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6.1.1.5 Biological Description of the Cortisol Responses to Corticotropin-Releasing-Hormone (CRH) Stimulation. An Optimization and Simplification of the Test

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ABSTRACT

For the corticotropin-releasing-hormone stimulation test the number of samplings and measurements are reduced to two - and the ratio between concentrations at 60 min and 0 min is calculated. The difference between the information given by absolute concentrations and ratio is negligible, but by using ratio, the influence of bias is eliminated. The test becomes simpler and the costs are reduced. The use of only two measurements factitiates the evaluation of quality specifications.

CLINICAL SITUATION

In the diagnostic assesment of Cushing's syndrome the measurement of cortisol in serum and urine is advocated. In addition cortisol measurements after corticotropin-releasing-hormone (CRH) stimulation is useful both in the evaluation and differential diagnostic classification of Cushing's syndrome (1, 2, 3).

Any measured result must be compared to some reference in order to be interpreted. The reference intervals of cortisol in other laboratories and departments are known, although the investigations on Cushing's syndrome and the biochemical components related to this syndrome have revealed the necessity of each laboratory making its own reference interval (1, 2). Imperfect analytical standadization may be an explanation (4).

The aim of this study was to establish a model/method making the results independent of analytical standardization by establishing a general reference interval applicable to all laboratories independent of analytical bias. Further we wanted to make the **signal** (measured component) more distinct compared to the **noise** (the disturbing factors confusing the signal).

METHOD

10 healthy men (age range 22-40) were in a randomized and standardized sequence tested to placebo and stimulation with human CRH. Bloodsamples were drawn at -15, 0, 10, 20, 60 and 90 min., 1 microgram of human-CRH pr. kilo bodyweight or the same volume of saline was given intravenously at time zero. Cortisol was analysed with a radio-immunoassay (Farmos), all samples from one person in the same run, in duplicate.

MODELS FOR THE EVALUATION OF QUALITY SPECIFICATIONS

In the model for improvement of signal-to-noise ratio, the steps are the following:

A. The traditional presentation of time related cortisol-response from the stimulation test (Fig. 1) as mean +/- 2 standard deviations, for both placebo and CRH-stimulation.

B. Elimination of the inherent between-subject biological variation of the initial concentration by calculation of ratios of each measured concentration at time (t), X_t, to the initial concentration at zero time, X_0: $R_t = X_t/X_0$ and presentation of data as function of time (Fig. 2) (comparable to A). Hereby, the effect of proportional bias is eliminated and the signal-to-noise ratio is improved.

C. From A and B the optimum separation between placebo and stimulation is found to occur between 30 and 60 minutes.

D. By investigation of distributions for placebo and stimulation at 30 and 60 minutes for situations A and B, respectively, the interpretation of data is reduced to a simple bi-modal concept (Fig. 3) (4). The best separation (for both A and B) is at 60 minutes. This procedure simplifies a time consuming procedure with a complicated curve evaluation to a two-point sampling with a clear interpretation, whereby, also the costs are reduced considerable.

EVALUATION OF QUALITY SPECIFICATIONS

The quality specifications are evaluated by a graphical method (4). The clinical situation of separating placebo from stimulation may be considered as a test capable of separating stimulation from no stimulation. As the prevalence may be considered arbitrary, 0.5 is chosen, and the optimization is simply defined as the minimum fraction of misclassified (FN+FP) where the FN and the FP are given the same weight.

The within-run analytical coefficient of variation, CV_{WA}, in the measurements is 0.052 (5,2%) and the total (within- plus between-run) CV_{TA} is 0.07 (7%). Based on these informations the (isolated)

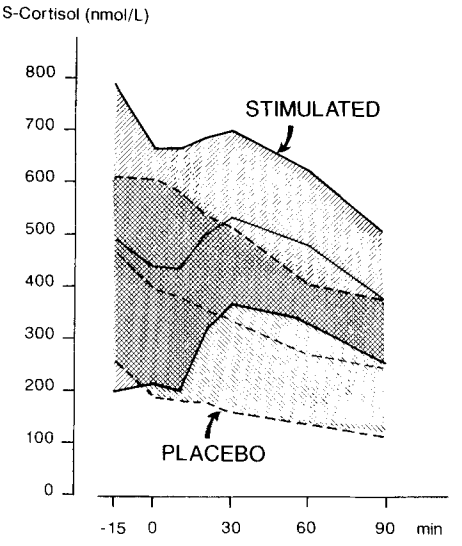

Fig. 1. Concentrations of S-cortisol as function of time for placebo and h-CRH-stimulation, showing the two mean causes with 0.95 statistical coverage intervals for expectation of new individual results for both groups.

