Immunotherapy: Open Access

Dreborg et al., Immunotherapy (Los Angel) 2016, 2:1
DOI: 10.4172/2471-9552.1000109

Research Article OMICS International

Evaluation of Skin Reactivity during (Immuno-) Therapy. Validation of Methods for Estimation of Changes in Skin Reactivity and Correlation to Shock Organ Sensitivity

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Received date: Jan 4, 2016; **Accepted date:** Jan 25, 2016; **Published date:** Feb 3, 2016

Abstract

Background: Parallel line bioassay (PLBA) has been acknowledged to be the gold standard for estimation of changes in reactivity, e.g., in RAST and ELISA inhibition tests.

Objective: To study correlations between two simple methods for evaluation of changes in skin prick test (δSPT), using the slope of the allergen dose response (drr_a) in relation to PLBA.

Methods: Skin prick test data from two published immunotherapy trials were used. In a *D. farinae* trial we used duplicate tests with three fixed ten-fold concentrations and in a *P. judaica* trial three tenfold individually chosen allergen concentrations causing wheals of similar size to that of histamine dihydrochloride 10 mg/mL, tenfold lower and tenfold higher concentration. Evaluation of the δSPT by PLBA, and two simple methods were correlated. In the *D. farinae* trial δSPT was compared to the change of conjunctival threshold concentration.

Results: The δSPT as measured by both the simple methods gave similar results to that of PLBA ($p<0.001$). The δSPT was around 30-fold, i.e., about 3% of the pre-treatment reactivity. The δSPT correlated with the δCPT threshold concentration.

Conclusions: Estimation of the δSPT during therapy expressed as change in concentration using simple methods based on the slope of the drr_a correlated well to changes by PLBA and CPT and should therefore be used both in clinical research and in practice.

Keywords: Skin prick test; Allergen; Histamine; Method; Allergy; Immunotherapy; Conjunctival provocation test; Threshold concentration; Wheal area; Wheal diameter; Dose response

Abbreviations A: Wheal area; A_a: Wheal area induced by allergen SPT; A_{h1}: Wheal area induced by allergen of the same size as that induced by histamine dihydrochloride 1 mg/mL; A_{h10}: Wheal area induced by allergen of the same size as that induced by histamine dihydrochloride 10 mg/mL; a: The intercept of the drra with the Y-axis; b: The slope of the allergen dose response relationship (model: A=a+b log conc.); C: Concentration; C_a: Concentration of allergen; δC_a: Change of allergen concentration; C_{h1}: Concentration of allergen eliciting a wheal of the same size as that of histamine dihydrochloride 1 mg/mL; C_{h10}: Concentration of allergen eliciting a wheal of the same size as that of histamine dihydrochloride 10 mg/mL; C.l.: Confidence limits; CPT: Conjunctival provocation test; D: Wheal diameter; D_a: Mean wheal diameter induced by a given allergen concentration; drr_a: Allergen dose response relationship; D_{h1}: Mean wheal diameter induced by histamine HCl 1 mg/mL; D_{h10}: Mean wheal diameter induced by histamine HCl 10 mg/mL; PLBA: Parallel line bioassay; r: The coefficient of variation; SCIT: Subcutaneous immunotherapy; SPT: Skin prick test; δSPT: Change in skin sensitivity as measured by change in SPT threshold concentration; X: X indicates methods used as x variables; Y: Y indicates methods used as y variables

Introduction

Some decades ago, most allergists used endpoint titration by intradermal skin testing as a measure of skin reactivity. During the 1980's European manufacturers started delivering extracts for SPT in one concentration. Since then results have been reported in terms of wheal diameter, or in some scientific reports in terms of wheal area. It has not been possible to compare changes in skin test wheal sizes with that of bronchial, nasal or conjunctival provocation test threshold concentrations. Furthermore, due to the flat allergen wheal dose response [1-4], a 10-fold increase in skin sensitivity roughly corresponds to an increase in wheal diameter from 3 mm to 4.65 mm,

from 4.65 mm to 7.2 mm, or from 7.2 mm to 11.1 mm in diameter [5]. Thus, wheals 3 mm and 11 mm in diameter represent a thousand-fold difference in reactivity. However, data on change in threshold concentrations are, with few exceptions, not used in clinical studies.

