

The reproducibility of bolus allergen challenges; power calculations for clinical trials

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The inhalation of allergen in atopic asthmatics causes an early asthmatic response (EAR) due to mast cell and basophil activation. A late asthmatic response (LAR) occurs in a proportion of subjects due to TH2 inflammation involving lymphocyte and eosinophil activation [1–3]. This challenge model has been used to study novel drugs for the treatment of asthma [3–6].

A common method used by ourselves and other investigators is the bolus allergen challenge method; a calibrated dosimeter delivers a controlled allergen quantity per inhalation [4–6]. An incremental allergen challenge test is performed at screening [4–6], during which ascending quantities of allergen are administered until an EAR and a subsequent LAR are observed. The total quantity of inhaled allergen required to cause a LAR is calculated, and administered as a single bolus dose during the treatment phases [4–6]. There is good agreement between incremental and bolus allergen challenge responses [7].

Many allergen challenge clinical trials use a crossover design to assess the effect of active treatment compared to placebo; this has the theoretical advantage of reduced variability compared to parallel group studies, as each subject acts as their own control. Accurate sample size calculations are necessary to adequately power these trials. Using the intra-subject standard deviation (SD) in the observed EAR and LAR is an appropriate way to estimate the sample sizes required for crossover studies, as the within-subject variation will influence the sample size. In contrast, parallel group studies require the between-subject SD for power calculations, as the variability between subjects will alter the sample size.

There are no publications that have defined the intra-subject variation of the bolus allergen challenge method; we have therefore studied the reproducibility of the bolus dose allergen method to calculate this parameter.

Fourteen asthma patients not using inhaled corticosteroids were recruited (11 male and 3 female, mean age 38, mean FEV1 % predicted 89.5 %). Written informed consent was obtained and the local ethics committee approved the study. Subjects demonstrated an EAR and LAR in an inhaled incremental challenge to either cat, grass or house dust mite (Aqua-gen, ALK, Horsholm, Denmark). Two bolus allergen challenges were performed 3 to 6 weeks apart (Challenges 1 and 2), using the same concentration of the same allergen. Allergen challenges were performed using the Mefar dosimeter (Mefar, Brescia, Italy) as previously described [5].

EAR and LAR were expressed as area under the curve of percentage change in FEV1 during first two hours (AUC)_{0–2} and from 4 to 10 h (AUC)_{4–10} respectively. The maximum fall in FEV1 during the EAR and LAR (EAR max and LAR max respectively) were calculated. The reproducibility of the EAR and LAR were assessed using the intra-class correlation coefficient (r_i) using SPSS version 15.0 (r_i was calculated using two-way mixed ANOVA model). The intra-subject SD was calculated using both the difference between the means and ANOVA, and used for power calculations using n-Query version 7 software. The software uses the formula $n = 1 + C(S/D)^2$; n =sample size, S =intra-subject SD, D =smallest difference to be detected, C =constant (for 90 % power $C=10.5$) [8].

Table 1 shows no significant differences between challenges for the EAR and LAR parameters measured ($p>0.05$ by paired t-tests for comparisons between challenges). The r_i values were mostly > 0.8 , indicating very good agreement between challenges. The intra-subject SD was lower using ANOVA compared to the difference between the means. Power calculations using the larger estimate (difference

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Table 1 Reproducibility of bolus allergen challenge

	First Allergen Challenge Mean (SD)	Second Allergen Challenge Mean (SD)	Mean Difference (95 % CI)	Within Subject SD (calculated from difference of means)	Within Subject SD (calculated from ANOVA)	Intra-Class Correlation Coefficient (95 % CI)
EAR (AUC) _{0–2} ΔFEV1%. hr	28.4 (17.0)	31.4 (20.1)	3.0 (−3.1, 9.2)	10.6	7.6	0.84 (0.57–0.94)
Max EAR (ΔFEV1%) _{0–2}	31.0 (13.2)	33.7 (13.8)	2.7 (−1.5, 6.8)	7.2	5.3	0.85 (0.60–0.95)
LAR(AUC) _{4–10} ΔFEV1%.hr	96.7 (77.7)	82.7 (76.9)	14.0 (−10.3, 38.3)	42.1	30.3	0.85 (0.60–0.95)
Max LAR (ΔFEV1%) _{4–10}	27.6 (16.9)	24.8 (13.3)	2.8 (−8.5, 2.9)	9.8	7.0	0.79 (0.48–0.93)

Table Reproducibility of Bolus Allergen Challenge: All shown data was normally distributed and presented as mean (standard deviation). Also presented are within-subject standard deviations for each studied variable. Agreement between measures of EAR and LAR (Maximum Change in FEV1 and area under curve) for the first and second allergen challenge is presented as (r_i)

between means) for the LAR (AUC)_{4–10} showed that sample sizes of $n=12$, 17 and 28 are required to demonstrate 50 %, 40 % and 30 % reductions respectively, with 90 % power in a cross-over study design.

A comparison of incremental and bolus allergen challenges previously reported r_i values from 0.55 to 0.78 [7]. This is lower than our current study, and suggests some loss of reproducibility due to differences between the incremental and bolus methodologies. Allergen challenges performed using the constant breathing method have lower reproducibility than our current study, e.g. r_i 0.61 for the LAR AUC, compared to 0.85 in the current study [9, 10]. Nevertheless, power calculations for the constant breathing method are similar to those presented here.

In summary, we show that the bolus allergen challenge method has very good reproducibility, and present the within-subject SD that can be used to power future clinical trials.

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