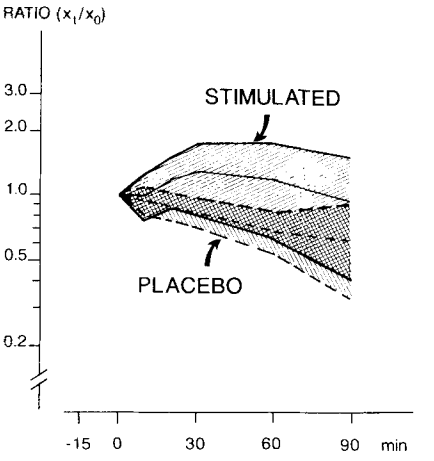

Fig. 2. Ratio-plot of the same data as in fig. 1, showing mean causes with 0.95 statistical coverage expectation intervals for both groups.

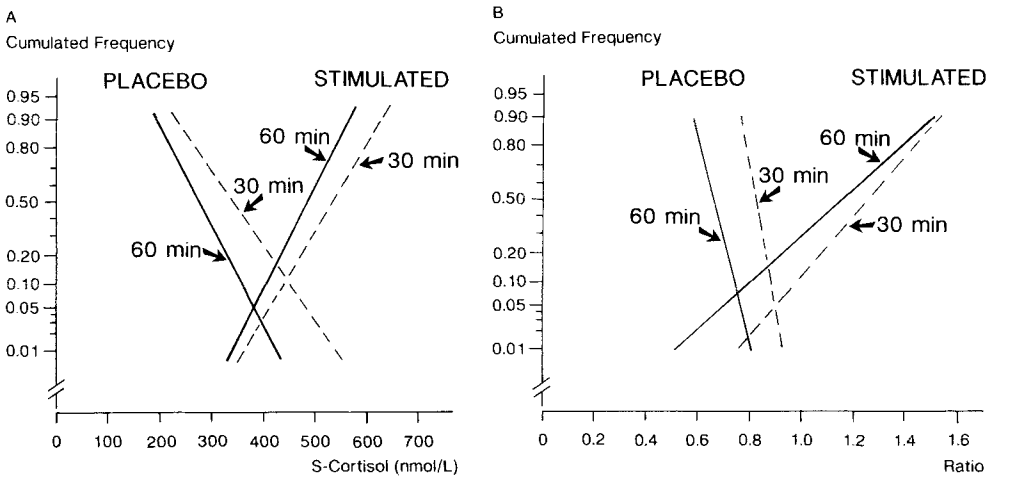

Fig. 3. Probit-plots of the two situations in fig. 1 and 2 at 30 and 60 min (measured concentrations (A) and ratios (B)).

biological standard deviations or coefficients of variation, can be estimated for each by the formulas:

1) concentrations:

$$s_{B(conc)} = \sqrt{s^2_{calculated} - s^2_{TA}}$$

2) ratio:

$$s_{B(ratio)} = \sqrt{s^2_{calculated} - s^2_{WA}}$$

For the ratio, s_{WA} is calculated from the equation: $s_{WA} * 2/2$ due to two measurements in same run and means of duplicate.

Fig. 4 gives the isolated distributions at 60 min together with assumed analytical precision at CV_A's of 0.10, 0.20 and 0.30.

