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Multivariate Regression of Liver on Intestine of Mice: A Chemotherapeutic Evaluation of Plant Extracts (*Pp. 322-333*)

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Abstract

Mice infected with 50 cercariae of Schistosoma mansoni in unequal group sizes were challenged with some doses of Zingiber officinale extracts, Cremophore and Praziquantel. Data were available for the number of male and female parasites, weights of liver and intestine, and egg per gramme liver and intestine tissues. Using an analysis of covariance model, the effects of the extracts and compound on intestine were predicted. The findings revealed, with the aid of likelihood-ratio statistic, a marked improvement in the precision of the estimates of the effects on intestine when the liver was taken into consideration.

Key Words: *Schistosomiasis*; Multivariate Regression; Likelihood-Ratio Statistics.

Introduction

Schistosomiasis (Bilharziasis) is one of the world's major public health problems for rural and agricultural communities living near slow-moving water in the tropics and subtropics. Schistosomiasis is a disease caused by digenean blood flukes of the genus *Schistosoma* (Brackenbury, 1998).

There are three major species of schistosomes, *S. haematobium*, *S. mansoni* and *S. japonicum*, which are principally parasitic in man. In Nigeria, two of

these species infecting man are important namely *S. haematobium* (the cause of urinary schistosomiasis) and *S. mansoni* (the cause of intestinal schistosomiasis) (Adewunmi, Gebremedhin, Becker, Dorfler and Adewunmi, 1993).

Statistics provide protection from the fortuitous result because no two observations can be relied upon to agree exactly, and their difference may arise by chance or as a result of a difference in treatment. Researchers have made efforts, to date, in using some techniques for evaluating potential antischistosomal drugs.

In the analysis of all data, the three replicates of the five experiments of Austin and Frappaolo (1973) revealed that a strong, direct correlation exists (as high as $r=0.9$) between the number of cercariae that penetrate and the number of worms in the resulting interaction. The curve then best fits the scatter diagram of the data.

The statistical significance of the data of Andrade and Azevedo (1982) was evident when submitted to Pearson's χ^2 tests. However, since the sample is rather small it is convenient to use the Yate's correction factor, and by doing that the statistical significance is not maintained.

Cheever (1986) regressed the variables examined on the number of worm pairs recovered. Linear, semi-logarithmic and logarithmic-logarithmic (log-log) regressions were performed. He chose the log-log curves because its variance was more uniform.

The statistical comparison of different regression models (linear and stepwise linear), the likelihood ratio test was used (Engels, Sinzinkayo, De vlas and Gryseels, 1997).

The statistical analyses of Karanja, Colley, Nahlen, Ouma and Secor, (1997) for comparisons of groups were performed by a non-parametric (Mann-Whitney) t-test. Linear regression analyses were performed by calculation of the Pearson correlation coefficient.

The thrust of this paper is to examine the extent to which the effect of the extracts and compounds on the liver can predict the effect on intestine as organs in a mouse.

Methods and Materials

Some data obtained from the laboratory on experimental mice exposed to some classified plant extracts and compounds were used.

The experimental plan consists of an experiment in which an unequal number of mice were subjected to some plant extracts and compounds.

Mice of both sexes, 20-22g, 6 to 8 weeks of age were used in all experiments, and were infected each with 50 cercariae of *S. mansoni* (Puerto Rican strain) by tail immersion (Andrade *et al.*, 1982; Cheever, 1986). The mice were kept in groups in Makrolon Bayer cages and had free access to feed (ssniff – R – pellets, Intermax GmbH) and water.

In this study, the mice were used 48 to 55 days after infection. Because of the slow onset of the antischistosomal action of some of these extracts, evaluation of the chemotherapeutic activity by autopsy of the treated mice was performed some weeks after drug administration (Cheever, 1986). Killing mice of the seven groups in the experimental group then determined the effect of treatment. The animals were usually sacrificed between 08.00 and 10.00 hours. They were not fasted the night before they were sacrificed (Austin *et al.*, 1973).

