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# STATISTICAL METHOD OF ASSIGNING FIDUCIDAL LIMITS TO A PROTECTIVE EFFECT ESTIMATED FROM BIOLOGICAL ASSAYS WHICH COMPARE THREE GROUPS OF RESULTS

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Abstract

A statistical method of assigning fiducidal limits to a protective effect is proposed. The method is based on a theorem of mathematical statistics given by Fieller, and may be applied to all experimental procedures which measure an adequate quantitative response in three groups of animals: intoxicated animals, intoxicated and treated animals, and control animals.
A numerical example of assigning fiducidal limits to the antilipidical effect of the mucopolysaccharide extracted from Mytilus galloprovincialis in the intoxication with Triton, is also given.

The problem of estimating the protective effect of drugs against experimentals intoxications appears frequently in pharmacological researches.

To estimate the protective effect, an experimental procedure using three groups of animals is needed: a standard group (C) a group (I) of animals which have received the toxic substance, and a group (T) of animals which have received both the toxic substance and the protective preparation. Further one measures an adequate quantitative responde and let $\bar{C}$, $\bar{I}$ and $\bar{T}$, be the arithmetic means of the responses in the above mentioned groups. Then, the protective effect may be estimated by the formula:

$$E = \frac{\bar{I} - \bar{T}}{\bar{I} - \bar{C}} \quad \text{or} \quad E\ \% = 100\,\frac{\bar{I} - \bar{T}}{\bar{I} - \bar{C}} \qquad (1)$$

which was first proposed by Bianchini (1958) in his studies concerning the antilipidical effect of some heparinoid substances in the intoxication with Triton.

Newbould (1963) uses formula (1) to estimate the antiinflammatory effect in experimental inflammations.

The same formula has been used by some of us a series of papers: Molnar (1963, 1966, 1970), Winter (1971), Simionovici (1971), Molnar (1975). For instance, by means of formula (1), Molnar (1975), establishes the antilipidical effect of the sulphomucopolysaccharide extracted from Mytilus galloprovincialis in the intoxication with Triton.

In all these papers, the significance of the difference between the arithmetic means $\bar{I}$ and $\bar{T}$ has been tested by means of the Student distribution, but no fiducial limits were assigned to the protective effect, because, the protective effect being not a random variable with normally distributed errors, the usual formula $E \pm ts_E$ is no more applicable.

In this paper a statistical method of assigning fiducial limits to the protective effect, E, is proposed. The method is based on a theorem concerning the fiducial limits of a ratio, which was given by Fieller and further developped by Finney (1964).

Let $I_i$ ($i = 1, 2, \ldots, n_1$); $T_j$ ($j = 1, 2, \ldots, n_2$); $C_k$ ($k = 1, 2, \ldots, n_3$) be the individual responses (e.g., the turbidity of rat serum) obtained in the above mentioned groups of animals, and let x, y and z be the corresponding random variables. Then, each individual value $I_i$ represents an estimate of x, and similarly each $T_j$ and $C_k$ represents an estimate of y and z respectively. Of course, instead of the variables x, y and z, one may consider a number of $n_1 + n_2 + n_3$ variables, i.e., $n_1$ identical variables $x_1 = x_2 = \ldots = x_{n1}$, $n_2$ identical variables $y_1 = y_2 = \ldots = y_{n2}$, and $n_3$ identical variables $z_1 = z_2 = \ldots = z_{n3}$ respectively. In this approach, the individual values $I_i$ $(i = 1, 2, \ldots, n_1)$; $T_j$ $(j = 1, 2, \ldots, n_2)$; $C_k$ $(k = 1, 2, \ldots, n_3)$ will represent estimates of the corresponding variables $x_i$ $(i = 1, 2, \ldots n_1)$; $y_j$ $(j = 1, 2, \ldots, n_2)$ and $z_k$ $(k = 1, 2, \ldots, n_3)$ respectively.

We shall suppose that $x_i$, $y_i$, $z_k$ are random variables with normally distributed errors, and we shall consider the following variables:

$$\alpha = (\sum_1^{n_1} x_i)/n_1 - (\sum_1^{n_2} y_j)/n_2; \quad = (\sum_1^{n_1} x_i)/n_1 - (\sum_1^{n_3} z_k)/n_3 \qquad (2)$$

$$\mu = \alpha/\beta \qquad (2')$$

$\alpha$ and $\beta$ being linear functions of normal random variables, will be also normal random variables. Therefore, $\mu$ represents the ratio of two normal random variables.

