

MORPHOMETRY IN SKIN-TEST METHODOLOGICAL STUDIES - VALIDATION OF THE POINT-COUNTING TECHNIQUE FOR PRECISE AREA DETERMINATION

A. Antico, MD⁽¹⁾

SUMMARY:

Background: The methods for delimiting and measuring cutaneous responses can significantly influence the result of skin tests. For this reason, when skin tests are used for purposes of clinical research, the use of computerized methods is recommended to reduce the measurement error of traditional methods. In the present study we evaluated the degree of precision and accuracy of the point counting technique, employed in morphometry for measuring plane images.

Methods: The degree of precision and accuracy of the point counting technique were studied using a panel of geometric figures (circles and ellipses) of scalar size and some series of wheals and flares of various sizes, from very small dimensions to greater and irregularly-shaped ones, which can represent the different degrees of intensity of cutaneous responses. The efficiency of the method was compared with the efficiency of the traditional one based on the measurement of diameters.

Results: Under the various experimental conditions the average measurement error by the point counting technique was contained within the limit of 8%, with a variation coefficient of about 7%. Unlike the traditional method based on the length of diameters, the point counting technique proves to be very precise and accurate to estimate both areas of small dimensions and very large and irregularly-shaped ones.

Conclusions: The point counting method proved to be reliable and to possess a satisfactory degree of accuracy and precision in the measurement of cutaneous responses of any type. When computerized programmes are not available, the point counting technique can advantageously be used for purposes of clinical research and for methodological studies on prick test procedures.

Key-words: Skin-prick test - Skin-test areas measurements - Morphometry - Point-counting technique.

INTRODUCTION

The methods for outlining and measuring cutaneous responses can significantly influence the result of skin tests (1-4). To diagnose allergy the criteria of positivity are based on a semi-quantitative evaluation of the size of cutaneous responses compared with a histamine reference weal (5) or on a fixed cut-off limit (6, 7).

Quantitative measurement methods, as precise and accurate as possible, are required in the studies of reproducibility of the method and when the prick test is used for purposes of clinical research or for purposes different from allergy diagnosis, - as for instance the biological titration of extracts, the monitoring of immunotherapy or the efficacy studies of anti-allergic drugs (4, 8, 9). A measurement of areas by computer scanning has been recommended to reduce the errors of traditional methods (10).

The use of a computer and a tracing device such as a digitizer pad or a scanner, however, require rather expensive equipment and/or programmes, which are not always available to all researchers. So the use of the methods of morphometry, and in particular of the point-counting technique, has recently been suggested (11). The method is quick, cheap and scientifically approved of (12), but so far it has not been applied or validated for clinical studies in allergy.

The purpose of the present study was to evaluate the degree of precision and accuracy of the point counting method for the measurement of cutaneous responses also with a view to a possible application for purposes of clinical research and for methodological studies on prick test procedures.

METHODS

The performances of the point counting technique were analysed using a panel of geometric figures of scalar size and some series of wheals and flares of various dimensions, which can represent the different degrees of intensity of cutaneous responses, from very small to larger and irregularly-shaped ones.

(1) Azienda Istituti Ospedalieri 'C.Poma' - Mantova - Servizio Autonomo di Allergologia - Ospedale di Asola, MN (Italia).

Point-counting morphometry

Morphometry defines the methods used to obtain quantitative data from the measurement of plane (bi-dimensional) images (12). Areas of regular geometric figures (such as squares, circles or ellipses) can easily be calculated by an appropriate formula. However, no equations exist for irregularly-shaped areas.

Precise measurement methods require the use of a planimeter, a device which is not easily available today, or of computerized techniques of image analysis.

A simple solution to obtain precise enough estimates is to draw the outlines of the area on a paper sheet with a square grid. The total area of the figure will equal the sum of the squares that predominantly lie in the outline. Even though some squares are not completely included in the figure, its outlines will occupy a part of the adjacent squares, so the errors tend to average out.

To make counts easier we can indicate the area of each square with a dot placed exactly at its centre and then eliminate the grid. This obviously makes the work much easier, as no qualitative decision has to be made about whether a square is predominantly in or outside the outline. In this way, to calculate the surface, it will suffice to count the number of points inside the figure under examination and multiply them by the square area. This is the origin of the expression 'point counting' technique in morphometry. In fact a grid of points of the needed dimensions printed on a transparency is put over the figure under examination.

The precision of the method depends on the size of the squares: the smaller the size, the more precise the estimation. In the present study we used a 3mm grid (areas of 9 mm squared per point), an average size compared with the size of the wheals and flares that can be observed in the routine practice of skin tests.

