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CORRECT WAYS OF USING REGRESSION FOR METHOD COMPARISON STUDIES - DETERMINATION OF LDL-CHOLESTEROL[1]

Maša ISLAMČEVIČ[a], Jan MOCAK[b2], Darinka BRODNJAK-VONČINA[a] and Pavol BLAŽÍČEK[c]
[a]Faculty of Chemistry and Chemical Engineering, University of Maribor, SI–2000 Maribor, [b]Department of Analytical Chemistry, Slovak University of Technology, SK–81237 Bratislava, [c]Hospital of the Ministry of Defence, SK–83331 Bratislava

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Statistical comparison of two laboratory methods, measuring the same objects, is usually performed by regression. However, the results achieved are often incorrect due to application of ordinary least squares linear regression, which should not be used for this purpose. A statistically correct decision whether two laboratory methods provide concordant or discordant results is reliable only when using regression techniques which respect the errors of both compared variables. Another correct possibility is to apply a robust regression where the errors of compared variables are not influencing the calculation of regression parameters

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[2] To whom correspondence should be addressed.

and their standard deviations. A survey of appropriate regression techniques and their use for comparison of real-life clinical methods is given in this paper.

Introduction

Correct statistical comparison of two quantitative laboratory methods is a frequent task performed in analytical laboratories and represents also a necessary step in validation of a new analytical method. This task is usually fulfilled by means of *linear regression*, in which a series of results obtained by the *investigated* and the *reference* methods, measuring the same objects, is used. Then the measurement results made by the reference method are usually plotted on the horizontal axis (X) and the results of the investigated method are plotted on the vertical axis (Y). In the next step, the *slope* of this regression dependence is statistically compared with respect to the theoretical value 1.0 (assumed by the null hypothesis) by the t-test, and a similar t- test is applied to find whether the *intercept* is or is not significantly different from 0.0 (assumed by the null hypothesis). A *proportional systematic error* is indicated when a significant difference between the observed and theoretical slopes is found. Similarly, a *constant systematic error* is indicated when a significant difference is proved between the observed and theoretical intercepts. In case of discordant results it is not possible to state just on the basis of the performed tests which of the compared two methods is incorrect. However, a possible and useful statistical output is to suggest in which way the results of two compared methods can be harmonized.

The main goal of this work is to evaluate agreement/disagreement of results obtained by two automatic analyzers, HITACHI 911 and KONELAB 20, used for analytical determination of LDL-Cholesterol. Both of these analyzers are frequently used in clinical biochemical laboratories for the mentioned purpose; they utilize enzyme analysis combined with a final spectrophotometric determination but differ in details of the applied analytical procedure. In our study, the calculation results obtained by several advanced regression techniques and several applicable software packages are compared and discussed.

Theory

Ordinary Least Squares Regression and Advanced Linear Regression Methods

For method comparison studies, the use of ordinary linear least squares regression (*OLS*) is not justified since the obligatory *OLS* assumption on the error-free independent variable is violated in this case. Consequently, the *OLS* application may cause a considerable bias in the calculated regression parameters. This is the

main reason why more sophisticated, advanced regression techniques have to be applied. The following advanced regression techniques are used in practice: Deming method (*DM*), orthogonal regression (*OR*), bivariate least squares (*BLS*) and Passing-Bablok (*PB*) rank regression method.

In the case of *DM* and *BLS*, the ratio of the variances of both variables (i.e. compared laboratory methods) must be known. In both cases, this ratio (denoted commonly as λ) is needed in calculation of regression parameters and their standard deviations. *OR* is a special sub-case of *DM* when the variance ratio $\lambda = 1$ and the effect of random errors of both variables is equal.

PB regression is based on a robust statistical approach, independent of any assumptions on the error distribution. Due to its statistically robust algorithm, the change of the variance within the considered concentration range (hetero-scedasticity) is allowed without a need to use statistical weights. Moreover, *PB* regression is well applicable also when outliers are present in the data set since it is not sensitive to their occurrence.

