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RELATIVE POTENCY OF TWO PREPARATIONS WITH CORRELATED OBSERVATIONS

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Summary

An estimation method of a test preparation potency in relation to a standard preparation administered to the same experimental units is presented in this paper. The responses for the doses of preparations on the same units are correlated. Moreover, a special structure connected with allocation of the doses on experimental units is considered. A model for the observations and a method of estimation of parameters of the model are presented. A test function for testing a hypothesis about parallelism of regression lines for the standard and test preparations is given. When the hypothesis about parallelism fails to reject, then the formula for the estimator of the relative potency can be defined, which is the main goal of the paper.

Key words and phrases: relative potency, parallel-line assay, correlated observations, testing a hypothesis, tuberculin-test

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1. Introduction

Some biological experiments are conducted in order to compare the effects of two applied preparations. In general, the effect of one preparation, a standard one,

is known but the effect of a new one, a test one, is unknown. One of the statistical methods of such a comparison is estimation of their relative potency. The estimation of the potency assesses the dose of the test preparation which gives the same effect as unit dose of the standard preparation. The method of estimation of the relative potency has been described in many papers and in a fundamental book by D.J. Finney under the title *Statistical Method in Biological Assay*. Two preparations can be compared by the relative potency if at least two different doses of each preparation are administered to experimental units. The most useful model for describing dependences of responses on logarithm of the doses is a parallel-line assay. In general, responses of the experimental units getting the dose of preparation are statistically independently distributed. In the paper we consider a case, where the doses of both preparations are simultaneously administered to the same experimental units. Then, the responses are correlated and the estimation of relative potency of preparations should be proper to such a model.

2. Description of the experiment

Tuberculosis of cattle is the most frequent bacterial infection which could be transmitted on humans. One of the method of diagnosis of tuberculosis is a tuberculin-test. In Poland two preparations: *Bovituberculin* and *Avituberculin* are produced in Biowet, Pulawy, Ltd. The main component of the preparations is tuberculin protein having different derivation. New series of preparations, before sending it into a market, has to be compared to standard international preparations. To a statistical comparison of the standard and the test preparations, the relative potency in parallel-line assays is recommended. To estimate relative potencies of test preparations to standard ones, the experiments on homogeneous allergic guinea-pigs are conducted in the laboratory of The National Veterinary Research Institute in Pulawy. On each guinea-pig with the same cause of allergy the injection of two preparations in three dilutions are done after 4 weeks. After 24 hours the diameters of reaction are measured (with precision $\pm 0.5\ mm$). The injection of all doses of the standard and the test preparations are replicated at least on nine guinea-pigs. Moreover, the doses of the preparations are administered in a special way. The first four animals are injected in such a way that the doses of the standard preparation are placed on the left side, cyclically changing the place on the body from the back and the head. The sketch of such an allocation of the injections is presented in Table 1, where u_{S1}, u_{S2} and u_{S3} denote doses of the standard preparation while u_{T1}, u_{T2} and

u_{T3} denote doses of the test preparation. The next four animals are injected in a similar way but starting from the right side, and so on.

Table 1. Arrangement of injection of doses of preparations to animals

First animal		Second animal		Third animal		Fourth animal	
Left	Right	Left 0	Right	Left	Right	Left	Right
u_{S1} $-$ u_{S2} u_{S3}	$-$ u_{T1} u_{T3} u_{T2}	$-$ u_{S3} u_{S1} u_{S2}	u_{T3} $-$ u_{T2} u_{T1}	u_{S3} u_{S2} $-$ u_{S1}	u_{T2} u_{T3} u_{T1} $-$	u_{S3} u_{S2} $-$ u_{S1}	u_{T2} u_{T3} u_{T1} $-$

Let us note that the experiment doses of preparations are injected to the same animal, so the responses are correlated. To describe a model of observations we have to consider a model with some structure of covariance matrix. Moreover, in a model we have to consider some effects concerning the place of injection on the body, namely, an effect of the side of animal (left or right), an effect of the row (the distance from the back) and an effect of the column (the distance from the head).

3. Model of observations

Let us assume that the response for the dose of standard or test preparation is linear in relation to logarithm of the dose of preparation, ie. $E(y_{ij}) = \alpha_i + \beta_i x_{ij}$, where $i = S,T$ denotes a standard (*S*) or a test preparations (*T*), α_i and β_i denote an intercept and a slope, for the standard or the test preparation, respectively, x_{ij} denote the *j*th dose of *i*th preparation, $j = 1,\cdots,v$ and *v* is the number of doses the same for the standard and the test preparation. To consider parallel-line assay, the slopes for the standard and the test preparation have to be the same. Then, logarithm of relative potency of preparations is estimated by the ratio $\frac{\hat{\alpha}_S - \hat{\alpha}_T}{\hat{\beta}}$, where $\hat{\alpha}_S$, $\hat{\alpha}_T$ and $\hat{\beta}$ denote estimators of intercepts and common slope for both preparations.