Autopsy was done on all mice 8 weeks after infection; they were particularly examined for worms in the hepatic portal and mesenteric veins. Their liver and intestine were examined between glass plates for worms and eggs. This method of worm determination was used instead of perfusion because with it more counts were possible (Cheever, 1986). Tissue specimens, including the liver, colon and small intestine, were taken at necropsy for histological examination.

Notations

We define the following terms and partitions:

$Y_1 = \ln z_1/x_1$, natural logarithms of egg load per worm pair per weight of liver.

$Y_2 = 1/x_1$, reciprocal of the weight of liver.

$Y_3 = \ln 1/x_1$, natural logarithms of the reciprocal of the weight of liver.

$Y_4 = 1/x_1 \ln 1/x_1$, the product of Y_2 and Y_3 .

$Y_5 = \ln z_2/x_2$, natural logarithms of egg load per worm pair per weight of intestine.

$Y_6 = 1/x_2$, reciprocal of the weight of intestine.

$Y_7 = \ln 1/x_2$, natural logarithms of the reciprocal of the weight of intestine,

$Y_8 = 1/x_2 \ln 1/x_2$, the product of Y_6 and Y_7 .

$U'_1 = \{Y_1, Y_2, Y_3, Y_4\}$, vector of liver variables with mean vector μ'_1

$U'_2 = \{Y_5, Y_6, Y_7, Y_8\}$, vector of intestine variables with mean vector μ'_2

$U' = \{Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8\}$, vector of variables partitioned into U'_1 and U'_2

i.e. $U' = \{U'_1, U'_2\}$ is the partition into U_1 and U_2 .

Let μ be mean vector of U , partitioned such that $\mu' = (\mu'_1, \mu'_2)$

Let $\Sigma_{8 \times 8}$ be variance – covariance matrix of U

Let $\Sigma_{11}, \Sigma_{12}, \Sigma_{21}$ and Σ_{22} be the partitions of Σ .

Suppose

$$U \sim N_8(\mu, \Sigma)$$

Then the conditional distribution of U_1 given $U_2 = u_2$ is normal with

$$E(U_1|U_2 = u_2) = \mu_1 + \Sigma_{12} \Sigma_{22}^{-1}(U_2 - \mu_2)$$

and

$$\text{Var}(U_1|U_2 = u_2) = \Sigma_{11} - \Sigma_{12} \Sigma_{22}^{-1} \Sigma_{21} = \Sigma_{4.4}$$

where

$E(U_1|U_2 = u_2)$ is the regression function of U_1 on U_2 ,

and $\text{Var}(U_1|U_2 = u_2)$ is the conditional covariance matrix.

Estimation of Regression Parameters

We write our regression model as

$$\Omega: E(Y_1, Y_2, Y_3, Y_4) = E(U_1) = 1\mu_1^1 + GM \quad \dots (1)$$

where

$$G = [U_2 - 1\mu_2^1] \quad \text{and} \quad M = (\beta_1, \beta_2, \beta_3, \beta_4)$$

with

$$\beta_j^1 = [\beta_{j.5}, \beta_{j.6}, \beta_{j.7}, \beta_{j.8}] \quad \text{for } j=1, 2, 3, 4 \quad \dots (2)$$

The subscript j is associated with a particular liver variable. It can be established that the least – squares estimates of the regression co-efficient are exactly the same as those obtained by considering the separate regression of each component of the dependent variable on the regressors, and this fact enables a conceptual understanding to be based on univariate experience.

The covariance structure is specified by

$$\text{Cov}(Y_j, Y_k) = \sigma_{jk.4} I_n \dots\dots\dots(3)$$

j, k=1,2,3,4.

where j, k refer to a particular liver variable j and the corresponding intestine variable k and where, we note,

$\sigma_{jk.4}$ is the (j, k)th element of $\Sigma_{4.4}$

Suppose estimates for mean vector μ and variance-covariance matrix Σ are given by

$$\hat{\mu}' = (\mu_1, \mu_2) \quad \text{and} \quad \hat{\Sigma} = S = \begin{pmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{pmatrix}$$

on n-1 degrees of freedom

The matrix of sums of squares and product derivations is simply (n-1)S with the jk^{th} of the 64 elements denoted by $S_{jk.4}$.