Reference geometric areas

Two series of geometric figures of scalar size, one of circles and the other of ellipses, were obtained with a stencil. Fifteen circles, whose diameters ranged from 4 to 34 mm, and 13 ellipses with longer diameters ranging between 5 and 40 mm were drawn. For each of them we calculated the area by the corresponding mathematical formula. The value obtained was considered as "true value" to validate the method statistically.

Skin prick test areas

The prick tests were performed with a standardized technique, using a disposable puncturing device in

acrylic copolymer, with a 1-mm pyramidal tip (Laboratorio Farmaceutico Lofarma, Milan). The tests were carried out, after the patients' informed consent, in three healthy volunteers with six different concentrations of histamine in a glycerol-saline solution (0.1, 1, 2, 3, 5, 10%) to obtain cutaneous responses of scalar size. We performed 18 prick tests, 6 per subject, into forearms. Ten minutes after the prick test the weal and flare outlines were marked with a permanent ink felt pen, transferred onto transparency sheets, and then photocopied onto paper.

In this way we obtained three series of prick tests of scalar size (longest diameter ranging from 3.5 to 12 mm), which well represented different degrees of intensity of the cutaneous responses. Finally we used a further series of ten particularly wide cutaneous responses with very irregularly-shaped wheals. They were obtained from seven different patients sensitized to the common allergens which had caused exaggerated cutaneous responses during the normal procedures of allergy diagnosis by skin prick test.

Cutting and weighing method

This method was used to establish the "true value" of the areas of the cutaneous responses that we obtained with histamine and the common allergens, by the procedures previously described in literature (4).

The outline of the skin responses, recorded as described before, were enlarged four times by photocopying and cut with a scissor. The areas of the wheals and flares were then weighed on a laboratory precision balance (precision degree of $\pm 0.1 \mu\text{g}$).

Three reference areas of 100 mm were similarly enlarged, cut and weighed. The average weight was used to calculate the areas of the skin tests in square millimetres, assuming a uniform thickness of the photocopy paper.

To estimate the inaccuracy of the method we repeated, three times, the procedures of "cutting and weighing" of the same series of skin tests, including some very small and very large, irregular cutaneous responses.

Size determination by diameters

The size of the skin responses was recorded as the mean of the product of the longest and the midpoint orthogonal diameters of both wheal and flare, according to the EAACI's recommendations (6, 7). By analogy with the cutting and weighing method, in order to minimize possible inaccuracies, we measured the

diameters with a magnifying glass (4x) equipped with a millimetric scale graded at 0.5 mm.

When it was necessary for the statistical analysis of the data, we calculated the areas of the wheals and flares by the formula $\pi \cdot Dm/2$ (where Dm is the arithmetical mean of the sum of the longest diameter and of the one perpendicular to its midpoint), as in previous studies on the topic (4).

Statistics

The accuracy of the method was evaluated by comparing the measurements of the geometric areas and of the prick tests, estimated by the point-counting technique, with their "true value", obtained respectively by formula or by the cutting and weighing method. Measuring agreement was also assessed by plotting the data according to Bland and Altman's method (13, 14).

The degree of precision of the method was calculated by repeatedly measuring the areas of the cutaneous responses of three prick tests, two of which were very wide and irregular. Seven different operators, who had previously been instructed in the theoretical principles of the method and who had been given a short practical demonstration of the use of the point-counting technique, performed the measurement operation at different times.

In order to make a comparison between the overall efficiency of the point-counting technique and the efficiency of the traditional method based on the measurement of diameters under the different measurement conditions, we calculated the correlation straight line

of the results obtained by each of the two methods and the ones of the reference system (mathematical formula or cutting and weighing method). As the use of the correlation coefficient can be misleading (13, 14), the agreement between the two methods was evaluated again by Bland and Altman's method, after converting the linear measurements of the diameters into areas, as previously described.

RESULTS

With a point grid of 3 mm (9 mm per point), the point counting technique proved to possess a satisfactory degree of accuracy under the different experimental conditions provided for by the study protocol (table 1). The measurement of regular geometric areas demonstrated a good correspondence of the values with an average percentage error of more or less about 5%. Also the estimate of the areas of the cutaneous responses, related to both the wheals and flares, was satisfactory. The average percentage error was similar and was contained within the limits of about $\pm 8\%$, in spite of the presence of very small areas, inferior to 30 mm, which are prejudicially subject to a higher measurement error (4).

