0.5mg (Figure 1). Dose by dose, the highest values are found in groups E, H, and D, which represent all the hyper-active subjects of rhinopatics in the population. Comparing the TNR responses to the different

Table 1. Total Nasal Resistance (TNR), average (M) and standard diviation (SD) before and after Non-Specific Nasal Provocation Test (NSNPT) with histamine in the tested groups.

Group	Baseline values	Histamine (mg) 0.2	0.3	0.4	0.5	0.6	0.8	
A	0.32	0.36	0.41	0.51	0.72	0.97	1.35	M
	0.12	0.11	0.11	0.09	0.42	0.71	1.25	SD
B	0.36	0.36	0.40	0.46	0.52	0.57	0.61	M
	0.12	0.10	0.09	0.10	0.06	0.11	0.08	SD
C	0.23	0.35	0.43	0.62	1.19	1.91	3.07	M
	0.05	0.16	0.16	0.11	0.54	0.61	0.83	SD
D	0.29	0.66	1.56	2.15	3.63	4.33	5.08	M
	0.14	0.44	2.07	2.66	3.88	4.21	4.25	SD
E	0.28	0.78	1.71	2.84	4.96	6.47	7.54	M
	0.15	0.48	2.17	2.96	3.99	4.00	3.38	SD
F	0.33	0.41	0.54	0.60	0.63	0.63	0.65	M
	0.11	0.12	0.18	0.17	0.17	0.13	0.13	SD
G	0.34	0.39	0.47	0.53	0.58	0.60	0.64	M
	0.11	0.11	0.14	0.13	0.12	0.12	0.10	SD
H	0.27	0.72	1.53	2.52	4.42	5.82	6.90	M
	0.12	0.32	1.16	1.62	2.30	2.31	2.11	SD

Classification of the Groups:
group A = 10 healthy control subjects;
group B = Group A minus 3 hyper-reactive subjects noticed in this group (10 - 3 = 7 subjects);
group C = hyper-reactive subjects in the control group (3 subjects);
group D = group of the rhinopathics patients (26 subjects);
group E = hyper-reactive patients of the group D (18 subjects);
group F = normo-reactive patients of the group D (8 subjects);
group G = normo-reactive subjects of groups A and D, group B + group F (15 normo-reactive subjects);
group H = hyper-reactive subjects of groups A and D, group E + group C (21 hyper-reactive subjects).
TNR after NSNPT increased two-fold in comparison with the baseline values (that are reliable indexes of a minimal positive response to the test) among the various groups at the histamine dosages as shown:
Group A at 0.5 mg; Group B not increased; Group C at 0.3 mg; Group D at 0.2 mg, Group E at 0.2 mg; Group F not increased; Group G not increased; Group H at 0.2 mg.

Table 2. Comparison among the groups showed in Table 1 at the single dosages of histamine: statistical importance.

Comparison among the groups	Baseline values	Histamine (mg) 0.2	0.3	0.4	0.5	0.6	0.8	
A *vs* D	ns	2.18	ns	6.14	2.35	2.49	3.23	t
		.05		.001	.05	.05	.01	p<
E *vs* F	ns	2.17	ns	2.11	3.02	4.08	5.68	t
		.05		.05	.01	.001	.001	p<
B *vs* C	ns	ns	ns	2.32	2.42	6.07	8.49	t
				.05	.05	.001	.001	p<
A *vs* E	ns	2.30	ns	2.05	3.32	4.27	5.53	t
		.05		.05	.01	.001	.001	p<
B *vs* E	ns	2.29	ns	2.10	2.90	3.84	5.33	t
		.05		.05	.01	.001	.001	p<
B *vs* F	ns	ns	ns	ns	ns	ns	ns	
C *vs* E	ns	ns	ns	ns	ns	ns	2.23	t
							.05	p<
H *vs* G	ns	4.44	3.41	4.49	6.42	8.73	11.43	t
		.001	.001	.001	.001	.001	.001	p<