The skin response to SPT in an individual depends on the technique applied but also other factors, such as medication, allergen extract total allergenic potency and composition [6,7]. The difference in wheal size between investigators can be minimized by adjusting the allergen wheal size to that of the histamine wheal size [8]. Methods for evaluation of allergen skin reactivity by estimating the allergen concentration inducing wheal reaction of the same size as that of the histamine reference have been developed [9].

In the 1993, EAACI position paper on skin tests and allergen standardization [6], it was proposed that methods for estimation of change in concentration eliciting a wheal of the same size as that of histamine should be used, but without reference to published papers.

The aim of the present study was to evaluate two simple methods [9] for the estimation of skin reactivity to allergens, expressing the results as the change in "threshold concentration" from before to after (immuno-) therapy.

Material and Methods

Patients

Skin test data from two previously published subcutaneous immunotherapy trials (SCIT) were used:

Mite, *D. farinae*. Thirty-two Swedish adults with chronic rhinitis, positive SPT, CPT and *in vitro D. farinae* specific IgE (Phadebas RAST®, Pharmacia) responses were included. Twenty-one [21] patients were allocated active treatment with *D. farinae extract* (Pharmalgen®, Pharmacia) for 12 months. Eleven [11] patients served as open controls [10].

Wall pellitory, *Parietaria judaica*. Twenty-four [11] Spanish adults with seasonal worsening of their respiratory symptoms during the wall pellitory season were studied. They all had positive SPT and CPT responses to a freeze-dried wall pellitory (*P. judaica*) allergen extract (Pharmalgen® In-Hose Reference, IHR, Pharmacia) and positive *in vitro P. judaica*-specific IgE test results (Phadebas RAST®) [12]. Half of the patients were treated with a *P. judaica* pollen extract (Abelló Madrid, Spain) for four months, while the other half was given histamine placebo in a double-blinded manner. The original trials were approved by the local ethical committees.

Test solutions and other materials for SPT and CPT

Freeze-dried, partly purified, standardized allergen preparations of wall pellitory, *Parietaria judaica*, and house dust mite, *Dermatophagoides farinae*, (Pharmalgen®, Pharmacia) were used.

Positive reference in the *D. farinae* trial was histamine dihydrochloride 1 mg/mL (5.43 mmol/L or 0.63 mg/mL histamine base) (Pharmacia) and in the wall pellitory trial histamine dihydrochloride 1 mg/mL and 10 mg/mL (Pharmacia). In the wall pellitory trial Albumin diluent®, and in the *D. farinae* trial Glycerol diluent® (50% glycerol in saline, Pharmacia), was used for reconstitution and as negative SPT control. For CPT Albumin diluent® was used for reconstitution, dilution for CPT and as negative control.

In both trials, the same batch of freeze-dried extract was used before and after immunotherapy. The freeze-dried extracts were reconstituted with Albumin diluent® (0.03% human serum albumin and 0.4% phenol in saline) (Pharmacia).

One hundred thousand Nordic BU [13] (100,000 BU/mL) contains about 100 µg/mL of major allergen/mL, ± a factor 2 [14]. However, for the purpose of this communication, the unit does not matter. The wall pellitory extract was the Pharmacia Diagnostics IHR [12], freeze-dried in one concentration. The freeze-dried *D. farinae* extract used for SPT and CPT was delivered freeze-dried in three concentrations, 100,000, 10,000 and 1,000 BU/mL after reconstitution with Albumin diluent for SPT, and was further diluted for CPT to 1 million to 10 BU/mL in half ^{10}log steps.

