Repeat Measurement of Case-Control Data: Corrections for Measurement Error in a Study of Ischaemic Stroke and Haemostatic Factors

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Bashir S A (International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France), Duffy S W and Qizilbash N. Repeat measurement of case-control data: Corrections for measurement error in a study of ischaemic stroke and haemostatic factors. International Journal of Epidemiology 1997; 26: 64–70.

Background. Haemostatic factors are suspected to be involved in the aetiology of cerebrovascular events.

Methods. In a case-control study of 105 cases of transient ischaemic attack and minor ischaemic stroke, and 241 controls, data were available on levels of the haemostatic factors—von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI), tissue plasminogen activator (TPA) and factor VII (FVII). These are subject to measurement error and within-person fluctuation of true levels, which may bias relative risk estimates. For all subjects, two determinations were performed on the same blood sample, which allowed estimation of pure measurement error. For estimation of within-person fluctuation, levels were measured from a repeat blood sample on 81 of the controls one year later.

Results. The pure measurement error accounted for a very small proportion of the total variation in all cases. Uncorrected for within-person fluctuation, the odds ratio estimates associated with exceeding the median of vWF, PAI, TPA and FVII respectively were 1.88, 0.87, 1.30 and 0.93. After correction for within-person fluctuation odds ratios were 3.56, 0.80, 1.41 and 0.91. Because the PAI determination was not robust to storage conditions, it was estimated that 75% of the variation in this factor was within-person rather than between-persons. Thus, estimates of relative risk relation to PAI cannot be regarded as reliable in this study.

Conclusions. It is likely that elevated levels of vWF are associated with increased risk of ischaemic stroke, but interpretation must be tentative, due to relatively large within-person fluctuation of vWF levels.

Keywords: case-control studies, misclassification, repeated measures, ischaemic stroke, risk estimation, odds ratio

Various haemostatic factors are considered to be potentially involved in the aetiology of stroke. In this paper we report on the measurement of certain haemostatic variables, their within-subject fluctuation and an assessment of the associated relative risk in the context of a case-control study of transient ischaemic attack (TIA) and minor ischaemic stroke. The haemostatic variables examined were von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI), tissue plasminogen activator (TPA) and factor VII (FVII).

Elevations or reductions of these haemostatic factors may result in an increased or decreased tendency to thrombosis. The von Willebrand factor is the carrier protein for factor VII in plasma which mediates adhesion between collagen and platelet glycoprotein Iib. Factor VII is a key coagulation component. Thus increases in these factors may be expected to increase the tendency to clot formation. Plasminogen activator inhibitor-1 is the major inhibitor of plasminogen activator and thus, elevated levels of PAI should be associated with depression of fibrinolysis. Tissue plasminogen activator is the natural plasminogen activator which causes physiological fibrinolysis. Thus, depressed tPA levels may be associated with an increased tendency to thrombosis and other circulatory events.

In this field, as in other areas of epidemiology, measurement error is a problem, both as a result of pure laboratory error and from random variation of the ‘true’ value within individuals. In the latter case, a person who usually has high levels of a factor, for example, may happen to have a lower level than usual when the blood sample is taken.

The present study investigates the effect of these four factors on risk of TIA and minor ischaemic stroke in the context of a case-control study, along with the implication of measurement error and temporal fluctuation of levels of these factors. Minor ischaemic stroke and TIA were used as surrogate models for major ischaemic
stroke as they are less likely to cause post-attack changes in haemostatic variables and hence invalidate retrospective inference. Major ischaemic stroke is likely to cause such changes, and may also introduce difficulty in obtaining valid pre-morbid histories due to comprehension and speech.

DATA DESCRIPTION
A summary of the design of the case-control study used in this analysis is given below. Further details about the design of this case-control study have been published previously. This study was designed to assess several biochemical variables as potential risk factors for ischaemic stroke.

Cases of TIA and minor ischaemic stroke and controls were recruited to the study in 1986 and 1987 from the Oxfordshire Community Stroke Project (OCSP) and from a neurology clinic. There were 105 cases and 294 (community) controls. In all, 241 (82.6%) controls provided adequate blood samples for analysis. The remaining 53 either were unwilling to provide a sample, or the sample contained insufficient plasma for analysis.

A second measurement of the haemostatic variables was provided by a random sample of 81 controls one year after the original measurement using identical procedures. This was not performed for cases, as many were undergoing therapy in the months after the stroke which would change their blood chemistry. It had originally been planned to have 100 such repeat measurements. The number with repeated measurements was less than 100 due to non-response and furthermore varied between the haemostatic variables due to breakages and inadequate plasma. Figure 1 shows recruitment details for controls and Figure 2 the corresponding details for cases.