Similar results were observed as far as the measurement of the areas of particularly wide and irregular wheals and flares was concerned (about $\pm 7\%$). A very good agreement between point counting and measurements resulting from formula or the cutting and weighing method, both regarded as the gold standard, was also shown by Bland and Altman's test (figure 1). Repeated counts of the same areas, perfor-

Reference areas		Point counting		
Type	N	Size (mm_) (range)	Maximum Error (range)	Average Error (mean $\pm$ sd)
Circles	14	12,56 — 855,30	8,49 — 6,46	1,09 $\pm$ 4,13
Ellipses	13	8,25 — 816,81	9,09 — -8,26	0,43 $\pm$ 4,83
Wheals (*)	18	19,4 — 56,6	8,11 — -8,30	-2,77 $\pm$ 5,32
Flares (*)	18	220,9 — 816,3	7,25 — -6,64	1,98 $\pm$ 4,37
Wheals (°)	10	69,0 — 127,9	4,35 — -7,12	2,43 $\pm$ 3,58
Flares (°)	10	928,4 — 1482,1	10,10 — -6,55	2,95 $\pm$ 4,94
All	83	8,25 — 1482,1	10,10 — -8,30	0,28 $\pm$ 4,88

Table 1: Cumulative results of the measurement of the reference areas by the point counting technique.

Size: variation interval of the size of the reference areas considered (true value).

(*) Series of cutaneous responses of scalar size obtained in healthy volunteers with growing concentrations of histamine.

(°) Series of particularly wide cutaneous responses with irregularly-shaped wheals.

med by different operators at different times, show that the method is very precise, with a variability coefficient (CV) inferior to 8% (table 2).

To calculate the "true value" of the cutaneous responses we used the cutting and weighing technique. It is a method validated to this purpose in literature, but it is liable to slight inaccuracies resulting from the cutting operations. The smaller and the more irregular the surfaces under examination, the greater the possibility of error. To minimize it, it is useful to enlarge the areas four times before the cutting operation. With this obvious precaution the method shows a very good degree of precision, with a CV inferior to 4%.

The study of the linear regression shows the linearity of the point counting technique compared with the reference system and the good overall performance of the method (table 3).

Also the usual estimation method based on the product of diameters demonstrates a good correlation with the reference system, with coefficients similar to the ones obtained by the point counting technique (table 3). However, when this method is used for the estimation of very irregular areas, there is a significant shift from linearity ($r = 0,629$), as already documented in literature (8).

Bland and Altman's test shows that there is a systematic overestimation error of the size of the areas, with significant differences (over 20%), unacceptable in the studies where a very high degree of precision is necessary (figure 2).

In the present study, however, we observed no significant differences between the two methods in the estimation of very small areas. We think that this result was due to the preliminary enlargement of the areas to be measured, as previously described.

DISCUSSION

The results of our study demonstrate that the point counting technique is reliable and can be profitably used to measure the areas of the responses obtained by skin tests.

Using a 3-mm grid we obtained a very satisfactory degree of accuracy and precision in the measurement of both wheals and flares, under all experimental conditions. The precision of the point counting method obviously depends on the size of the squares used: the smaller the square, the more precise the estimates. The same principle is used in computers, where each digital image is composed of a high number of pixels,

	Area (mm)	Counts (7x each) (mean $\pm$ sd)	CV (%)
1t wheal	43,1	36 $\pm$ 0,0	0
1t flare	476	478,28 $\pm$ 19,04	3,98
2d wheal	96,9	95,14 $\pm$ 4,81	5,05
2d flare	1195,9	1276,71 $\pm$ 58,19	4,56
3d wheal	123,2	120,86 $\pm$ 8,78	7,26
3d flare	1454	1596,86 $\pm$ 58,52	3,66

Table 2: Precision degree of the point counting method. Results of seven repeated counts of three cutaneous responses (wheal and flare, including two very large and irregular ones).

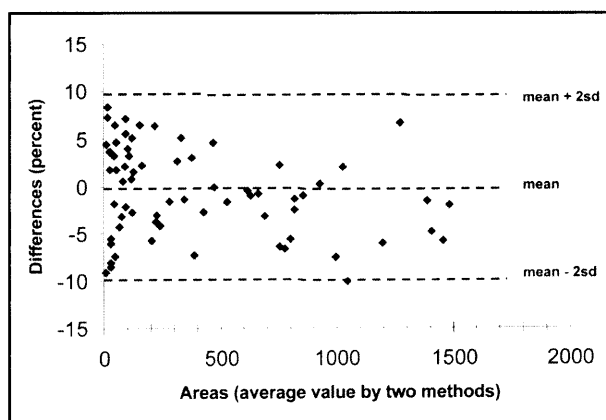


Figure 1: Evaluation of agreement between point counting and measuring reference methods according to Bland and Altman's test.