The least squares estimates of the parameters are given by the normal equations [9]

$$\hat{\mu}_1 = \bar{u}_1 \dots (4)$$

$$(U_2 - 1\bar{u}'_2)' (U_2 - 1\bar{u}'_2) \hat{M} = (U_2 - 1\bar{u}'_2)' (U_1 - 1\bar{u}'_1) \dots\dots\dots (5)$$

or

$$\hat{\mu}_1 = \bar{u}_1 \dots (6)$$

$$S_{22} \hat{M} = S_{21} \dots (7)$$

where

$$S_{22} = (U_2 - 1\bar{u}'_2)' (U_2 - 1\bar{u}'_2) / (n - 1)$$

and

$$S_{21} = (U_2 - 1\bar{u}'_2)' (U_1 - 1\bar{u}'_1) / (n - 1)$$

We note as follows:

- (i) The parameters to be estimated for model (1) are μ_1 and M.
- (ii) The least squares estimate for μ_1 is given as

$$\hat{\mu}_1 = \bar{u}_1$$

This is obtainable from $n - 4$ –dimensional random variables.

\bar{U}_{1r} , $r = 1, 2, \dots, n$ where observations appear in the data matrix U_1 . In fact

$$\hat{\mu}_1 = \left[\sum_{r=1}^n \frac{Y_{1r}}{n}, \sum_{r=1}^n \frac{Y_{2r}}{n}, \sum_{r=1}^n \frac{Y_{3r}}{n}, \sum_{r=1}^n \frac{Y_{4r}}{n} \right]'$$

{iii} The least squares estimate for M is given from a transposition of (5) as

$$\begin{aligned} \hat{M}' &= U_1'(U_2 - 1\bar{u}_2')S_{22}^{-1} / (n - 1) \\ &= U_1' L \dots \dots \dots (8) \end{aligned}$$

where

$$\begin{aligned} L &= (U_2 - 1\bar{u}_2')S_{22}^{-1} / (n - 1) \\ &= (L_5, L_6, L_7, L_8) \end{aligned}$$

{iv} The total sum of squares is

$$(U_1 - 1\bar{u}_1')'(U_1 - 1\bar{u}_1') = (n - 1)S_{11}$$

(v) The vector of fitted values $\hat{E}(U_1|U_2)$ is given by

$$\hat{E}(U_1|U_2) = \bar{u}_1 + (U_2 - 1\bar{u}_2')\hat{M}$$

and the uncorrected sum of squares $SS(\Omega)$ of fitted values is

$$\begin{aligned} &(U_1 - 1\bar{u}_1')'(U_1 - 1\bar{u}_1') + (U_1 - 1\bar{u}_1')(U_2 - 1\bar{u}_2')\hat{M}'\hat{M} \\ &= (U_1 - 1\bar{u}_1')'(U_1 - 1\bar{u}_1') + (n-1)\hat{M}'S_{22}\hat{M} \\ &= (U_1 - 1\bar{u}_1')'(U_1 - 1\bar{u}_1') + (n-1)\hat{M}'S_{21} \\ &= (U_1 - 1\bar{u}_1')'(U_1 - 1\bar{u}_1') + (n-1)S_{12}S_{22}^{-1}S_{21} \\ &= (n-1)S_{11} + (n-1)S_{12}S_{22}^{-1}S_{21} \end{aligned}$$

{vi} The residual SSPM matrix (sum of squares and product matrix) $R_{4,4}$ is then given by

$$R_{4,4} = (n - 1) (S_{11} - S_{12} S_{22}^{-1} S_{21})$$

This is a 4x4 matrix on n - 5 d.f. It provides the estimate $\hat{\Sigma}_{4,4}$ of Σ given by

$$R_{4,4} / (n - 5)$$

{vii} The corresponding regression SSPM (Regression) matrix is

$$\begin{aligned} & (n - 1) S_{11} - (n - 1) S_{4,4} \\ & = (n - 1) S_{12} S_{22}^{-1} S_{21} \end{aligned}$$

{viii} It is easy to deduce from the normal equation (5) and equation (3) that the variance structure can be estimated as

$$\text{Cov} \left(\hat{\beta}_j, \hat{\beta}_k \right) = \sigma_{jk,4} S_{22}^{-1} / (n - 1)$$

{ix} The multivariate analysis of variance (MANOVA) table follows from the analogous one for the univariate and it is given as Table 1.