A lancets of the Østerballe type [15] was used for SPT. The SPTs were performed and recorded according to the EAACI position paper [6].

Materials for immunotherapy

In the *D. farinae* trial the same preparation as for diagnosis was used for SCIT [10].

The *P. judaica* extract was partly purified, characterized and freeze-dried (Abelló, Madrid, Spain). A histamine placebo preparation was given to the controls in a double-blind fashion.

Depot diluent® (aluminum hydroxide 0.2%, Pharmacia Diagnostics AB) was used in the *D. farinae* trial.

Skin prick test methods

In the *D. farinae* trial duplicate tests with three ten-fold concentrations of allergen were used.

The method described by Østerballe and Weeke [18] was employed. In principle, the criteria set out in the EAACI position paper on skin testing were followed [6], e.g., when indicated, the allergen wheal size was accommodated for the wheal response to the negative control, although this was not needed since we used Østerballe needles with a single, one mm tip, that induces minimal trauma.

Methods for evaluation of changes of skin reactivity

For all methods the data before and after immunotherapy were compared. Immunotherapy was given for one year in the *D. farinae* trial [10] and during four months in the *Parietaria* trial. The letters A-C identify methods and the number attached to the method letter indicates the number of replicates with the same concentration used for calculations. Thus, B2 means two replicates using method B, etc.

Parallel line bioassay (both trials): Parallel line bioassay was performed according to Finney [16,17]. In principle the regression line estimating the allergen dose response relationship (drr_a) was calculated before and after immunotherapy and tested for parallelism.

When the null hypothesis for parallelism could not be rejected, then the relative change in concentration of allergen needed to elicit a wheal of the same size, before and after immunotherapy, was calculated for each patient. The principle is illustrated in (Figure 1a). Changes were expressed as change in allergen concentration, δC_a.

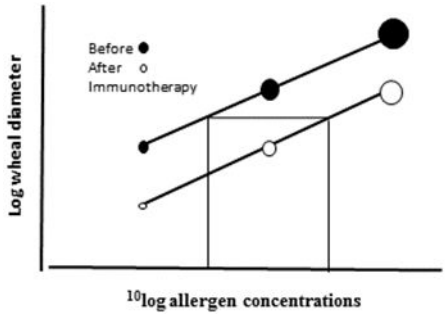

Figure 1a: PLBA method [1,16,17], method A. The vertical lines indicate the concentration of allergen that elicits a wheal response of the same size, the horizontal line, the difference in concentration eliciting the same skin response before and after therapy. Filled circles indicate before therapy and open circles after therapy.

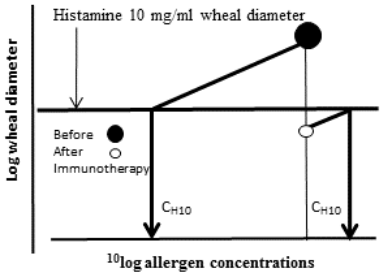

Figure 1b: Method of Bjorkten et al., method B [9,18]. The thin vertical line indicates the concentration of allergen tested. The slope of the drr of allergens 0.196 (diameter) [2,4,18] is used to calculate the concentration of allergen eliciting a wheal response of the same size as that of histamine, indicated by the two vertical arrows, the horizontal line the size of the histamine weal. Filled circles indicate before therapy and open circles after therapy

Modified method according to Björkstén, F et al. (P judaica trial): One and the same concentration of allergen was used for all patients, i.e., in some cases the wheal area was much larger and in some cases was much smaller than that of the histamine wheal [18]. The model log D=a+b logC (4), or $C_{h1}=(D_{h1}/D_a)^{5.07}$ (xC_a) [log A=a+b log C (4), or $C_{h1}=(A_{h1}/A_a)^{2.54}$ (xC_a)] was applied to determine the concentration eliciting a wheal response of the same size as that of histamine dihydrochloride 1 mg/mL. The slope of the dose response relationship of allergens, i.e., 0.196 using wheal diameters (0.394 for areas). The principles are shown in Figure 1b. Changes are expressed as change in allergen concentration, δCa. The method is further described in Appendix 1 of Dreborg and Holgersson [9].

