POTENCY RATIO ESTIMATION IN THE PRESENCE OF A COVARIABLE

J. Hermans ¹⁾, H. Mattie ²⁾ and B.A.C. Dijkmans ²⁾

Summary

A case study of a parallel-line potency ratio estimation in the presence of a covariable is presented. The estimation is performed using a multiple regression analysis, in which a dummy variable presents the two drugs involved.

1. Theoretical considerations

The estimation of a potency ratio in a parallel-line assay is a well established technique in medical research. The statistical evaluation of such an experiment can be found in several textbooks, e.g. Finney (1978), while special computer programs are available, like Finney (1976). The present paper reports a case study of a potency ratio estimation in the presence of a covariable. The analysis will be formulated in terms of a multiple regression with a dummy variable, which formulation has the advantage of referring to generally available multiple regression computer programs.

- Department of Medical Statistics Leiden University Wassenaarseweg 80 2333 AL Leiden, Netherlands Telephone (071) 148333, ext. 5148/5009
- Department of Infectious Diseases University Hospital, Leiden, Netherlands

The application concerned the potency ratio of two antimicrobial drugs active against Bacteroides fragilis in an experimental infection. This ratio was derived from the regression of the log number of outgrown Bacteroides fragilis (y) versus the log dosis of the drugs (x). A substantial part of the variability of this regression was due to the presence of a covariable, the log number of outgrown E. coli (z). Co-inoculation with this bacteria was necessary to establish an experimental B. fragilis infection in mice. The case study concerned three dosage levels for each of the two drugs, with (maximal) six mice at each level (Dijkmans et al.; 1984). Attention will be stressed to potency ratio estimation in the presence of a covariable. However, to illustrate clearly the principle we will first describe the situation without a covariable.

1.1. Potency ratio estimation without a covariable

Let y = response variable, i.e. the log number B. fragilis, and $x = log_{10}$ dosis. The dummy variable u is defined by

 $u = < \begin{bmatrix} 0 \\ 1 \end{bmatrix}$ when the first drug is applied when the other drug is applied

To be able to investigate an interaction between dosis and drugs, the product u.x is included in the multiple regression equation

$$y = b_0 + b_1 x + b_2 u + b_3 (u.x)$$
(1)

Equation (1) corresponds for the two drugs in the y-x-plane to the following two lines:

for u = 0: $y = b_0 + b_1 x$ (1a)

and for
$$u = 1$$
: $y = (b_0 + b_2) + (b_1 + b_3)x$ (1b)

Presence of an interaction between dosis and drugs, so $b_3 \neq 0$ in eq. (1), corresponds to different slopes for the two drugs, see Fig. I. The potency ratio of two drugs is on the log dosis scale equivalent to the difference of the two log doses (say m) necessary to obtain the same response y, see Fig. II. As will be clear from Fig. I and II, the concept of a potency



Figure I. Dosis-response lines for the two drugs (u = 0,1) in the presence of a dosis x drug interaction.



Figure II. Parallel dosisresponse lines for the two drugs (u = 0,1).

ratio is only meaningful (i.e. independent of the response) in case the two lines have the same slope, or equivalently, the interaction is zero $(b_3=0)$. Algebraically this follows simply from eq.s (1a) and (1b):

$$m = x_{u=0} - x_{u=1} = \frac{y - b_0}{b_1} - \frac{y - (b_0 + b_2)}{b_1 + b_3}$$
(2)

If and only if $b_3=0$ the right hand side of eq. (2) becomes independent from y:

$$m = x_{u=0} - x_{u=1} = \frac{y - b_0}{b_1} - \frac{y - (b_0 + b_2)}{b_1} = \frac{b_2}{b_1}$$
(3)

So the following procedure can be applied:

(a) fit the multiple regression equation (1); (b) test for $b_3=0$ (the absence of interaction or, equivalently, parallel lines); (c) if $b_3=0$ is not rejected, fit the reduced model

$$y = b_0 + b_1 x + b_2 u$$
 (4)

and finally (d) calculate the quotient of the regression coefficients $m = \frac{b_2}{b_2}$ and then the potency ratio can be estimated as 10^m .

Approximate lower and upper confidence limits $(m_L \text{ and } m_U)$ are obtained from

$$m_{L}, m_{U} = \frac{b_{2}}{b_{1}} \pm \frac{t}{b_{1}} \left\{ var(b_{2}) - 2\frac{b_{2}}{b_{1}} covar(b_{1}, b_{2}) + \left(\frac{b_{2}}{b_{1}}\right)^{2} var(b_{1}) \right\}^{2}$$
(5)

where t has n-3 degrees of freedom, see Finney (1978). Exact confidence limits can be obtained using Fieller's theorem, see again Finney (1978).