In parallel-line assays, where doses of preparations are administered to different units, the problem of estimation of the relative potency has been considered by many authors- namely: Finney (1952), Hubert (1984), Park and Kshirsagar (2002). In this paper we consider a problem of estimation of the relative

potency in assays, where responses are correlated and depend on the place of the dose injection. Thus we consider the assays with the effects of two sides, two rows and two columns. After putting the usual restrictions on the effects of the allocation, we can take only plus (+) or minus (-) effect, for example: $+\omega$ denotes the effect on the left side, while $-\omega$ denotes the effect on the right side of the animal. Let us assume that the response of animal for the dose of preparation is described as follows

$$y_{ijk} = \alpha_i + \beta_i x_{ij} \pm \omega \pm \kappa \pm \tau + e_{ijk} \tag{3.1}$$

where $i = S,T$, $j = 1,\cdots,v$, $k = 1,\cdots,n$ and n is a number of tested animals, ω, κ, τ denote the side, the row and the column effect, respectively. Then, the response of kth animal $(k = 1,\cdots,n)$ can be described as $\mathbf{y}_k = [y_{S1k},\cdots,y_{Svk},y_{T1k},\cdots,y_{Tvk}]'$. We assume that $\mathbf{y}_k$ has multivariate normal distribution, $N_{2v}(E(\mathbf{y}_k),\boldsymbol{\Sigma})$, where $\boldsymbol{\Sigma}$ is $(2v\times 2v)$ covariance matrix, the same for each animal. Under such assumptions vector of all responses can be described by the following model

$$\mathbf{y} = \mathbf{XB} + \mathbf{e}, \tag{3.2}$$

where $\mathbf{y} = [\mathbf{y}_1',\mathbf{y}_2',\cdots,\mathbf{y}_n']'$, $\mathbf{X} = [\mathbf{D}_\omega,\mathbf{D}_\kappa,\mathbf{D}_\tau,\mathbf{I}_2\otimes\mathbf{1}_{nv},\mathbf{I}_2\otimes\mathbf{x}]$, $\mathbf{B} = [\omega,\kappa,\tau,\alpha_S,\alpha_T,\beta_S,\beta_T]'$, $\mathbf{e} = [\mathbf{e}_1',\mathbf{e}_2',\cdots,\mathbf{e}_n']'$, $\mathbf{D}_\omega$, $\mathbf{D}_\kappa$ and $\mathbf{D}_\tau$ are $(2nv\times 1)$ vectors having elements -1 or 1, connected with the allocation on the animal, $\mathbf{I}_2$ is identity matrix of the size 2, $\mathbf{1}_n$ is unit vector of the size n and $\mathbf{x} = \mathbf{1}_n \otimes [\log u_1,\cdots,\log u_v]'$, $u_1,\cdots,u_v$ denote doses the same for the standard and the test preparations. In general, in the univariate bioassay proportional doses of preparations are administered. This allows to transform log of doses to get $0,\pm 1,\pm 2$ and so on in the case of odd number of doses or $\pm 1/2,\pm 3/2$ and so on in the case of even number of doses.

4. Estimation of log relative potency

In this section we consider a problem of estimation of log of relative potency defined in the previous section. As the response vector **y** described in (3.2) has covariance matrix of the form $\mathbf{V} = \mathbf{I}_n \otimes \mathbf{\Sigma}$ so the estimator of the parameter vector **B** has the following form

$$\hat{\mathbf{B}} = (\mathbf{X}'\mathbf{W}\mathbf{X})^{-1}\mathbf{X}'\mathbf{W}\mathbf{y}, \tag{4.1}$$

where $\mathbf{W} = \mathbf{V}^{-1} = \mathbf{I}_n \otimes \mathbf{\Sigma}^{-1}$.