One individually chosen concentration of allergen giving a similar wheal size to that of histamine dihydrochloride 10 mg/mL and histamine dihydrochloride 10 mg/mL (P. judaica trial): One of the three tested ten-fold concentrations of allergen giving a similar wheal size to that of histamine dihydrochloride 10 mg/mL was chosen. Originally quadruplicate tests were made, applied to the subject's back, as described by Dreborg et al. [1,12]. By choosing an allergen concentration eliciting a wheal with a size close to that of histamine the influence of variation of the slope (b) between patients and test occasions was minimized. The principles are shown in (Figure 1c). Changes are expressed as change in allergen concentration, δCa. The method is further described in Appendix 2 of Dreborg and Holgersson [9].

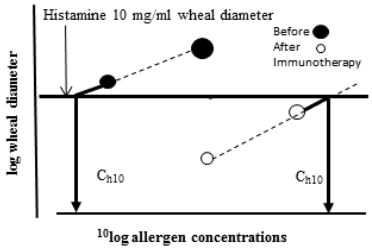

Figure 1c: Method C (4). Three tenfold concentrations were tested. The allergen response similar to that of histamine was selected from for calculations. The slope of the drr of allergens (b using the area 0.4) [1] was set for calculation of the allergen concentration eliciting an allergen wheal response of the same size as that of histamine, indicated by the two vertical arrows, the horizontal line the size of the histamine wheal. Filled circles indicate before therapy and open circles after therapy.

Conjunctival provocation tests

The CPT's were performed in the *D. farinae* trial as previously described [19,20]. In summary, the conjunctivae were inspected to make sure they were free from irritation. Albumin diluent® was then instilled into one eye. If no reaction occurred the lowest concentration of allergen, 10 BU/mL (about 1 ng of major allergen/mL), was instilled and the dose was increased by half ^{10}log steps every 10 minutes, using alternate eyes. A combination of 50% reddening of the sclera and itching was regarded as a positive result (threshold concentration). Changes were expressed as change in threshold concentration, δC_a.

Statistical analysis

Logarithmic transformation of all variables was performed prior to calculation of the relationships described below.

The different methods were compared in terms of correlation coefficients calculated by the model X=a+bY, as estimated by the method of least squares, where X=change in skin sensitivity as measured by method X(A-C) and Y=change in skin sensitivity as measured by method Y(A-C). The variable "Change in skin sensitivity" was measured as the relative change in allergen concentration (methods A-C-).

For calculation according to method B and C, a common slope (b=0.40) was assumed for the drr_a [1-3,9] since the wheal area, A_a, was used in both trials.

Results

P. judaica trial

The results obtained with methods A4, B4 and C4 showed a high correlation (r>0.92; P<0.0001). The slopes of the drr_a we're not different from 1 (P<0.05) in all cases. The simplified methods B2 and C2 also correlated to the gold standard method A4 (r>0.75; P<0.0001) (Table 1).

All correlations between methods, based on duplicate tests, were significantly different from 0 (p<0.05) (Table 1).