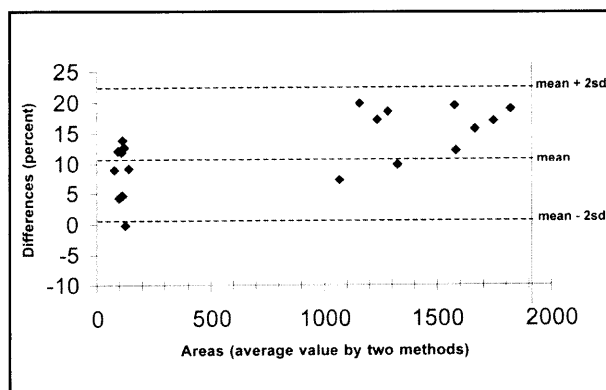


Figure 2: Evaluation of agreement between size of irregular flares and wheals calculated by diameters and true value of areas according to Bland and Altman's test.

Figures	Linear regression	r - value	Straight line formula (y = a + bx)
Circles	Dxd/2 Vs True Area	0,999	$y = -1,32 + 0,64 x$
	Point C Vs True Area	0,999	$y = 1,41 + 0,99 x$
Ellipses	Dxd/2 Vs True Area	0,999	$y = 0,03 + 0,64 x$
	Point C Vs True Area	0,999	$y = -0,86 + 1,00 x$
Wheals (*)	Dxd/2 Vs True Area	0,962	$y = -4,12 + 0,71 x$
	Point C Vs True Area	0,986	$y = -0,13 + 0,98 x$
Flares (*)	Dxd/2 Vs True Area	0,986	$y = -23,54 + 0,78 x$
	Point C Vs True Area	0,998	$y = -23,89 + 1,08 x$
Wheals (°)	Dxd/2 Vs True Area	0,629	$y = 25,63 + 0,41 x$
	Point C Vs True Area	0,984	$y = 7,54 + 0,90 x$
Flares (°)	Dxd/2 Vs True Area	0,905	$y = -90,56 + 0,92 x$
	Point C Vs True Area	0,965	$y = 13,06 + 102 x$

Table 3: Correlation between measurements obtained by the point counting method (Point C) and on the basis of the length of the diameters (Dxd/2) and the ones obtained by the reference methods (True Area: true value of areas by formula or cutting and weighing method).

(*) Cutaneous responses of scalar size obtained in healthy volunteers with increasing concentrations of histamine.

(°) Series of particularly wide cutaneous responses with irregularly-shaped wheals.

which are tiny squares that can be seen very well when the image is highly enlarged. A precise dimension of the image can be obtained by counting the single pixels that compose it, obviously at computer speed.

The use of very small grids would require time and an unbearable workload with manual techniques; therefore it is necessary to come to a reasonable compromise between the size of the grid and the time necessary to measure the areas. In morphometry it was, however, demonstrated that the number of points required to reach a high degree of accuracy is surprisingly low, so a comparatively coarse grid might be employed.

In confirmation of this methodological assumption, in our study we obtained a high degree of accuracy using a 3-mm grid, relatively coarse to measure areas of very small wheals with the longest diameter inferior to 5-6 mm. This could have caused a significant loss of the method performance, which did not occur in our series, showing once again that potential estimation errors, due to relatively wide grids, tend in practice to balance out.

It is possible to estimate the precision of the point counting method by calculus. For ease of use nomograms have been published which allow reading off the number of necessary points at a given error level (15, 16).

We empirically calculated that, to contain the measurement error within and not beyond the limit of 12%, it would be necessary to use grids with a diagonal distance between two points inferior to half the longest diameter of the area to be calculated (the diagonal can be obtained by multiplying the horizontal distance between two points by 1,41).

A preliminary choice of grids suitable to the size of the areas to be measured is to be recommended for scientific studies, where the relatively longer time needed for the calculation can be justified.

We think that a 3-mm grid like the one we used is suitable to measure, with precision, the areas elicited under ordinary conditions by skin tests. In case of very small areas, inferior to 8 mm of diameter, where the estimation error could be over 12%, it is useful and advisable to enlarge the areas three or four times by photocopying. This procedure is used in various circumstances in literature and has proved, also in the present study, suitable to reduce the estimation error to negligible levels.

The estimate of the size of the wheals and flares, based on the measurement of the longest diameter and of the orthogonal one at its mid-point, as recommended by

the EEACI, was, as foreseen, satisfactory and reliable to measure average size areas. The performance of the method was good also when the areas were very small, thanks to the use of a magnifying glass equipped with a millimetric scale. We think that with these simple precautions and on condition that the areas to be measured are round and regular enough, the measurement of cutaneous responses on the basis of orthogonal diameters is in any case a useful, reliable method also for purposes of clinical research.