4.1. Hypothesis about the same slopes

In order to estimate the common slope, first, we should test the hypothesis about the same slopes for both preparations (to have a parallel-line assay). This hypothesis can be described as follows

$$H_\beta^0 : \mathbf{c}'\mathbf{B} = 0 \quad \text{tested against} \quad H_\beta^1 : \mathbf{c}'\mathbf{B} \neq 0, \tag{4.2}$$

where $\mathbf{c}' = [0, 0, 0, 0, 0, 1, -1]$. To test the hypothesis $H_\beta^0 : \mathbf{c}'\mathbf{B} = 0$ we use Wilks' lambda statistics given by the following formula

$$\Lambda = \frac{1}{1 + SSH / SSE},$$

where $SSE = (\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}})'\hat{\mathbf{W}}(\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}})$, $SSH = (\mathbf{c}'\hat{\mathbf{B}})'\left[\mathbf{c}'\left(\mathbf{X}'\hat{\mathbf{W}}\mathbf{X}\right)^{-}\mathbf{c}\right]^{-1}(\mathbf{c}'\hat{\mathbf{B}})$ and $\hat{\mathbf{W}} = \mathbf{I}_n \otimes \hat{\mathbf{\Sigma}}^{-1}$.

The hypothesis H_β^0 is rejected if $F^0 = [N - r(\mathbf{X})]\frac{1-\Lambda}{\Lambda} > F_{1,N-r(\mathbf{X});\alpha}$, where $r(\mathbf{X})$ denotes the rank of the matrix **X**, $N = 2\nu n$, and α is a known significant level (see, Krzyśko, 2000, p.188). We say that the effect of the test preparation is similar to the effect of the standard preparation if H_β^0 is not rejected.

4.2. Model of parallel-line assay

When we assume that the hypothesis $H_{\beta}^{0} : \mathbf{c}'\mathbf{B} = 0$ in (4.2) is true, then the model (3.2) can be described in the form

$$\mathbf{y} = \tilde{\mathbf{X}}\tilde{\mathbf{B}} + \mathbf{e},$$

where $\tilde{\mathbf{X}} = [\mathbf{D}_{\omega}, \mathbf{D}_{\kappa}, \mathbf{D}_{\tau}, \mathbf{I}_2 \otimes \mathbf{1}_{nv}, \mathbf{1}_2 \otimes \mathbf{x}]$, $\tilde{\mathbf{B}} = [\omega, \kappa, \tau, \alpha_S, \alpha_T, \beta]$, and $\mathbf{D}_{\omega}$, $\mathbf{D}_{\kappa}$, $\mathbf{D}_{\tau}$ and $\mathbf{x}$ remain the same as in the model (3.2). The estimator of the new parameter vector is described by

$$\hat{\tilde{\mathbf{B}}} = \left(\tilde{\mathbf{X}}'\mathbf{W}\tilde{\mathbf{X}}\right)^{-1}\tilde{\mathbf{X}}'\mathbf{W}\mathbf{y}.$$

Log relative potency can be calculated from the formula

$$\hat{\mu} = \log\hat{\rho} = \mathbf{c}_1'\hat{\tilde{\mathbf{B}}} / \mathbf{c}_2'\hat{\tilde{\mathbf{B}}},$$

where $\mathbf{c}_1' = [0, 0, 0, 1, -1, 0]$ and $\mathbf{c}_2' = [0, 0, 0, 0, 0, 1]$.
To calculate the relative potency $\hat{\rho}$ we have to calculate antilog of $\hat{\mu}$.

5. Estimation of log relative potency for tuberculin-tests

We consider estimation of relative potency of *Bovituberculin* and *Avituberculin* to international standards. The doses of injections were 32, 6.4 and 1.28 of international units (IU). For the data sets the estimates of covariance matrix $\boldsymbol{\Sigma}$, test functions for the hypothesis in (4.2) and estimates of the log potencies in (4.1) are shown in Table 2. The linear regression for both preparations are illustrated in Fig. 1 and Fig. 2. The regression lines presented in Fig. 1 show parallelism of the standard and the test preparation of *Bovituberculin*. The regression lines presented in Fig. 2 show that standard and test preparations of *Avituberculin* produce almost the same responses, so we can conclude that the standard and test preparations have the same potency. Moreover, the estimate of the relative potency for *Bovituberculin* presented in Table 2 is equal to 0.82. It means that the test preparation is more potent than the standard preparation. It means that using 0.82 of the dose of the test preparations gives the same effect as one dose

of the standard preparation. The estimate of the relative potency of *Avituberculin* is equal to 0.98. We can conclude that *Avituberculin* has the same potency as the standard preparation.