Deming Regression Method

DM is based on a *structural model*, in which the *observed* (measured) variables X_i and Y_i are composed of latent *expected* ("true") values x_i and y_i and corresponding random errors u_i and e_i, respectively. The errors u_i and e_i are supposed to be independent and normally distributed with a zero mean value and a constant standard deviation [1]. Then the dependences between observed and latent variables are given by equations

$$X_i = x_i + u_i \tag{1}$$

$$Y_i = y_i + e_i \tag{2}$$

The linear regression model is expressed by two equivalent relationships

$$y = \beta_0 + \beta_1 x = \bar{y} + \beta_1 (x - \bar{x}) \tag{3}$$

where $\bar{y}$ and $\bar{x}$ express the means of all coordinates y_i and x_i, respectively, and the regression parameter b_0 is given by relationship

$$\beta_0 = \bar{y} - \bar{x}\beta_1 \tag{4}$$

The estimates b_0 and b_1 of the regression parameters β_0 and β_1 are calculated by means of the variance ratio λ or its reciprocal value δ, defined by equations

$$\lambda = \frac{V(u)}{V(e)} = \frac{s_u^2}{s_e^2} \tag{5}$$

$$\delta = \frac{V(e)}{V(u)} = \frac{s_e^2}{s_u^2} \tag{6}$$

The case when $\lambda = \delta = 1$ and $V(e) = V(u)$ is of a special importance and is called *orthogonal regression*. The least squares minimization is made here in the direction perpendicular to the regression line.

The limiting case, when $\lambda = 0$ and $V(u) = 0$, is identical to the *OLS* method, which is a special case of Deming regression. Another limiting case happens when $\delta = 0$ and $V(e) = 0$, in this case the *OLS* method is valid for the inverse $X = f(Y)$ dependence.

If an auxiliary variable L is defined as $L = (S_{YY} - \delta S_{XX})/(2S_{XY})$, where S_{YY} and S_{XX} are the respective variances of variables Y and X, and S_{XY} is the corresponding covariance, then the calculation of b_0 and b_1 is simplified

$$b_1 = L + (L^2 + \delta)^{1/2} \tag{7}$$

$$b_0 = \bar{Y} - b_1\bar{X} \tag{8}$$

More complicated and in literature hardly accessible is *calculation* of standard deviations of regression parameters, s_{b1} and s_{b0}. Nevertheless, assuming (a) normally distributed measurement errors of both variables, and (b) homoscedasticity conditions (constant variances), Fuller [1] derived unambiguous estimates of s_{b1} and s_{b0}, which, as we have proved [2], is consistent with the results obtained by *Analyse-it* software [3]. The mentioned conditions need not be met when a non-parametric alternative of the standard deviations estimates is made

by the *jackknife* method, which is described in detail in Ref. [4]. *Method Validator* [5] as well as *CBstat* [6], both available on the web, use only this calculation approach.

Bivariate Least-Squares Technique

Bivariate Least-Squares Regression (*BLS*) is the generic name for a set of techniques used for regressing *bivariate* data, i.e. whenever a regression method is applied to data containing errors in both axes [7]. *BLS*, derived in Refs [7,8] and based also on the approach described in Ref. [9], performs *minimization* of the sum, S, of *weighted residuals*, $w_{Ri} R_i$, defined as

$$S = \sum_{i=1}^{n} w_{Ri} R_i^2 \qquad i = 1, 2, \ldots, n \tag{9}$$

$$R_i = \left[Y_i - f(X_i, b_j) \right] \qquad j = 1, 2, \ldots, m \tag{10}$$

$$w_{Ri} = \frac{1}{s_{Ri}^2} = \frac{1}{s_{Yi}^2 + s_{Xi}^2 - 2 b_1 cov(X_i, Y_i)} \tag{11}$$

The residuals were originally expressed for m regression parameters [9] but the weights w_{Ri} have to be expressed for $m = 2$ in case of method comparison. The minimisation leads to nonlinear equations therefore the algorithm is based on iterative calculations [7–10]. *BLS* is effectively used in software package *Calibro 2000* [10,11] and its MATLAB code is available in Ref. [8]. Despite the *BLS* definition in Ref. [7], by which Deming method can be also considered a *BLS* technique, only the variants published in Refs [7–10] are designed in the way described here. *BLS* can treat even a heteroscedastic case (with non-constant variance) so that different weights can be put not only for each regression variable but also for every individual point i in *Calibro 2000*, if desired. On the other hand, the determination of appropriate weights in many practical situations is difficult and even obtaining the variances ratio for the compared variables is often cumbersome. In such situations, *OR* is applicable, which means that equal weights are adopted in Deming regression, $\lambda = \delta = 1$.

