

Short communication

A computer program based on parallel line assay for analysis of skin tests

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A computer program for the analysis of differences or changes in skin sensitivity has been developed. It is based on parallel line assay, and its main features are its ability to conduct a validation process which ensures that the data from skin tests conform to the conditions imposed by the analysis which is carried out (regression, parallelism, etc.), the estimation of the difference or change in skin sensitivity, and the determination of the 95% and 99% confidence intervals of this estimation. This program is capable of managing data from independent groups, as well as paired data, and it may be applied to the comparison of allergen extracts, with the aim of determining their biologic activity, as well as to the analysis of changes in skin sensitivity appearing as a consequence of treatment such as immunotherapy.

**S. Martín¹, P. Cuesta², P. Rico¹,
C. Cortés¹**

¹Medical Department, Alergia e Inmunología Abelló SA, Madrid; ²Centro de Proceso de Datos, Universidad Complutense de Madrid, Madrid, Spain

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S. Martín
Medical Department
Alergia e Inmunología Abelló SA
Miguel Flea, 19
28037 Madrid
Spain

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Quantitative skin tests in which cutaneous response is objectively measured in response to several concentrations of an allergen extract have been used in a wide variety of applications; e.g., to evaluate patient sensitivity before immunotherapy (IT) or provocation tests; to monitor IT; to evaluate the efficacy of pharmacologic treatments; to evaluate the standardization of allergen extracts; and, to a lesser extent, to aid in epidemiologic studies.

The parallel line assay (PLA) (1) has been recommended for the estimation of relative activities of allergen extracts (2) and for the analysis of changes in skin sensitivity which may appear during IT (3). However, PLA is rarely used, probably due to the large amount of calculation and statistical background involved. We have developed an application based on PLA methodology which is specifically designed for use in the analysis of differences in cutaneous responses, either in the comparison of groups of allergic patients, or arising as a consequence of treatment. In this context, there are many publications in which a decrease in skin sensitivity has been associated with IT (4).

Statistical principles

PLA was developed to quantify the activity of a substance in a preparation by comparing the

biologic activity of this test preparation (T) with one of a reference (standard [S]). This is done by analyzing the responses of each preparation at different dilutions; that is, their dose-response curves. They must be linear when plotted against the logarithm of the doses and parallel between them to make analysis by PLA possible. The horizontal distance between the parallel lines determines the difference in dose in the two preparations needed to obtain the same response, meaning the relative activity of the T in comparison with the S preparation (Fig. 1).

Although this analysis can be effected graphically, there are some important drawbacks which make use of the statistical approach preferable:

- 1) it is not possible to validate the conditions imposed by the PLA analysis (i.e., when the dose-response curves corresponding to the S and T preparations are in fact straight, parallel lines)
- 2) the estimated values of difference in activity may be inexact
- 3) it is not possible to establish the confidence limits of this estimation.

Statistical analysis of assay data overcomes these problems by providing procedures for the calculation of best-fitted lines and differences,

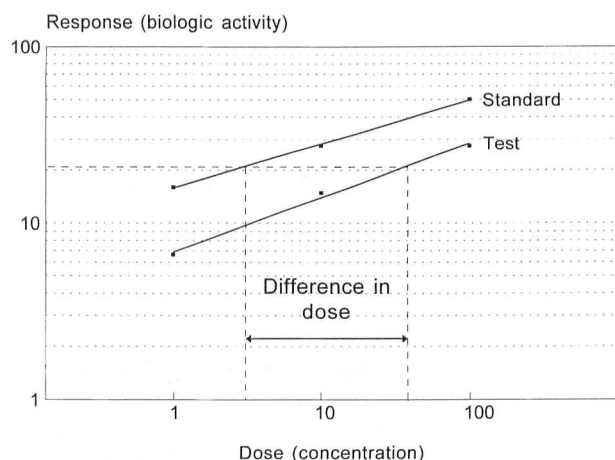


Fig. 1. Principle of PLA: difference in dose needed for same response in test substance as in standard one is measure of relative activity of this substance.

together with the analysis of factors contributing to the validation and precision of the results obtained.

PLA usually commences with an adequate transformation of the data. The aim of this transformation is dual: first, to obtain straight lines, and, second, to normalize the distribution of data and homogenize the variances along the response interval.

The validation of the assay ensures that only data which fulfill the needed requirements are analyzed. The validation is carried out by standard statistical procedures (normality, variance homogeneity) and by specific procedures based on the analysis of variance (ANOVA). This method provides an adequate mode of isolating the different components which influence the assay, and determining whether their effect is significant. The major components of variation are regression, parallelism, linearity, and nature of preparations. Associated with these is a *P* value which indicates their statistical significance.

Preparations. This component measures whether the mean responses of the S and T preparations are different.

Regression. This component determines whether subject responses change with varying doses.

Parallelism. The measurement of the horizontal distance between the S and T dose-response curves is possible only when this distance is kept constant all along the response interval. In geometric terms, this means that the lines must be parallel. Parallelism is undoubtedly a required condition for PLA analysis, but, under some circumstances, a good choice of doses and/or transformation of data may

correct an apparently nonparallel assay and make its analysis by PLA possible.