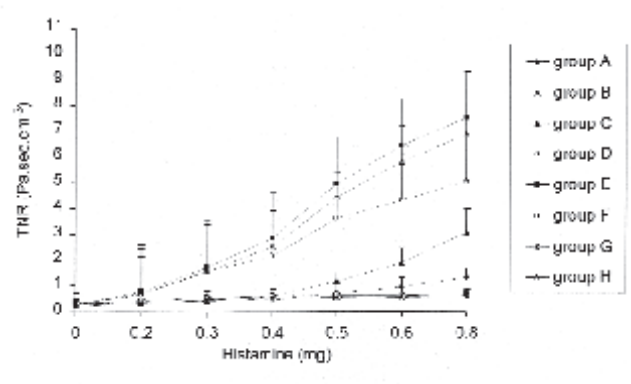

Figure 1. Average (M) and Standard Deviation (SD) of total nasal resistance (TNRs) before and after non-specific nasal provocation test (NSNPT) with histamine in the tested groups.

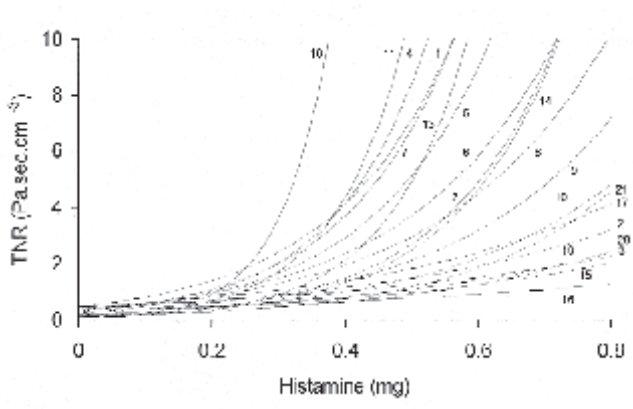

Figure 2. Nasal reactivity curves of all hyper-reactive patients (21 patients).

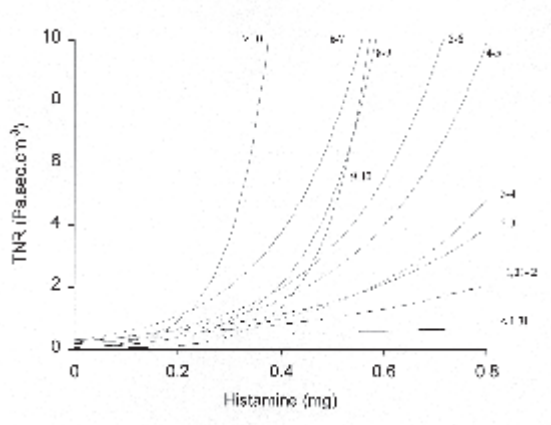

Figure 3. Nasal reactivity curves expressed in reactivity coefficient units.

doses of histamine (Table 2), the differences become statistically significant starting from 0.4 mg (A vs D), while the comparison of group H with group G is already significant starting from a dose of 0.2 mg. Such differences among the compared groups are highly significant up to a dose of 0.8 mg of histamine.

The single reactivity curves are illustrated in Figure 2. If we group those curves having similar reactivity coefficients, we obtain a curve distribution as indicated in Figure 3, where it is evident that the difference between the various curves is important when the respective reactivity coefficients differ at least one unit.

Table 3. Coefficient of the nasal reactivity to histamine (regression b coefficient) calculated by using the empirical equation 1 (see text). There are also showed the correlation coefficient (R^2) between TNR and the dosage of histamine.