Passing–Bablok Rank Regression

In *Passing–Bablok* regression (*P–B*), slopes of straight lines between any two points of the set of n regression points are calculated. The number of possible slopes is $N \leq n(n-1)/2$, since from the total number of possible slopes $n(n-1)/2$ those with $\pm\infty$ and 0 values are subtracted. All N slopes are calculated by equation

$$S_{ij} = \frac{y_i - y_j}{x_i - x_j} \qquad \text{for } 1 \leq i < j \leq N \tag{12}$$

and sorted in increasing order. The *P–B* slope b_1 is then calculated as *shifted median* in the following two ways

$$b_1 = S_{(N+1)/2+K} \qquad \text{for } N \text{ odd} \tag{13}$$

$$b_1 = \exp\frac{\log S_{N/2+K} + \log S_{N/2+1+K}}{2} \tag{14}$$

for N even (geometric mean)

where K (representing the shift) is the number of S_{ij} values less than -1. The *intercept* b_0 is successively obtained by calculating the median of all $(y_i - b_1 x_i)$ values. The confidence intervals for the slope and the intercept are derived by a nonparametric calculation procedure as the appropriate indices indicating the upper and lower confidence limits in the set of sorted values, as described in Ref. [12]. The standard deviations of regression coefficients are not obtained.

Passing–Bablok regression is applicable without the need to use any variance estimates (no λ or δ is needed). Its original version tests for the agreement between two laboratory methods [12]; therefore, this variant is called *PB agreement*. It works well even if the errors of variables are not normally distributed and a non-constant variance over the data range does not influence the results. However, *Linnet* [13] claims that the *PB* results are biased. Despite it, *PB* regression is frequently used mainly in biochemical and clinical chemistry literature. An extremely useful feature of *PB* regression is its robustness with respect to the outliers.

Experimental

Due to high costs of direct LDL-cholesterol (*LDL*) quantitative analysis, *LDL* used to be determined in clinical laboratories indirectly, which is a considerably cheaper way. The corresponding *LDL* values were therefore obtained from the results of analyses of total cholesterol (*Chol*), HDL-cholesterol (*HDL*) and triacylglycerols (*Tg*) in the blood of 300 patients according to Friedewald formula

$$LDLcalc = Chol - HDL - \frac{Tg}{2.2} \quad (\text{mmol l}^{-1}) \tag{15}$$

All the data were measured by means of automatic analyzers Konelab 20 and Hitachi 911. In this way, two series of the calculated values resulted: *LDLcalc_K* for Konelab and *LDLcalc_H* for Hitachi. The determinations of *Chol* and *Tg* (*Roche*) and *HDL* (*Genzyme*) in blood serum were based on enzymatic assays with a spectrophotometric final measurement.

Results and Discussion

Regression Results for Different Regression Techniques and Software

We performed an extensive comparison of the results of indirect LDL determination received by advanced regression techniques as well as by *OLS* reg-

Table I Regression parameters, their standard deviations and 95 % confidence intervals for the regression dependence $LDLcalc\_H = b_0 + b_1 LDLcalc\_K$ (model: $y = b_0 + b_1 x$) [a)]

Way of calculation	b_1	b_0	s_{b1}	s_{b0}	b_{1L}	b_{1U}	b_{0L}	b_{0U}
OLS (Analyse-it)	0.9814	0.1203	0.0090	0.0315	*0.9636*	*0.9992*	*0.0583*	*0.1824*
Deming (Analyse-it)	0.9937	0.0794	0.0092	0.0319	0.9756	1.0117	*0.0165*	*0.1422*
Deming (Meth. Valid.)	0.994	0.0794	-	-	0.973	1.015	*0.0097*	*0.1492*
Deming (CBstat)	0.9937	0.0794	0.0106	0.0354	0.9729	1.0144	*0.0099*	*0.1489*
Deming (Quick Basic)	0.9937	0.0794	0.00916	0.03193	0.9757	1.0117	*0.0165*	*0.1422*
BLS (Calibro 2000)	0.9937	0.0794	0.00907	0.03165	0.9758	1.0115	*0.0171*	*0.1417*
P-B (Analyse-it)	0.993	0.0927	-	-	0.973	1.012	*0.0360*	*0.166*
P-B (Meth. Validator)	0.993	0.0927	-	-	0.973	1.012	*0.0366*	*0.1662*
P-B (CBstat)	0.9931	0.0925	-	-	0.973	1.0123	*0.0365*	*0.1661*
P-B (MedCalc)	0.9931	0.0927	-	-	0.973	1.0122	*0.0366*	*0.1662*

