

Methodological cutoff of basophil activation test and basophil activation test diagnostic value



To the Editor:

Salas et al¹ published an article in this journal on the value of the basophil activation test (BAT) in the evaluation of amoxicillin-clavulanic acid allergic reactions. Their conclusion is that the sensitivity of BAT is 62% and that BAT is a promising complementary technique.

Before discussing sensitivity, the limit between positive and negative test results, that is, the cutoff of the method, must be established.

A cutoff limit should differentiate between those with positive reactions according to the method and those in whom the test/stimulation is not different from the background. Normally, the cutoff of *in vitro* tests is defined by the mean of blank tests + 3.3 SD.² *In vivo* tests are limited by the highest concentration of allergen available that is not toxic/locally irritating in normal subjects. BAT with aeroallergens can be performed using a wide range of concentrations of allergen and the results are given as the concentration inducing 50% of maximum activation with anti-IgE. The principle is the same as that used for histamine release tests and for *in vivo* challenge tests defined by their threshold concentrations. This is not possible for drug-stimulated BAT. Much higher concentrations of drugs are needed for basophil activation than are used with aeroallergens. Slightly higher concentrations are cytotoxic, leaving a narrow window, excluding the possibility to use for titration and calculation of threshold concentrations.

The sensitivity of BAT depends on the cutoff of the test. Salas et al¹ use a stimulation index (SI), that is, the ratio of percent basophils activated by allergen (>5% activation)/percent basophils activated spontaneously. They regard a ratio of 1.5 as positive, but such a cutoff is not well documented. As late as in 2016 European researchers agreed on a more realistic cutoff limit of SI more than 2.³ Using the accepted, but not well-documented, cutoff (SI > 2) instead of an SI of 1.5 would decrease the sensitivity (Figure 1, A). The cutoff of 1.5 for the SI is said to be based on a receiver-operating characteristic curve presented as Figure E2 in the article by Salas et al.¹ This involves circular reasoning in that they use a proposed new cutoff and then confirm it in a receiver-operating characteristic curve based on the sensitivity and specificity resulting from the proposed cutoff.

Three concentrations were used, with 10 and 25 times difference between the highest and the lowest concentration of amoxicillin and clavulanic acid, respectively.¹ This difference is far lower than the variation in sensitivity using *in vivo* provocation tests, *in vitro* IgE tests, or histamine release tests.⁴ The variation is at least 4 magnitudes.

A value less than the cutoff limit is negative. Negative data can only be presented as negative. Thus, the median cannot be given a value less than the cutoff 2 (or 1.5); it is just negative. In Figure 1, A, all dots and rings lower than 2 (1.5) should be removed (Figure 1, B). The number of negative test results is presented as a number below the graph in Figure 1, B. For the purpose of statistical analysis, negative results are sometimes given a value lower than the cutoff. In this case SI = 1 would be suggested.

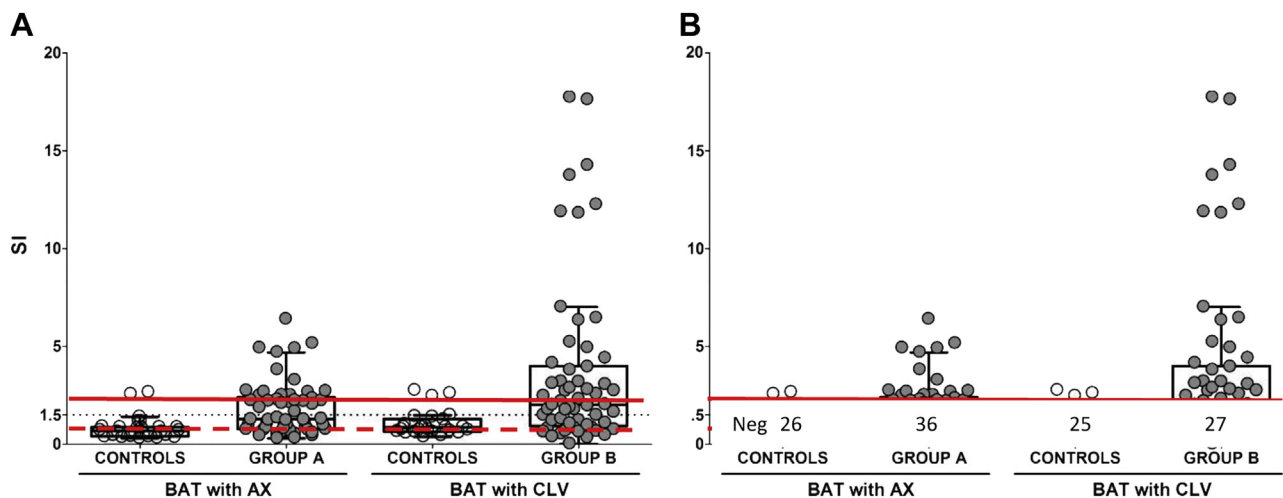


FIGURE 1. A, A copy of Figure 2, A, in the original communication.¹ The solid red line indicates an SI of 2 and the broken red line an SI of 1. Observe that some of the controls, and even drug-stimulated basophils, have an SI below 1; ie, the spontaneous activation of basophils was higher than the activation by drugs. The number of positive and negative test results is highly influenced by changing the cutoff from 1.5 to more than 2. B, The same data as in Figure 1, A. Numbers indicate the negative test results. Data are approximate because I do not have the original data at hand. To better illustrate the results, the Y-axis should have a log-transformed scale, because the data are not normally distributed (like most biological data). AX, Amoxicillin; CLV, clavulanic acid. Modified with permission from Salas M, Fernández-Santamaría R, Mayorga C, Barrionuevo E, Ariza A, Posadas T, et al. Use of basophil activation test may reduce the need for drug provocation in amoxicillin-clavulanic allergy. *J Allergy Clin Immunol Pract* 2018;6:1010-8.¹

Normal criteria for documentation of background and degree of sensitivity of BAT cannot be established before a well-documented BAT method has been agreed upon.