SPT method		Pair	n	r	pm	b	P
X	Y						
A 4	B 4		22	0.92	<0.0001	0.84	<0.05
A 4	C 4		22	0.95	<0.0001	0.93	<0.05
B 4	C 4		24	0.93	<0.0001	1.05	<0.05
A 4	B 2	1	22	0.82	<0.001	0.96	<0.01
A 4	B 2	2	22	0.8	<0.001	0.74	<0.01
A 4	C 2	1	22	0.76	<0.001	0.87	<0.01
A 4	C 2	2	22	0.79	<0.001	0.84	<0.05
A 4	A 2	1	21	0.85	<0.001	0.93	<0.05
A 4	A 2	2	22	0.87	<0.001	1.12	<0.01

Table 1: The relationship between changes in skin sensitivity as measured by methods A, B and C, i.e., the parallel line bioassay method and the two simplified methods employed, using data from the Parietaria trial. Pairs: Pairs of tests used for calculations according to methods E and F. Two of the quadruplicate tests (tests 1 and 2) with each allergen concentration in each patient were chosen by random and designated as pair number 1, while the two remaining tests (tests 3 and 4) became pair 2. All pair 1 and pair 2 values, respectively, were used for the calculations according to the respective methods B and C. n denotes the number of eligible patients, r=the coefficient of correlation, pm denotes the significance of correlations between methods, b=the slope and p denotes the significant difference of b from 1. All slopes were significantly different from 0 at the P<0.00001 level.

After four months, the within group decrease in skin sensitivity, as measured by methods A, B, and C (with four or two randomly selected replicates), was about 20% of the pre-treatment level among actively treated patients (P<0.0001). That means a 5-fold reduction in skin sensitivity. On the other hand, the controls demonstrated about three-fold increased skin sensitivity, as measured by method A4 (Figure 2a). There was therefore an approximately 15-fold difference in post-treatment levels and a substantial difference in change between the actively treated and the placebo group.

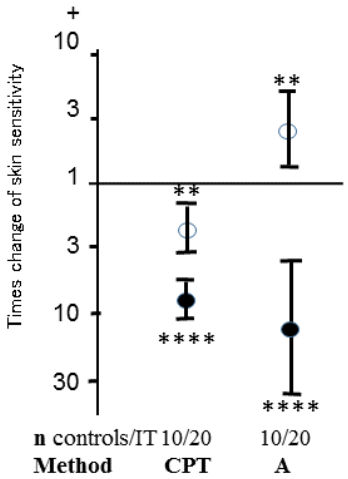

Figure 2a: *D. farinae* trial. Open controls are marked with open circles and the active group by filled circles. The number of eligible controls/actives. CPT, or SPT according to method A. The change in allergen sensitivity is given in percentage (Y-axis) of the histamine wheal size. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. n.s. no significant change. The vertical bars illustrate the c.l.

D. farinae trial

(Figure 2b) illustrates that the degree of change was much less using conjunctival threshold concentrations than the parallel line bioassay, method A. From before to after treatment the sensitivity as measured by CPT decreased in the placebo group (P<0.01) but the improvement was stronger in those actively treated (P<0.0001) and there was a significant difference in change between the groups.

However, when skin tests were used there was significantly increased sensitivity in the control group, most marked when using the gold standard method A.

Histamine reactivity

In the *P. judaica* trial there was a significant reduction in the size of the histamine wheal areas after four months of immunotherapy (P<0.001) (56 mm^2 to 38 mm^2, 95% c.l. 50 mm^2-62 mm^2 and 32 mm^2-44 mm^2, respectively) that may indicate differences in SPT techniques between the two test occasions or may be due to a change in skin reactivity.

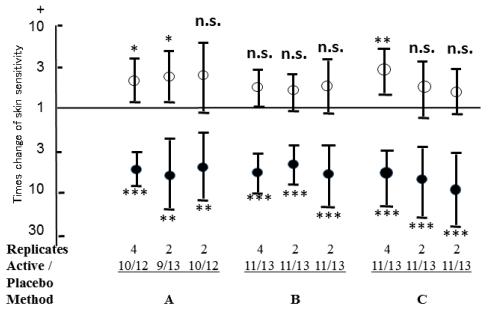

Figure 2b: The *P judaica* trial. Double-blinded controls are marked with open circles and the active group by filled circles. The number of replicates, the number of eligible p controls/actives and the methods A, B and C for evaluation of C_a, are shown beneath the diagram. The change in allergen sensitivity is given in percentage (%) (Y-axis) of the histamine wheal size. The vertical bars illustrate the c.l. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. n.s. no significant change.