[a] The symbols b_{1L} and b_{1U} denote the limits of the confidence interval for the slope, b_{0L} and b_{0U} are used to confine the intercept confidence limit. Variances ratio $\lambda = 1$ was used for *Deming* and *BLS* methods. The number of tabulated decimal digits is given by respective software. *Italics* indicate that the slope or intercept is different from the theoretical value, which is valid for concordant results of the compared laboratory methods

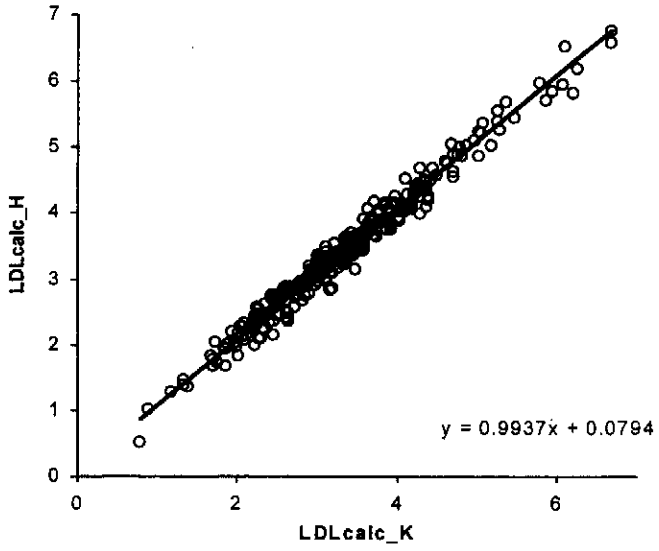

Fig. 1 Comparison of two ways of indirect determination of LDL-cholesterol by Deming regression using the variance ratio $\lambda = 1$. H – Hitachi analyzer, K – Konelab analyzer. Software Analyse-it

ression. For this purpose commercially available software and our own programs in Quick Basic (composed according to the above theoretical relationships) were used. Since the measurements performed for indirect determination of LDL-cholesterol were made in the hospital without replications, the ratio of the variances corresponding to the Hitachi and Konelab analyzers was not known and Deming regression was therefore made with the variance ratio $\lambda = 1$, recommended in such a case [14]. In fact, this is the case of orthogonal regression but practically in all software packages it has to be used under Deming regression.

The results of performed calculations are summarized in Table I. Close inspection of this table reveals a constant systematic error in the regression slope confirmed by all applied regression techniques. However, only the *OLS* regression indicated also a systematic error in the intercept (its confidence interval does not contain zero). It is also evident that the confidence intervals in Deming regression found by *Method Validator* and *CBstat* packages differ from *Analyse-it*, our Quick Basic program performing Deming regression as well as *BLS*. It is caused by different ways of calculation; *jackknife* robust statistics is used in the first two mentioned packages while *Analyse-it* and our program utilize a less-known classical statistical approach. A good agreement between the values of regression parameters found by Deming regression and *BLS* on one side and Passing–Bablok regression on the other side is a proof that outliers do not influence the results

achieved by the former group of methods. The same conclusion follows from the calculated regression residuals in Deming regression accessible in *Analyse-it*. All results received by our program and *Analyse-it* software were in a full agreement.

In addition to performed calculations we have also examined graphical possibilities of all mentioned software packages. Exhibits of two graphs chosen from a large collection of prepared figures are shown in Figs 1 and 2.