Groups	Reactivity (b) coefficient	R^2
Normo-reactive(*) :	1.30 ± 0.12 (maximum value)	0.91
Patients :		
1	4.15 ± 0.48	0.97
2	2.53 ± 0.29	0.94
3	1.41 ± 0.57	0.61
4	6.60 ± 0.75	0.88
5	5.41 ± 0.81	0.94
6	5.33 ± 0.54	0.96
7	6.64 ± 1.06	0.93
8	4.21 ± 0.73	0.89
9	4.11 ± 0.35	0.97
10	3.97 ± 0.39	0.96
11	9.58 ± 1.03	0.84
12	5.73 ± 2.45	0.81
13	8.86 ± 4.42	0.76
14	5.77 ± 1.20	0.85
15	4.16 ± 1.30	0.86
16	1.31 ± 0.40	0.96
17	2.41 ± 0.40	0.96
18	20.83 ± 4.93	0.64
Hyper-reattive subjects of the control group:		
19	1.35 ± 0.43	0.97
20	1.31 ± 0.39	0.95
21	2.55 ± 0.31	0.95

(*) = 7 normal healthy control subjects + 8 normo-reactive subjects of the group of patients. 76.2% of the hyper-reactive subjects showed regression b coefficient 6, with contained SD of the mean values, while 23.8% (corresponding to the hyper-reactive patients with a strong nasal response to the NSNPT) show that such coefficients are widely distribuited, with consistent SD of the mean values.

The regression b coefficients (indexes of the reactivity to histamine of equation 1) of all the subjects that underwent the NSNPT (Table 3) shows that for normo-reactive subjects the maximum value is 1.3±0.12 while for hyper-reactive subjects it varies according to the entity of the rhinomanometric response from 1.31±0.40 to 20.83±4.93. In Figure 3 it can also be noted that, starting from a dose of 0.5mg histamine (the minimum, statistically most relevant dose) the reactivity curves already differ from each other, except those having 2 to 3 and 3 to 4 coefficients, which differ only with a higher, 0.8mg dosage. Considering that those reactivity curves with reactivity coefficients bigger than 6 correspond to such high TNR values that the nose can be considered practically totally obstructed. Therefore, with the same clinical response, nasal reactivity categories can be proposed, as is shown in Table 4 and the statistical relevance of which is expressed in Table 5.

A little over 76% of the hyper-reactive subjects have a regression b coefficient inferior to 6, while 23.8% (corresponding to the hyper-reactive patients with a strong nasal response to the NSNPT) show that such coefficients are widely distributed and

Table 4. Nasal reactivity coefficient (regression b coefficient, average and SD) of the patients in the single reactivity classes; % incidence in comparison to all hyper-reactive subjects studied. There are showed both type of reactivity and the values of correlation coefficient (R^2) between TNR and dosages of histamine according to equation 1.

Class of reactivity	N° of subjects	Reactivity (b) coefficient	% incidence	Type of reactivity	R^2
1 : from 1.31 to 3	7	1.93±0.63	33.33	Light	0.97
2 : from 3.1 to 5	5	4.12± 0.09	23.81	Medium	0.94
3 : from 5.1 to 6	4	5.56±0.22	19.05	Consistent	0.93
4 : > of 6	5	10.50±5.92	23.81	Strong	0.95
5 : < of 1.31	15	1.30 ±0.12		Normal	0.91

The mean TNR after NSNPT with histamine tend to double to one class of reactivity to another starting from a dose of 0.5 mg (the averages and the SD are respectively: 2.0±0.3 at 0.5 mg ; 2.2±0.8 at 0.6 mg ; 2.4±1.9 at 0.8 mg).

Table 5. Comparison among classes of the hyper-reactive subjects in relation to the reactivity coefficient (regression b coefficient).

Comparison among classes	t	p<
1 *vs* 5	9.92	.001
2 *vs* 5	38.60	.001
3 *vs* 5	53.85	.001
4 *vs* 5	12.36	.001
1 *vs* 2	7.69	.001
1 *vs* 3	11.03	.001
1 *vs* 4	3.56	.01
2 *vs* 3	14.31	.001
2 *vs* 4	2.41	.05
3 *vs* 4	1.64	ns