(x) The likelihood – ratio statistic for testing the independence of U_1 and U_2 is given by the wilks Λ - test (Testing $H_0: M = 0$ or equivalently $\Sigma_{12} = 0$ against $H_1: M \neq 0$ or $\Sigma_{12} \neq 0$) with

$$\Lambda_{4,4,4,n-5} = \frac{|S|}{|S_{11}||S_{22}|} = \frac{|(n - 1)S|}{|(n - 1)S_{11}|| (n - 1)S_{22}|} \dots\dots\dots (9)$$

Numerical Computations

Ordering the variables according to our eight – dimensional measurement given by

$$\bar{U}' = \{U_1', U_2'\} = \{\text{liver; intestine}\}$$

After the necessary additional calculations we have:

$$\bar{U}' = \{5.230, .478, -.751, -.343; 5.521, .328, -1.149, -.357\}$$

We now consider the regression of U_1 on U_2 .

The test for significance of regression is given by (4.9) which is

$$\Lambda_{4.4, 4, 51} = \frac{1.8536 \times 10^{10}}{(1.333 \times 10^6)(3.005 \times 10^5)} = 0.054$$

We shall use the distribution approximations to get

$$-(51 - 4 - 4 + 1) \ln \Lambda = \frac{149.910}{2}$$

$$\chi^2(16) = 32.00 \quad (\alpha = .01)$$

or alternatively

$$\frac{1 - \Lambda^{1/3} \cdot 295}{\Lambda^{1/3} \cdot 32} = 160.155$$

$$F(6, 147.5) = 2.04 \quad (\alpha = .01)$$

Both approximations indicate significance at 1% level, so providing strong evidence to reject the null hypothesis that $M=0$

We continue in the estimation

$$\hat{M} = S_{22}^{-1} S_{21} = \begin{pmatrix} .7549 & .0094 & .0179 & -.0002 \\ -24.8144 & -.7468 & -1.1291 & .1970 \\ 9.9034 & .4805 & 1.0189 & -.0648 \\ 64.8928 & 9.4629 & 25.2678 & .0801 \end{pmatrix}$$

Then the fitted regressions are

$$\hat{y}_{1r} - 5.22963202 = .7549(y_{5r} - 5.5213023) - 24.8144(y_{6r} - .32763969) + 9.9034(y_{7r} + 1.14854129) + 64.8928(y_{8r} + .35735107)$$

$$\hat{y}_{2r} - .47830876 = .0094(y_{5r} - 5.5213023) - .7468(y_{6r} - .32763969) + .4805(y_{7r} + 1.14854129) + 9.4629(y_{8r} + .35735107)$$

$$\hat{y}_{3r} + .75080681 = .0179(y_{5r} - 5.5213023) - 1.1291(y_{6r} - .32763969) + 1.0189(y_{7r} + 1.14854129) + 25.2678(y_{8r} + .35735107)$$

$$\hat{y}_{4r} + .34265681 = -.0062(y_{5r} - 5.5213023) + .1970(y_{6r} - .32763969) - .0648(y_{7r} + 1.14854129) + .0801(y_{8r} + .35735107).$$

The Residual matrix $R_{4.4}$ is

$$R_{4.4} = \begin{pmatrix} 1244.8 & 29.5 & 50.3 & 4.7 \\ 29.5 & 10.4 & 22.1 & 2.7 \\ 50.3 & 22.1 & 48.4 & 5.4 \\ 4.7 & 2.7 & 5.4 & 5.6 \end{pmatrix}$$

on 51 degrees of freedom

Hence

$$\hat{\Sigma}_{4.4} = \frac{R_{4.4}}{51} = \begin{pmatrix} 24.408 & .578 & .986 & .092 \\ .578 & .204 & .433 & .053 \\ .986 & .433 & .949 & .106 \\ .092 & .053 & .106 & .110 \end{pmatrix}$$

estimates $\text{Var}(U_1/U_2 = u_2)$, the conditional covariance matrix.