The Fieller's theorem gives the fiducial limits of a random variable which may be put as the ratio of two normal random variables, and consequently may be applied to the random variable $\mu$ given by eqs. (2) and (2'). Fieller's theorem states that upper and lower fiducial limits to $\mu$ are:

$$m_L, m_U = \left\{ m - g \frac{C(\alpha, \beta)}{V(\beta)} \pm \frac{t}{b} \left[ V(\alpha) - 2mC(\alpha, \beta) + m^2 V(\beta) - g \left( V(\alpha) - \frac{C^2(\alpha, \beta)}{V(\beta)} \right) \right]^{1/2} \right\} / (1 - g) \qquad (3)$$

where $m = a/b$ represents an estimate of $\mu$ (a, b being estimates of $\alpha$ and $\beta$ respectively), $V(\alpha)$ and $V(\beta)$ are the sample variances of $\alpha$ and $\beta$, and $C(\alpha, \beta)$ their sample covariance.

In formula (3), t is the ordinary t- deviate with f degrees of freedom (used to calculate the sample variances) and at the chosen probability level, and

$$g = t^2 V(\beta)/b^2 \tag{3'}$$

It is easy to see that the differences $\bar{I} - \bar{T}$, $\bar{I} - \bar{C}$ represent estimates of $\alpha$ and $\beta$ respectively, while the protective effect E (given by eq. (1)) represents an estimate of $\mu$. Therefore:

$$a = \bar{I} - \bar{T}; \quad b = \bar{I} - \bar{C}; \qquad m = E \tag{4}$$

Further, let us consider the most frequent case where only one error mean square, $s^2$, occurs in the analysis of variance of the data. Then, one may assign the same sample variance to each of the $n_1 + n_2 + n_3$ variables $x_i$, $y_j$ and $z_k$, i.e., $V(x_i) = V(y_j) = V(z_k) = s^2$, where:

$$s^2 = \frac{\sum_1^{n_1} (I_i - \bar{I})^2 + \sum_1^{n_2} (T_j - \bar{T})^2 + \sum_1^{n_3} (C_k - \bar{C})^2}{n_1 - 1 + n_2 - 1 + n_3 - 1} \tag{5}$$

Thus, usind eqs. (2) one gets:

$$V(\alpha) = (\sum_1^{n_1} V(x_i))/n_1^2 + (\sum_1^{n_2} V(y_j))/n_2^2 = (\frac{1}{n_1} + \frac{1}{n_2})\, s^2 \tag{6}$$

$$V(\beta) = (\sum_1^{n_1} V(x_i))/n_1^2 + (\sum_1^{n_3} V(z_k))/n_3^2 = (\frac{1}{n_1} + \frac{1}{n_3})s^2 \tag{7}$$

To calculate the sample covariance $C(\alpha, \beta)$ we shall start from the definition of a theoretical covariance:

$$Cov\ (\alpha, \beta) = M(\alpha, \beta) - M(\alpha)\ M(\beta) \tag{8}$$

where M( ) represents the expectation of the random variable written inside the bracket.

But :

$$= (\sum_{m=1}^{n_1} \sum_{p=1}^{n_1} x_m x_p)/n_1^2 - (\sum_{m=1}^{n_1} \sum_{p=1}^{n_2} x_m y_p)/n_i n_2$$

$$- (\sum_{m=1}^{n_1} \sum_{p=1}^{n_3} x_m z_p)/n_1 n_3 + (\sum_{m=1}^{n_2} \sum_{p=1}^{n_3} y_m z_p)/n_2 n_3 \qquad (9)$$

On the other hand, $x_i$, $y_j$ and $z_k$ are independent variables, and consequently:

$$M(\alpha\beta) = (\sum_{1}^{n_1} M(x_i^2) + \sum_{\substack{m=1 \\ (m \neq p)}}^{n_1} \sum_{p=1}^{n_1} M(x_m)\, M(x_p))/n_1^2$$

$$- (\sum_{m=1}^{n_1} \sum_{p=1}^{n_2} M(x_m)\, M(y_p))/n_1 n_2 -$$

$$- (\sum_{m=1}^{n_1} \sum_{p=1}^{n_3} M(x_m)\, M(z_p))/n_1 n_3 +$$

$$+ (\sum_{m=1}^{n_2} \sum_{p=1}^{n_3} M(y_m)\, M(z_p))/n_2 n_3 \qquad (10)$$

Similarly:

$$M(\alpha) = (\sum_{1}^{n_1} M(x_i))/n_1 - (\sum_{1}^{n_2} M(y_j))/n_2$$

$$(11)$$

$$M(\beta) = (\sum_{1}^{n_1} M(x_i))/n_1 - (\sum_{1}^{n_3} M(z_k))/n_3$$

Therefore:

$$M(\alpha)\ M(\beta) = \left(\sum_{1}^{n_1} M^2(x_i) + \sum_{\substack{m=1\\(m\neq p)}}^{n_1} \sum_{p=1}^{n_1} M(x_m)\ M(x_p)\right)/n_1^2$$

$$-\left(\sum_{m=1}^{n_1} \sum_{p=1}^{n_2} M(x_m)\ M(y_p)\right)/n_1 n_2 - \qquad (12)$$

$$-\left(\sum_{m=1}^{n_1} \sum_{p=1}^{n_3} M(x_m)\ M(z_p)\right)/n_1 n_3 + \left(\sum_{m=1}^{n_2} \sum_{p=1}^{n_3} M(y_m)\ M(z_p)\right)/n_2 n_3$$

It follows that:

$$\mathrm{Cov}\ (\alpha, \beta) = \sum_{1}^{n_1} (M(x_i^2) - M^2(x_i))/n_1^2 \qquad (13)$$

But $M(x_i^2) - M^2(x_i)$ represents the theoretical variance, $D(x_i)$, of $x_i$, and hence:

$$\mathrm{Cov}\ (\alpha, \beta) = \sum_{1}^{n_1} D(x_i)/n_1^2 \qquad (13')$$

To obtain the sample covariance, we shall replace $D(x_i)$ by the sample variance $V(x_i) = s^2$. It results:

$$C(\alpha, \beta) = s^2/n_1 \qquad (14)$$

Now, using eqs. (4), (6), (7) and (14), the equation (3) leads to the following formulae for the fiducial limits of the protective effect:

$$E_{L,U} = \left\{ E - g\frac{v_{12}}{v_{22}} \pm \frac{ts}{1-C} \left[ v_{11} - 2Ev_{12} + E^2 v_{22} - \right.\right.$$

$$\left.\left. - g\left(v_{11} - \frac{v_{12}^2}{v_{22}}\right)\right]^{1/2} \right\} /(1-g) \qquad (15)$$

where:

$$v_{11} = \frac{1}{n_1} + \frac{1}{n_1} \; ; \; v_{12} = \frac{1}{n_1} \; ; \; v_{22} = \frac{1}{n_1} + \frac{1}{n_3} \qquad (15')$$

When

$$g = t^2 s^2 v_{22} / (\bar{I} - \bar{C})^2 \qquad (3'')$$

is less than 0.1, eq. (15) simplifies to :

$$E_{L,U} = E \pm \frac{ts}{\bar{I}-\bar{C}} (v_{11} - 2Ev_{12} + E^2 v_{22})^{1/2} \qquad (15'')$$

Equation (15") is good enough for most practical purposes.

An example of assigning fiducial limits to a protective effect

To establish the antilipidical effect of the mucopolysaccharide extracted from Mytilus galloprovincialis in the intoxication with Triton, an experimental procedure using three groups of animals (rats) has been used an the data needed to apply eq. (15) are summarized below.

| Group of animals | Intoxicated $N_1 = 20)$ | Intoxicated and treated $(n_2 = 20)$ | Standard $(n_3 = 20)$ |
|---|---|---|---|
| Mean values | $\bar{I} = 0.94$ | $\bar{T} = 0.49$ | $\bar{C} = 0.06$ |
| Sum of squares | $\sum_1^{n_1} (I_i - \bar{I})^2 = 1.7899$ | $\sum_1^{n_2} (T_j - \bar{T})^2 = 0.2812$ | $\sum_1^{n_3} (C_k - \bar{C})^2 = 0.0025$ |
| Protective effect | $E = \frac{\bar{I} - \bar{T}}{\bar{I} - \bar{C}} = 0.51$ | | |
| Error mean square | $s^2 = (1.7899 + 0.2812 + 0.0025)/57 = 0.0364$ | | |
| $v_{11}, v_{12}, v_{22}$ | $v_{11} = v_{22} = 0.1; \; v_{12} = 0.05$ | | |

$s^2$ is based on f = 57 degrees of freedom, and the corresponding t-deviate for a probability of 0.05 is t = 2.00. Then, eq.(3") gives g = 0.019, and therefore the simplified eq.(15") may be used to assign fiducial limits to E. One gest: $E_L$ = 0.39, $E_U$ = 0.63, i.e., with a degree of confidence expressed by the 95 per cent. probability level, one may say that the protective effect is not less than 39%. and not greater than 63%.

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