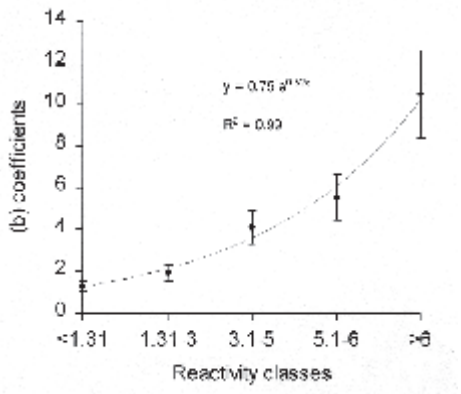

Figure 4. Average (M) and Standard Deviation (SD) of the nasal reactivity coefficients (regression b coefficients) of the patients of different groups of reactivity.

with consistent standard deviations, indicating the existence of a wide spectrum of responses. If we compare the increase of TNR after the NSNPT in all examined subjects with regression b coefficients starting from a dose of 0.5mg of histamine, we can notice that if we gather such coefficients in classes of reactivity, as indicated in Table 4, on average TNRs tend to double from one class of reactivity to another, demonstrating that a dose of 0.5mg of

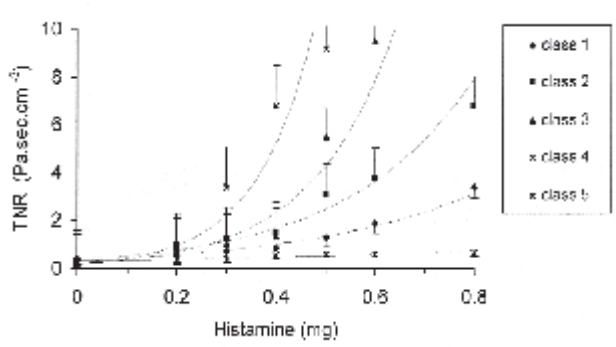

Figure 5. Average (M) and Standard Deviation (SD) of TNRs in patients of different groups of reactivity.

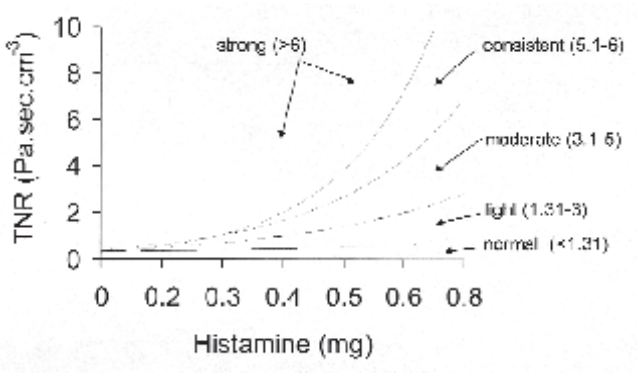

Figure 6. Staging of nasal reactivity: curves, areas and types of nasal reactivity.

histamine is the minimum provocative one capable to increase the nasal response after stimulation by 100%. On the basis of this observation it is possible to stage nasal reactivity in terms of intensity of response to the NSNPT in five classes (Table 4): normal, light, medium, consistent, and strong. Light hyper-reactivity affects 33% of the cases, inclusive the responses of the hyper-reactive subjects present among normal controls. In a variable range of percentage from 19% to 24% of the cases, hyper-reactivity results to be of mean, consistent, and strong degree.

The curve that represents the distribution of the regression b coefficients for the classes of reactivity (Figure 4) is exponential,

as generally the increase of TNR is. Indeed, correlating the distribution of TNRs by classes of reactivity with the doses of histamine (Figure 5), we can observe that the curves, expression of the equations, are exponential with very significant values of R^2. If we statistically correlate the classes of reactivity with the regression b coefficients (Table 5), we observe important differences between each class of reactivity, apart from comparison between classes 3 and 4, since there is such a wide spectrum of positive nasal responses in the latter.

With equation 1, by correlating the TNR variations to the doses of histamine used it is possible to mathematically draw the curves of response which delineate the areas of nasal reactivity including all the patients of the same class of reactivity (Figure 6), thus belonging to the same typology of reactivity.