Linearity. This component measures how well the data from the dose-response curves fit to a straight line.

Once it is known that there is no statistical evidence against the hypothesis on which PLA is based, establishment of the difference in activity between preparations is a simple arithmetic calculation, derived from the equations of the S and T lines.

The calculated difference in activity of preparations is of little value if it is not accompanied by a number indicating the precision of this estimation. In fact, the confidence interval for a given probability is the parameter which shows whether the calculated difference is significant, or can be ascribed to chance.

Application of PLA to skin tests

Cutaneous reactivity to allergens in allergic patients is a function of the concentration of the allergen extract used. This function is – at least in part of the response interval – of the form:

$$\log(w) = a + b \times \log(c)$$

w being the wheal area produced by a concentration *c* of the allergen extract, *a* the ordinate of origin (when the extract concentration equals 1 and thus its log is 0), and *b* the slope.

The logarithmic transformation of skin response normalizes the distribution of wheal areas and homogenizes their variances along the response interval. In this sense, skin tests are candidates for analysis by PLA. Validation procedures must be carried out in every assay in order to check that the theoretic assumptions are borne out. These include regression, parallelism, linearity, and preparations.

Description of the application

AIASA CRS PLA is an application which comprises several computer programs implementing varying statistical procedures – including PLA – for the analysis either of differences in skin sensitivity of several groups of patients or of changes in cutaneous sensitivity appearing as a consequence of a treatment such as IT.

The application is organized in a menu structure and has four options for the analysis of skin test data, together with a fifth for file management. Two of these options are based on PLA methodology, but are adapted to different assay configurations; another is designed to combine the results obtained

by the application of PLA to individual subjects; and the last deals with those assays which cannot be analyzed by PLA due to lack of adjustment to the validation criteria. The underlying principle of AIASA CRS PLA is to allow analysis of any properly designed skin test assay.

Discussion

Several methods have been used for the estimation of changes in skin sensitivity, including the comparison of wheal size and end-point titration as well as methods based on the median slope of the dose-response relationship in skin tests. Among these methods, PLA is the one recommended as the reference method (3, 5).

The principles of PLA methodology applied to cutaneous tests are based on the existence of a linear dose-response relationship. Dreborg et al. (6), studying the form of the equation that best represents this relationship, found that it was achieved with a double logarithmic transformation, obtaining regression lines with correlation coefficients higher than 0.97. They also found that linearity extended at least from 7 to 75 mm² of wheal area with timothy grass and dog dander extracts. Harris et al. (7) have also studied the dose-response curve of *Dermatophagoides pteronyssinus* extracts and histamine and have obtained similar results.

The slope of the regression line has been the subject of several studies. It is generally agreed that, when dealing with wheal areas, 0.4 is an average value for the slope, which is reduced to 0.2 when measuring wheal diameters. In their paper on standardization of allergen extracts, Turkeltaub et al. (8) reported that, with intradermal tests, the measurement of the erythema, instead of the wheal, leads to a steeper dose-response curve. Nevertheless, they observed that the equation which best fitted their data was of the form $y = a + b \times \log(x)$, y being the sum of erythema diameters. In this paper, and in later ones (9, 10), an approximation to PLA was used for the comparison of extract potencies. In Europe, Malling (11) has proposed several guidelines for the estimation of the biologic activity of allergen extracts by PLA. In addition to differences in patient selection criteria from the Turkeltaub et al. study, the results are measured here as wheal areas, and the fitted lines are of the form $\log(y) = a + b \times \log(x)$. In several IT studies, the PLA approach has been applied to estimate differences in the biologic potency of modified extracts, either alum-precipitated (12), modified with monomethoxypolyethylene glycol (13, 14), or formalinized allergoids (15).

In a bibliographic search of IT, taking into consideration 14 double-blind, placebo-controlled

IT studies in which skin sensitivity was monitored, we found that, in all but three, the cutaneous response showed a significant decrease (4). Several IT studies have applied the PLA methodology to confirm a reduction in skin sensitivity (14, 16–19). The main differences between the method of calculation applied in these studies and that of our proposal were the validation criteria, the expression of the results, and the determination of the precision of the estimation made. As regards validation of the assays, different criteria may be established. The use of analysis of variance allows the detection of the possible sources of nonvalidity: regression, parallelism, linearity, or heterogeneity of preparations. As there is a P value associated with every parameter, we presuppose the use of a 0.05 probability level for regression and non-parallelism, although the latter could be 0.01 when individual assays were being analyzed (8, 11).

The change in cutaneous sensitivity has been expressed in various ways. We have chosen the cutaneous tolerance index (CTI) as the factor by which it is necessary to multiply the extract concentration after IT in order to obtain the same response, measured as wheal area, as at the beginning. This index is inversely proportional to the change in skin sensitivity, increasing as sensitivity decreases. The determination of confidence intervals is of great importance because these give the significance of a change in skin sensitivity. In AIASA CRS PLA, this is effected by taking into account the whole of the information available from every subject and, when combining results, by weighting the individual estimations into a weighted average. All these factors together make it possible to obtain reliable, exact, and precise analysis of the cutaneous data.

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