Correlations between Changes in Skin Sensitivity and Conjunctival Threshold Concentrations

D. farinae trial

The change in skin sensitivity, as measured by the PLBA, method A, correlated to the change in shock organ sensitivity, as measured by change in conjunctival threshold concentration, using all methods.

The change in skin and conjunctival sensitivity between the two treatment groups using PLBA, method A, and CPT is shown in Figure 2b.

Discussion

This study shows that the two simple methods used for estimating changes in skin reactivity during SCIT correlate well with the gold standard, PLBA. Therefore, the simple methods, using the slope of the allergen dose response relationship (b-drr_a), are appropriate for estimating changes in skin histamine equivalent threshold concentrations during therapy or over time. In this example we used immunotherapy. However, the methods can certainly also be used to determine the change of skin sensitivity after administration of anti-histamines and other anti-allergic drugs or over time.

Moreover, good correlation was found between changes in shock organ sensitivity (CPT) and skin sensitivity. This makes it possible to follow the allergen-specific effect of immunotherapy using repeated skin tests, provided the precision of the SPT technique is good and differences in technique between testing personnel and occasions are minimized and by relating the allergen wheal response to that of the histamine reference, thus correcting for technique and possible changes in skin reactivity [8].

Skin threshold concentrations are comparable to the threshold concentrations of challenge procedures. The results are therefore more meaningful than reporting the size of allergen induced wheals. Methods A-C report continuous data.

In this communication we investigated the possibility of using simplified methods based on the slope b of the allergen drr_a for estimating changes in skin reactivity during (immuno-) therapy. The simple methods using the slope of the allergen drr_a, B4, C4 and B2, C2 correlated well with the parallel line bioassay method.

In the *D. farinae* trial, the reduction of skin reactivity in the active group was around tenfold, whereas the placebo group showed a threefold increase in skin sensitivity, i.e., a difference in change of skin sensitivity during therapy of around 30-fold, using a top dose about 100 µg of major allergen [14]. The reduction of shock organ/skin sensitivity was similar, i.e., 30-fold increased tolerance, to that reported by Dreborg et al. [21] in double blind trial using freeze-died timothy and mite extracts of similar potency.

Originally, the *D. farinae* trial used method A2 (Figures 1a and 2b). Method B2 is similar but uses just one concentration of allergen, i.e., the most common procedure in clinical practice nowadays. We could not detect any difference between methods A4, B2 and C2. Theoretically, however, method C2 that uses a concentration of allergen that elicits a wheal of similar size to that of histamine HCl 10 mg/mL should be better than method B2, which uses one and the same concentration of allergen in all patients, since the influence of possible variation of the slope (b) between patients using method C is minimized. Furthermore, method B2 may not induce a positive response in patients with low skin sensitivity, especially after (immuno-) therapy. Method C2 on the other hand guarantees a result in all patients and is expected to give more reliable individual data (to be further studied).

There was a decrease in histamine wheal size from before to after SCIT in the *P. judaica* trial. This has also been reported in other trials [22]. Stuckey et al. [23] found the size of histamine wheals to be correlated to total IgE and the number of sensitizing allergens. Bordignon and Burastero [24] found a correlation between the number of positive allergen skin prick tests and sensitivity to histamine (mono-sensitized versus poly-sensitized subjects: P=0.0015) [24]. A decreased sensitivity to the allergen used for SCIT may therefore explain the decrease in histamine wheal size. Another explanation may be a change of technique. Whatever the reason, the allergen response should be interpreted in relation to the skin reactivity on the same occasion [8].