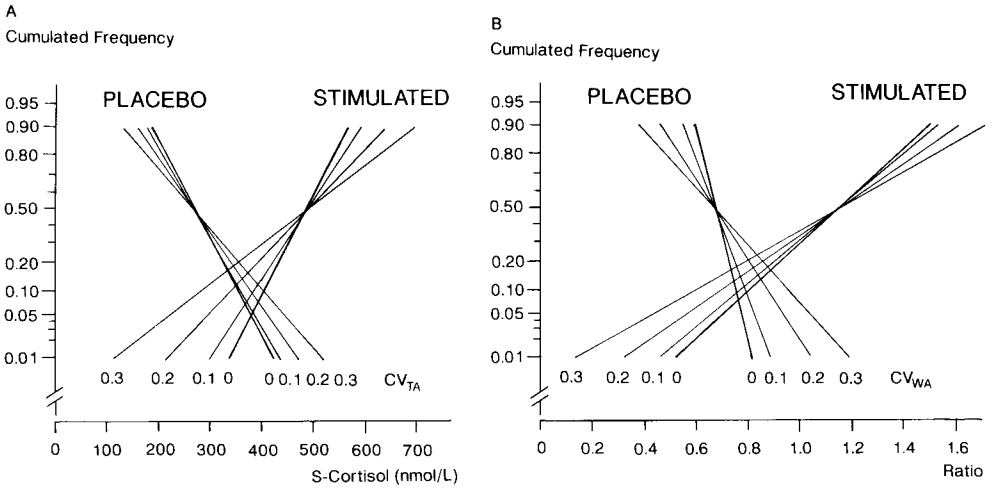

Fig. 4. Probit-plot of the situation in fig. 3 at 60 min (best discrimination). Isolated distributions and distributions with assumed values of precision.

The number of misclassifications as function of bias for different assumed precisions are shown in fig. 5, where also ratios are evaluated. For ratio the specifications for CV_{WA} are more demanding than for CV_{TA} in evaluation of concentration values, but the effect on ratio of proportional bias has no relevance, because the effect of bias is eliminated by calculation of ratios.

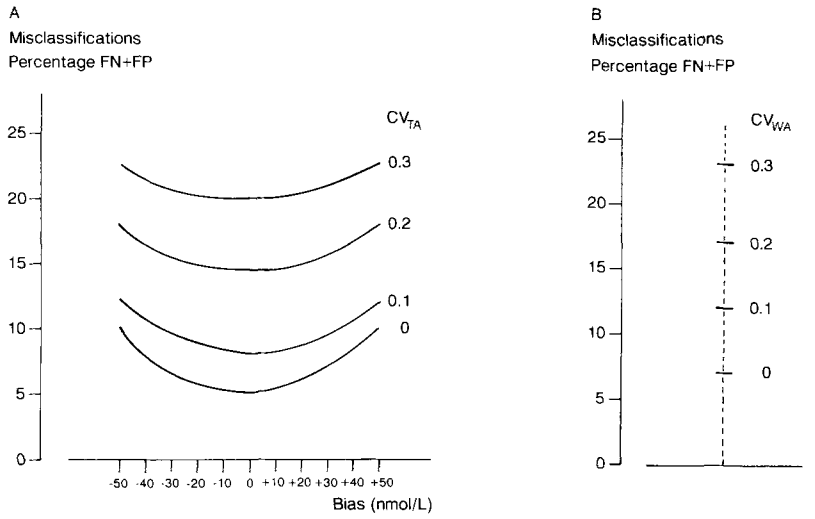

Fig. 5. Effect of analytical variation and bias on the estimated number of misclassifications, at a prevalence of 0.5. For the ratio there is no function of bias.

DISCUSSION

In this study we have shown, that it is possible to increase the signal-to-noise ratio by eliminating many disturbing factors (4, 5). The model is useful achieving independance of analytical standardization, as proportionally bias is without influence on ratios. However, the demand of a good CV_{WA} becomes more demanding aiming for an optimal outcome. In the unstimulated experiment the half-life of cortisol in serum is found to be about 90 minutes, which is in accordance with literature (6). This model may be useful also in the evaluation of other stimulation tests. The importance of a control system becomes less demanding concerning bias. The difference between the information given by absolute concentrations and ratio is negligible.

CONCLUSION

By use of ratio, the influence of bias is eliminated, but at the same time the demands for within-run precision are increased. Evaluations at a fixed time make the interpretations of results more clear -and the costs are at the same time reduced compared to the 5 to

7 samplings and measurements. Reference intervals on ratio can be applied everywhere. The quality specifications are evaluated graphically.

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