1.2. Potency ratio estimation with a covariable

To account for the presence of a covariable, say z, one has to extend equation (1) to

$$y = b_0 + b_1 x + b_2 u + b_3 z + b_4 (x.z) + b_5 (u.z) + b_6 (x.u) + b_7 (x.u.z)$$
(6)

Similarly to the procedure shown in equations (2) and (3) it can be shown that the difference $m = x_{u=0} - x_{u=1}$ is independent of the response variable y if and only if $b_6 = b_7 = 0$. Model (6) reduced with $b_6 = b_7 = 0$, leads for the log potency ratio m to:

$$m = x_{u=0} - x_{u=1} = \frac{b_2 + b_5 z}{b_1 + b_4 z}$$
(7)

Under this reduced model the concept of a potency ratio is again meaningful as m is independent of the response variable. However, the ratio depends on the value z of the covariable. How strong m depends on z can (and should) be investigated by substituting some relevant z values in eq. (7). The absence of <u>all</u> interactions in eq. (6), so $b_4 = b_5 = b_6 = b_7 = 0$, leads for the difference of log doses again simply to a quotient of two regression coefficients: $m = x_{u=0} - x_{u=1} = \frac{b_2}{b_1}$

The geometrical analogue of Fig.s I and II is as follows. The model without any interaction, which was assumed for eq. (8) corresponds in the (x,y,z) space to two parallel planes, one for each drug. The model underlying eq. (7) corresponds to two quadratic surfaces, whose intersections with a plane z = constant are two parallel lines like in fig. II, but with m dependent on the value of z. Eq. (6) corresponds to two quadratic surfaces, whose intersections with a plane z = constant are two parallel lines like in fig. II, but with m dependent on the value of z. Eq. (6) corresponds to two quadratic surfaces, whose intersections with a plane z = constant lead to two lines like in Fig. I, so no meaningful potency ratio can be defined.

If eq. (8) is justified approximate confidence limits can be calculated using eq. (5). Exact confidence limits can again be obtained from Fieller's theorem, which theorem has to be applied in case eq. (7) holds.

Validity testing focuses usually on testing for linearity. Some testing can be easily performed using the multiple regression technique. One might e.g. investigate whether a quadratic regression on log dosis fits better than a linear one after extending eq.s (1) or (6) with quadratic terms. Nonpolynomial deviations from linearity cannot be easily investigated with multiple regression techniques, but are unlikely to occur in bio assays.It should be stressed that when deviations from linearity occur, be they polynomial of non-polynomial, then the concept of potency ratio is no more meaningful.

2. An example

Mice were inoculated with an inoculum composed of B. fragilis and E. coli. Six hours later the drug was subcutaneously administered to each mouse in the neck as a single dose. Three dosage levels (D = 2.5, 5 or 10 mg/kg body weight; $x = \log_{10}D$) were used for each of the two drugs (u = 0,1), with maximal six mice at each level. For each mouse measurements on the log number of outgrown B. fragilis (y) and E. coli (z) became available. The regression coefficients together with the multiple R^2 and the standard deviation around regression for several models are summarized in table I. For the two models without covariable no dosis x drug interaction is present, see $R^2 = 0.57$ compared to 0.54. So eq. 4 fits as well as eq. (1); m = 0.15/-2.70 = -0.056 and the potency ratio becomes $10^{\rm m} = 10^{-0.056} = 0.88$. Application of eq. (5) to calculate approximate confidence limits form leads to m = -0.056 ± 0.199 . Expressed as potency ratio we obtain the interval 0.56 - 1.39.

spitter - States	without covariable		with covariable			
factor	with interaction	without interaction	all interactions	no dosis x drug interaction	no nteraction	
constant	7.89	8.27	-3.14	-1.86	3.89	
dosis (x)	-2.15	-2.70	7.39	5.28	-2.39	
drug (u)	0.92	0.15	2.89	1.07	0.26	
E.coli (z)			1.33	1.20	0.50	
dosis x E.coli			-1.15	-0.92		
drug x E.coli	1	a se-applied	-0.29	-0.12	(W)	
dosis x drug	-1.10	and	-2.31	1. 10 - 1	gaip ne	
dosis x drug x E.coli			0.22			
R ²	0.57	0.54	0.77	0.77	0.74	
s.d.	0.65	0.65	0.53	0.50	0.51	

Table I Regression coefficients together with multiple R² and standard deviation around regression for several regression models

However, taking into account the covariable, the model leads to a substantially better fit, see the corresponding R^2 values, and the lower standard deviation around regression. The dosis x drug interactions can be safely assumed to be 0 (equal R^2 values, so the corresponding F-statistics = 0), and the concept of potency ratio makes sense. Important practical implications result from the decision whether the interactions with the covariable can be assumed to be 0. If not, then the potency ratio depends on the level of the covariable, see eq. (7); else the potency ratio is independent of the covariable, see eq. (8).

Should one adopt the model with covariable, but without any interactions, we obtain m = 0.26/-2.39 = -0.109 and an estimated potency ratio of $10^{-0.109} = 0.78$. Application of equation (5) leads to m = -0.109 ± 0.180 while the confidence interval for the potency ratio becomes 0.51 - 1.18.

Preference for a model might be based on the R^2 value. A more appropriate measure in the present context might be the length of the confidence interval for the potency ratio. Without the covariable the length is 0.83; with the covariable the length is 0.67.

Should one adopt the model with interactions with the covariable, according to equation (7) the log potency ratio becomes:

$$m = \frac{1.07 - 0.12z}{5.28 - 0.92z}$$

The dependency of the potency ratio on the log number of outgrown E, coli (z) is for the observed range of z values as follows:

z	6.5	7	7.5	8	8.5	9
m	-0.452	-0.226	-0.127	-0.072	-0.036	-0.012
10 ^m	0.35	0.59	0.75	0.85	0.92	0.97

The two regression models with a covariable, one without dosis x drug interaction and the other with no interactions at all, can be compared with an F-test, which can be derived form the R^2 values and the corresponding degrees of freedom:

$$F = \frac{(0.77 - 0.74)/2}{(1 - 0.77)/17} = 1.11$$

and p > 0.10. So the regression model with covariable and no interaction terms can be accepted.

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