Instead, with irregular areas, above all wheals with pseudopods, we observed a great reduction of the method performances, with a significant shift from linearity. We must therefore agree that under the above experimental conditions it would be necessary to resort to other methods with better characteristics.

The point counting method proved to be reliable and possess a satisfactory degree of accuracy and precision in the measurement of cutaneous responses of any type. When computerized programs are not available, point counting techniques can profitably be used for purposes of clinical research and for methodological studies on prick test procedures.

Acknowledgements: We thank our friend Maria Luisa Zanolla MD, StatD, for her help with the statistical processing of the data.

References

1. Aas K. - Some variables in skin prick testing. *Allergy* 1980;35:250-252.
2. Voorhorst R. - Perfection of skin testing technique. A review. *Allergy* 1980;35:247-250.
3. Turkeltaub P.-C. - In vivo methods for standardization. *Clin Rev Allergy* 1986;4:371-387.
4. Poulsen L.-K., Liisberg C., Bindislev-Jensen C., Malling H.-G. - Precise area determination of skin prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993;23:61-68.
5. Aas K., Belin L. - Standardisation of diagnostic work in allergy. *Int Arch Allergy Appl Immunol* 1973;45:57-60.
6. Dreborg S. - Methods of skin testing. Position paper. *Allergy* 1989;44(Suppl 10):22-30.
7. Malling H.-J. - Methods of skin testing. Position paper. *Allergy* 1993;48(Suppl 14):55-56.
8. Ownby D.-R. - Computerized measurements of allergen-induced skin reactions. *J Allergy Clin Immunol* 1982;69:536-538.
9. Pijnemborg H., Nilsson L., Dreborg S. - Estimation of skin prick test reactions with a scanning program. *Allergy* 1996;51:782-788.
10. Dreborg S. - Histamine reactivity of the skin. *Allergy* 2001;56:359-364.
11. Bahmer F.-A. Letter to editor. *Allergy* 2001;56:1228.
12. Bahmer F.-A. - *Morphometry in clinical dermatology*. In: Wilhelm K.-P., Elsner P., Berradesca E., Maibach H.-I. Eds. Bioengineering of the skin: skin surface imaging and analysis. Boca Raton, FL: CRC Press, 1997:229-240.
13. Bland J.-M., Altman D.-G. - Statistical methods for assessing agreement between two methods of clinical measurements. *Lancet* 1986;1:307-310.
14. Bland J.-M., Altman D.-G. - Comparing methods of measurement: why plotting differences against standard method is misleading. *Lancet* 1995;346:1085-1087.
15. Gundersen HJG., Jensen E.-B. - The efficiency of systematic sampling in stereology and its prediction. *J Microscop* 1987;125:229-263.
16. Gundersen H.-J., Jensen EBV., Kieu K., Nielsen J. - The efficiency of systematic sampling in stereology - reconsidered. *J Microscop* 1998;193:199-211.

NEWS BRIEF

MOUSE ALLERGENS REDUCED IN HOMES OF ALLERGIC CHILDREN WITH ASTHMA

Arlington Heights, IL (April 20, 2004) – Mouse allergen levels were significantly reduced in inner-city Boston homes of allergic children with asthma using an integrated pest management intervention.

Wanda Phipatanakul, M.D., at Children's Hospital and Harvard Medical School in Boston, and colleagues, studied 18 homes of children with positive mouse allergen skin test results and at least mild persistent asthma in a report published this month in *Annals of Allergy, Asthma & Immunology*, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

Investigators randomly selected 12 intervention homes and 6 control homes from the 18 homes studied. All of the homes showed evidence of mouse infestation. During the 5-month intervention the homes received environmental pest-management controls consisting of filling holes with copper mesh, vacuuming, cleaning, and using low-toxicity pesticides and traps.

Results showed the intervention homes had a significant reduction in mouse allergens in the bedroom and kitchen, but less of a reduction in the living room compared to the control homes:

- 77.3 percent reduction in the bedroom, compared with an increase of 358 percent in control homes;
- 78.8 percent reduction in the kitchen, compared with an increase of 319 percent in control homes;
- 67.7 percent reduction in the living room, compared with a reduction of 32 percent in control homes.

Although an extremely high prevalence of mouse allergen has been demonstrated in inner-city homes of children with asthma, Dr. Phipatanakul and her team found no published studies to date that evaluate the potential role of environmental intervention in reducing mouse allergen.