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Reply



To the Editor:

Dreborg¹ has expressed concerns regarding our recent article on the value of basophil activation test (BAT) for the evaluation of amoxicillin-clavulanic acid (AX-CLV) allergy.²

His main concern is the cutoff used for a positive result. As he correctly stated, *in vitro* testing for drug allergy evaluation is quite different from that for aeroallergens,³ requiring much higher concentrations for basophil activation. It is therefore widely accepted that the cutoff used for *in vitro* tests for

many allergens, the mean of blank tests + 3.3 S.D, is not valid for drugs.

Thus, other approaches must be considered, and a stimulation index (SI) of more than 2 is often used.^{3,4} Dreborg expressed specific concerns with our usage of an SI of 1.5, implying that we had chosen it to increase the sensitivity of our test without reducing the specificity. However, because no previous studies have investigated a sensitivity/specificity cutoff for the AX-CLV BAT, we performed receiver-operating characteristic curve analysis, using a large number of well-characterized allergic patients and (exposed) tolerant individuals, to choose the optimal cutoff⁵ and to spark research in this area. Receiver-operating characteristic curve analysis has been widely used when evaluating drug reactions by BAT,^{3,6} to balance sensitivity and specificity. Nevertheless, given the concerns of Dreborg, we reran the analysis using an SI of 2. The overall sensitivity of BAT was reduced from 54% to 42%, the sensitivity for AX remained at 41%, and the sensitivity for CLV decreased from 62% to 52%. Specificity values remained the same. On combining skin test and BAT results, the sensitivity became 75.9% for AX-allergic patients and 84.5% for CLV-allergic patients.

These values should however be viewed in the context of their clinical utility: although false-positive results are of course undesirable, because they result in unnecessary drug avoidance, false-negative results can have dramatic consequences, especially when dealing with severe reactions. Thus, physicians must make a decision after balancing the risk-benefit ratio for each individual case. We would argue that this means that in some circumstances they would prefer a cutoff that sacrifices specificity in favor of increasing sensitivity. Therefore, we still contend that for the data presented in our study, an SI of more than 1.5 is optimal (Figure 1, A). Of course, many more studies are required in this area to establish an optimal clinically valid cutoff.

Regarding the presentation of values with an SI of less than 1, these data show that degranulation was sometimes lower with the drug than observed spontaneously. Dreborg suggested that we change these negative values to 1 for statistical analysis. We reanalyzed the data taking this into account and the differences

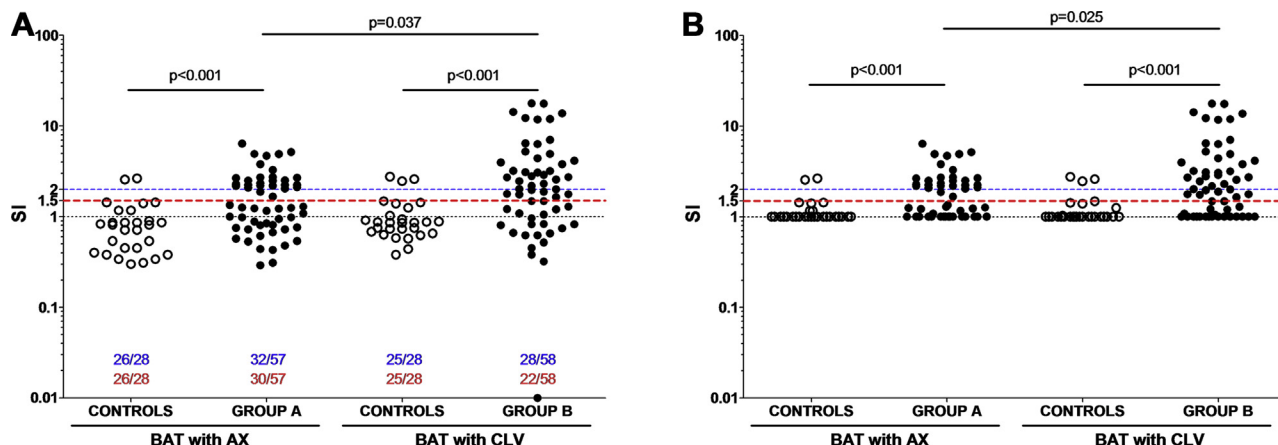


FIGURE 1. A, Copy of Figure 2, A, from the original communication.² Blue line indicates an SI value of 2 and blue numbers indicate negative results/total number using this cutoff. Red line indicates an SI value of 1.5 and red numbers indicate negative results/total number using this cutoff. B, Copy of Figure 2, A, from the original communication² assigning an SI value of 1 to all the individuals with values lower than 1. Blue line indicates an SI value of 2, and red line indicates an SI value of 1.5. Patients allergic to AX are represented as group A and patients allergic to CLV as group B. SI is represented on the Y-axis in logarithmic scale.

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