Table 2. Estimates of covariance matrix, model parameters, value of test and estimates of relative potency

Preparation	Covariance matrix	Parameter	F^0	p-value	Relative potency
Bovituberculin	$\begin{bmatrix} .25 & .30 & -.15 & .10 & .05 & .40 \\ .30 & .89 & -.22 & .07 & .26 & -.04 \\ -.15 & -.22 & 2 & -.37 & .37 & .63 \\ .10 & .07 & -.37 & .17 & -.13 & .02 \\ .05 & .26 & .37 & -.14 & 1.43 & 1.12 \\ .40 & -.04 & .63 & .02 & 1.12 & 2.99 \end{bmatrix}$	$\hat{\omega} = -0.017$ $\hat{\kappa} = 0.072$ $\hat{\tau} = 0.016$ $\hat{\alpha}_S = 11.018$ $\hat{\alpha}_T = 11.589$ $\hat{\beta}_S = 4.576$ $\hat{\beta}_T = 4.796$	0.42	0.48	0.82
Avituberculin	$\begin{bmatrix} .74 & .05 & .60 & .78 & .28 & .65 \\ .05 & 1.24 & 1.49 & -.03 & 0.26 & 0.68 \\ .60 & 1.49 & 3.31 & .94 & .93 & 1.94 \\ .78 & -.03 & .94 & 1.39 & .28 & .72 \\ .28 & .26 & .93 & .28 & 1.58 & 1.90 \\ .65 & .68 & 1.94 & .72 & 1.90 & 3.64 \end{bmatrix}$	$\hat{\omega} = -0.012$ $\hat{\kappa} = -0.081$ $\hat{\tau} = -0.022$ $\hat{\alpha}_S = 12.385$ $\hat{\alpha}_T = 12.338$ $\hat{\beta}_S = 3.908$ $\hat{\beta}_T = 4.106$	0.43	0.48	0.98

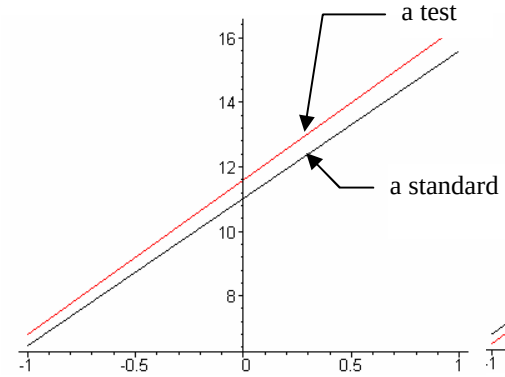

Fig. 1. Regression lines for *Bovituberculin*

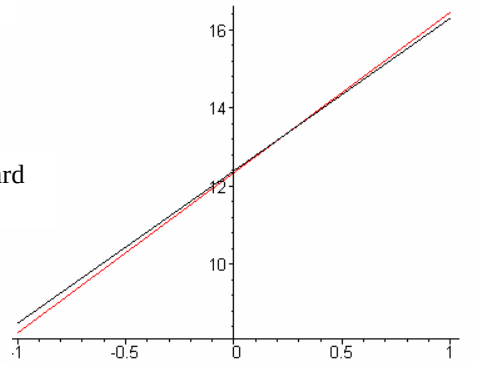

Fig. 2. Regression lines for *Avituberculin*

6. Conclusions

To estimate efficiency of new tuberculin-tests to standards the relative potency estimation should be used. However, we have to consider the model connected strictly with the experiment. For the data obtained for two series of the tuberculin-tests: *Bovituberculin* and *Avituberculin*, we have got the estimate of potencies related to standards very closed to units. This has confirmed that both series of tuberculin-tests should be accepted for sale.

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WZGLĘDNA MOC DWÓCH PREPARATÓW ZE SKORELOWANYMI OBSERWACJAMI

Streszczenie

Przedstawiono metodę estymacji względnej mocy preparatów stosowanych na tych samych jednostkach eksperymentalnych. Uzyskane obserwacje będące reakcjami jednostek eksperymentalnych na stosowanie dawek preparatów są skorelowane. Ponadto, rozważono specyficzny model do opisu układu eksperymentalnego, w którym dawki preparatów są rozmieszczone na jednostkach eksperymentalnych. Przedstawiono model dla obserwacji oraz pokazano metodę estymacji parametrów modelu. Ponadto, podano funkcję testową do weryfikacji hipotezy o równoległości prostych regresji dla preparatu standardowego i testowego. Przy prawdziwości hipotezy o równoległości podano wzór na estymator względnej mocy, co jest głównym celem pracy.

Słowa kluczowe: względna moc preparatów, doświadczenia liniowo równoległe**,** skorelowane obserwacje, testowanie hipotezy, testy tuberkulinowe

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