It has recently been shown that relating the allergen SPT wheal response to that of histamine in the same patient reduces the main shortcoming of the SPT method when comparing skin test results between testing personnel and clinics [8]. By relating the allergen wheal response to that of the histamine response in the same patient the influence on the result of variation in technique is reduced. Furthermore, a method based on estimation of the response to allergen SPT in terms of concentration has recently been published [9]. In that paper a formula for calculation of the skin sensitivity before and after therapy is given and an Excel file for this purpose is included (Table 2).

SPT method	n	r	p	b	P	a
A	29	0.53	<0.01	0.23	<0.001	0.6

Table 2: The relationship between changes in conjunctival sensitivity (Y-variable), and skin sensitivity, (X-variable) using data from the *D. farinae* trial. n denotes the number of eligible patients, r is the coefficient of correlation, P denotes the significance of difference of the slope from 0, b denotes the slope and a is the intercept with the Y-axis.

The correlations between changes in CPT threshold concentrations, the reference method A and the two investigated simple methods B and C using the common slope b of the drr_a were good. Skin testing can therefore be used as a surrogate for organ provocation (which is more complicated) and at the very least as a surrogate for bronchial allergen provocations, which carry some risk of severe allergic reactions. The estimation of change in C_a in daily routine can be easily made by introducing the simple formula given in Dreborg and Holgersson [9] in the computer program of any allergy clinic.

Based on these results, we propose the use of method B in daily practice to follow the skin sensitivity of patients, e.g., during SCIT, when evaluating the effect of anti-allergenic drugs or long-term follow up, expressing patient's sensitivity as δSPT. The magnitude of changes in skin sensitivity over time is better understood when using threshold concentrations than by using the size of the wheal [5].

At the time of the calculations for this paper in the autumn of 1986 [11] (paper 8), we were neither aware of the simultaneous publication of the Bland-Altman in the Lancet 1986 [25], nor the follow up publications by them in 1995 [26]. The original data have been destroyed by the sponsoring company why this analysis cannot be performed in these patients included in this communication. Verification of our findings using data from other studies including Bland-Altman analyses should be done. Bland Altman analysis means comparing the average of a standard method, in this case plba, with that of new methods, in this case, the two simple, dose response based methods, is the best way assuring similarity between a god standard method and new methods.

In conclusion, changes in SPT reactivity can be expressed in terms of change in allergen concentration eliciting wheals of the size of the histamine reference, minimizing the influence of differences in technique between test occasions and testing personnel and expressing the result in change of threshold concentration. This measure of skin sensitivity is well correlated to shock organ sensitivity. The methods described in [9] and used in this communication at two time points, should be used both in studies and in clinical practice.

Competing Interests

The birch and *D. farinae* trials, and the statistical work performed 25 years ago, were supported by Pharmacia Diagnostics AB, Uppsala, Sweden.

The diagnostic part of the wall pellitory trial was initially part of a Pharmacia Diagnostics biological standardization trial [1] and evaluation was performed with the same extracts. Immunotherapy was performed with a wall pellitory extract from Abelló, Madrid, Spain, who sponsored the SCIT part.

There was no financial support for this publication.

Authors' Contributions

The simple methods for estimation of histamine equivalent concentration of allergen used in this communication and applied in this paper and in Dreborg and Holgersson [7] were developed in collaboration with Margareta Holgersson, PhD, statistician.

Sten Dreborg designed the *D. farinae* trial together with the investigator, and evaluated the results and developed the evaluation methods together with Margareta Holgersson, PhD. Sten Dreborg also wrote the paper and drew the figures. Christian Möller co-designs the study and actively participated in the preparation of the original manuscript. Thorvald Löfkvist was the investigator in the *D. farinae* trial and Antonio Basomba was the investigator in the *Parietaria* trial. All authors contributed to the original manuscript and approved the final version. Sten Dreborg and Christian Möller designed the final version.

Acknowledgements

Our sincere thanks to the skin testing nurses who did the original skin testing, as well as to Anders Hansson, BSc, statistician, who performed the computer work.

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