DISCUSSION

The results of this study strongly emphasize/support the fact that NSNH is present in 67.5% of the vasomotor rhinopatics non-allergic to the NSNPT with histamine, while in 32.5% of the cases the test yielded a negative result. This could mean that NSNH is not present in the latter or that it could be provoked with other tests for example using the NSNPT with methacholine, with other drugs or with physical stimuli. By increasing the histamine doses beyond the quantities we have used, a conversion of some non responding patients into responding ones may occur. However higher doses could be non-physiological thus causing a higher plasmatic exudation and, may be, a forced response. We believe that the best provocative dose is the minimum relevant one, and the one which is effective in the distinction of normo-reactive and hyper-reactive subjects. Moreover, the minimum dose we propose is similar to the one which has been proposed in other studies (Plavec et al., 1994; Clement et al., 1983). Besides, the choice of the 0.5mg stimulation dose allows to obtain a more easily measurable nasal response in almost all the hyper-reactive patients, with reactivity coefficients smaller than 10 (Figure 3). The TNR increase with histamine NSNPT is exponential, according to Poiseuille's law, and the threshold of positivity to the test (doubling TNRs after stimulation) may be also adopted for the staging of the intensity of the response by the NSNH.

In agreement with the data provided by equation 1, this test made it possible to differentiate types of NSNH according to the intensity of the rhinomanometric response, through a classification of different stages. In case the intensity of the NSNH responseis to reflect the clinical gravity of the rhinopathy, staging may also have a prognostic value by using the nasal provocation test to histamine with a dosage of 0.5mg.

The use of equation 1 in evaluating nasal reactivity to histamine has made it possible to stage NSNH in five classes of reactivity with different regression b coefficients; the mean values between different classes were shown to double. As for the comparison between NSNPT using the sneeze parameter and the one using the nasal resistance parameter, we highlight that, although the first one is characterized by a very short sneeze it is affected by the phenomenon of tachyphlaxis. This is not present in the second case where, a stimulation after a congruous time has never modified the subsequent stage of the test, although the sensitiveness of the two tests is identical (Hellgren et al., 1997). Our test allows useful comparison with specific hyper-reactivity, which is mainly studied with rhinomanometric methods (Bachert and Keilman, 1988; Clarke, 1988; Salzano, 1997). Moreover, we believe that, practically, sometimes huge differences can exist between the symptoms caused in the patient and the real clinical objectiveness (e.g. small mucous reactions) that can be evaluated measuring the nasal flow (Hellgren et al., 1997).

In our opinion the dose-response curves are an elegant method to evaluate mathematically the nasal reactivity. These curves do not represent a routine method but can give useful, quick and easy results in studies of nasal sensitivity (threshold concentration of histamine).

In fact, by utilizing only the minimum relevant histamine dose (0.5mg) we can identify hyper-reactive subjects and, on the basis of the reactivity categories that were previously calculated on a sufficient number of patients in order to obtain a response standard, define the different types of nasal reactivity. Apart from having a prognostic and diagnostic value, the application of such test may be useful for monitoring the therapeutic effects of possible medical or surgical treatments directed to the control of NSNH.

Finally, the study of the behavior of NSNH in control subjects has clearly indicated the presence of hyper-reactivity in 30 % of the cases, even in only light manifestations. Such presence, which overlaps the response provided by some rhinopathic subjects, could be due to: 1. Inaccurate classification by application of the challenge test and RRM to some subjects that are only apparently normal; 2. NSNH of a lighter degree may constitute an expression of a perfectly normal response; 3. A certain NSNH is not a salient feature exclusively of rhinopathic patients. Further studies will be necessary to verify these and other possible hypotheses, since this paper proposes a mathematical model of study which is necessarily limited but also applicable, in a future perspective, to a larger number of patients.

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Giampietro Zambetti, M.D
Departement of Otorhinolaryngology
University of Rome “La Sapienza”.
Viale del Policlinico,
155 00151 Rome Italy
Tel./Fax:0774-363712