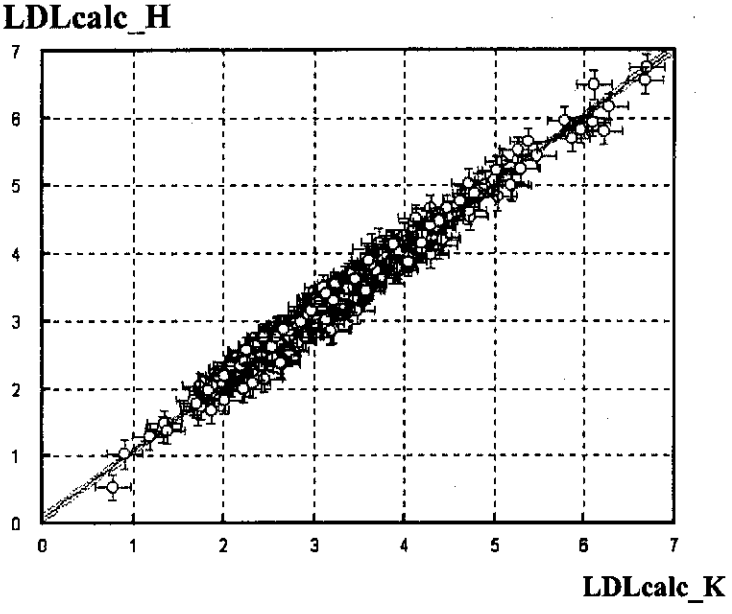

Fig. 2 Comparison of two ways of indirect determination of LDL-cholesterol by bivariate least squares regression using equal standard deviations of the compared methods, $s_H = s_K$. H – Hitachi analyzer, K – Konelab analyzer. Software *Calibro 2000*

Harmonization of Results

An important question is what kinds of correction can be made when a systematic error is indicated in the performed method comparison study. Such a situation is very common in clinical laboratories where often only one automatic analyzer is used if a lower number of samples is analysed but another analyzer is employed when the number of samples increases. In such a case it is necessary to *harmonize* the results achieved by a correction of the values obtained by one analyzer with respect to the other one. Such harmonization is made according to the calculated regression equation. Based on the regression coefficients found for *orthogonal* (*Deming*) regression or *BLS* regression, the LDL results received by means of the

Hitachi analyzer can be recalculated (i.e. corrected) to be valid for the Konelab analyzer by the following equation: $LDLcalc\_H = 0.0794 + 0.9937\,LDLcalc\_K$ (the regression coefficients used in this equation are shown in Table I). If the opposite recalculation is needed, then the inverse linear equation has to be applied. It can easily be obtained by rearranging the previous equation; the new intercept is $-b_0/b_1$ and the new slope is $1/b_1$.

Another possible approach for finding the mentioned correction is based on *bias plot* (*difference plot*) [15], well known in clinical chemistry, where the differences $(Y_i - X_i)$ are calculated for all pairs i, $i = 1, 2, \ldots, n$. This way of calculation is equivalent to the regression model $Y = b_0 + X$, with the fixed slope $b_1 = 1$, so that it can be used effectively when the systematic error exists in the intercept (b_0 significantly different from zero) but is not correct for the cases when the slope is significantly different from 1. Bias plot representation is optional in several statistical software packages, e.g . *Analyse-it*.

Conclusion

A statistically correct decision whether two laboratory methods measuring the same objects provide concordant results is obtained only by regression techniques which take into cnsideration the errors of both compared variables, or by those which are sufficiently robust. The overview of such techniques was given in this paper.

The application of all examined advanced regression techniques to the indirect LDL-cholesterol determination realized by two automatic analyzers, Konelab and Hitachi, exhibited consistently only a constant systematic shift while the *OLS* results were different. Under conditions when outliers are absent or eliminated, the most correct and consistent results are provided by *Deming* regression and bivariate least squares regression. Otherwise, a deeper study of residuals or Passing–Bablok regression are recommended. When the variance ratio λ is not known, the use of unity value is recommended for Deming regression, which corresponds to orthogonal regression.

The only violation of the obligatory assumptions on the least squares method described in this paper concerned the problem that both regression variables were subject to errors, which enabled us to concentrate solely a deeply on this problem. It can happen in practice, however, that other basic assumptions may also be violated. We have proved by means of software packages used that in comparison of two cholesterol analyzers only the mentioned type of violation was significant.

In this work a special attention was paid to comparison of software programs and packages commercially available for application of advanced regression techniques. With regard to comparison of laboratory methods the

performance of the following software products was evaluated: *Analyse-it*, *Method Validator*, *CBstat, MedCalc*, *Calibro 2000.*

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