We compare $\hat{\Sigma}_{4.4}$ with

$$\Sigma_{11} = \begin{pmatrix} 62.827 & .028 & -.124 & .167 \\ .028 & .284 & .566 & .090 \\ -.124 & .566 & 1.205 & .170 \\ .167 & .090 & .170 & .124 \end{pmatrix}$$

which estimates $\text{Var}(U_1)$, the unconditional covariance matrix.

We mention in passing that for the regression of U_2 (intestine) on U_1 (liver), it is only required to use the inverse transform on the fitted regression equation as follows:

$$\begin{pmatrix} y_5^1 \\ y_6^1 \\ y_7^1 \\ y_8^1 \end{pmatrix} = M^{-1} \begin{pmatrix} y_1^1 \\ y_2^1 \\ y_3^1 \\ y_4^1 \end{pmatrix}$$

Then we have

$$\begin{pmatrix} y_5^1 \\ y_6^1 \\ y_7^1 \\ y_8^1 \end{pmatrix} = \begin{pmatrix} 1.537 & .111 & .320 & -.009 \\ -20.50 & -32.435 & -95.554 & 2.418 \\ 3.301 & 11.975 & 35.341 & -.853 \\ 134.862 & -35.318 & -119.340 & 3.139 \end{pmatrix} \begin{pmatrix} y_1^1 \\ y_2^1 \\ y_3^1 \\ y_4^1 \end{pmatrix}$$

The fitted regressions are

$$\hat{y}_{5r} - .5213023 = 1.537 (y_{1r} - 5.22963202) + .111 (y_{2r} - .47830876) + .320 (y_{3r} + .7508061) - .009 (y_{4r} + .34265681).$$

$$\hat{y}_{6r} - .32763969 = -20.500(y_{1r} - 5.22963202) - 32.435(y_{2r} - .47830876) - 95.554 (y_{3r} + .7508061) + 2.4183(y_{4r} + .34265681)$$

$$\hat{y}_{7r} + 1.14854129 = 3.301(y_{1r} - 5.22963202) + 11.975(y_{2r} - .47830876) + 35.341(y_{3r} + .7508061) - .853(y_{4r} + .34265681).$$

$$\hat{y}_{8r} + .35735107 = 134.862(y_{3r} - 5.22963202) - 35.318(y_{2r} - .47830876) - 119.34(y_{3r} + .7508061) + 3.139(y_{4r} + .34265681).$$

Conclusion

It is clear that the reductions in variances and covariances are considerable. In the particular case of the first entry, corresponding to the effect of the extracts and compounds on the liver, the reduction in variance is as much as 61.15%.

This reveals a marked improvement in the precision of the estimates for the effects of the extracts and compounds, with the aid of likelihood-ratio statistic, on intestine when the liver is taken into consideration.

Furthermore, the regression prediction is that none of the pair of four variables in use has negative covariance. This is an indication that it is meaningful to have used the weight as a factor to be considered. In view of the reciprocal manner, the weight has been brought in the effect per unit weight appears to be inversely related to the size of the organs. A smaller sized organ is more likely to be cut off in terms of blood cells than a bigger sized organ.

Table 1: MANOVA Table for Multivariate Regression

Source	D.f.	SSPM	E(SSPM)
Regression	4	$(n - 1)S_{12}S_{22}^{-1}S_{21}$	$4\sum_{4,4} + (n - 1)M' S_{22} M$
Residual	$n - 5$	$R_{4,4}$	$(n - 5) \sum_{4,4}$
Total	$n - 1$	$(n - 1)S_{11}$	

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