Plasma samples stored at −28°C in EDTA containers for a median of 4 years (range 3–5 years) were thawed once and assayed. The vWF antigen was assayed with an enzyme linked immunosorbent assay (ELISA) (Dako patts A/S, Glastrup, Denmark); FVII antigen by ELISA (Diagnostics Stage, Asnières, France); and tPA antigen by ELISA (Biopool AB, Umeå, Sweden). All assays were performed in duplicate and blind to case-control status. The intra- and inter-assay coefficients of variation for vWF antigen were 3.8% and 4.2% respectively. The values in i.u./ml may be read as 1.00 i.u./ml of vWF antigen being equivalent to 100% of normal plasma.

STATISTICAL METHODS
For all of the haemostatic variables we have two assayed measurements taken from each of the blood samples (i.e. each blood sample is assayed twice). The 81 repeat-blood samples for controls were taken one year later and these also have two assayed measurements for each of the haemostatic variables. This enables us to
analyse the within-laboratory measurements (or within-year measurements) and the between-years measurements in an analysis of variance (ANOVA).

**Random effects ANOVA**

Random effects analysis of variance is used to estimate the proportion of the total variation that is attributable to each of the factors that have been specified in the model.

In a random effects analysis of variance we fit the model as given in equation (1).

\[ x_{ij} = \mu + A_i + B_{ij} + e_{ij} \sim N(0, \sigma^2) \]  

(1)

where \( i = 1, 2 \) represents the year, \( j = 1, 2 \) represents the measurement and \( k \) represents the subjects. In this model the \( A_i \) and \( B_{ij} \) are random variables with \( A_i \sim N(0, \sigma^2_A) \) and \( B_{ij} \sim N(0, \sigma^2_B) \). Due to this assignment of distributions, the \( A_i \)'s and \( B_{ij} \)'s are regarded as random effects.

In this model the variance component is split into \( \sigma^2 = \sigma^2_A + \sigma^2_B \), the between-years variability and \( \sigma^2_B \) the within-sample (or within-years) variability. These can be expressed as percentages of the overall variability of a single measurement.

**Coefficient of Variation**

The coefficient of variation is used to describe the variation in a population as a proportion of its mean and is defined as \( cv = \sigma/\mu \). However we use the sample estimate which is \( \hat{cv} = s/\bar{x} \). Below, the coefficient of variation will be expressed as a percentage (i.e. \( cv \times 100\% \)). Hereafter \( cv \), \( cv_A \) and \( cv_B \) will represent the between-persons, between-years and within-years coefficients of variation, respectively. Thus we describe variation between subjects, temporal fluctuation within subjects and pure measurement error.

**Risk Estimates**

For estimation of relative risks, the haemostatic variables were dichotomized at the medians of the controls (first year average). Measurements above the median were regarded as risk (confounding) factor present. The risk was assessed as the risk factor present relative to the risk factor absent. The extreme categorization by splitting at the median was used as a cutoff point because it is still unclear what role these haemostatic factors play in the aetiology of stroke, and we therefore would like to keep model assumptions as simple as possible.

Adjusted odds ratios were calculated using maximum likelihood techniques. Confidence intervals were calculated using variance asymptotic variance approximations (details available from SAB).

**Single Binary Risk Factor—Adjusting for Mismeasurement**

In a case-control study, suppose the proportion of cases with positive risk factor status is \( p_1 \) and the proportion of controls with positive status is \( p_2 \). Then the odds ratio is \( p_1(1-p_2)/(1-p_1) \). If \( p_1 \) and \( p_2 \) are the proportions observed positive using an imperfect measure with an error (either false positive or false negative) probability of \( 1-\alpha \), it can be shown that the true proportion positive is estimated as

\[ p_i = \frac{p_i^* - 1 + \alpha}{2\alpha - 1} \quad i = 1, 2 \]  

(2)

We assume that we have (external to the study) repeat determinations using the imperfect measure. The external assumption requires separation of the controls with the repeat measures from the remaining controls. Although this entails a loss of information, it enables us to obtain simple analytical estimates and it has been shown to give good agreement with more formal estimates and it also makes variance estimation easier. We estimate \( \alpha \) as

\[ \hat{\alpha} = \frac{1}{2} + \frac{1}{2} \left( \frac{N - 2n}{N} \right)^{1/2} \]  

(3)

where \( N \) individuals are measured twice and there are \( n \) disagreements between the first and second measurements. The variance can be estimated as

\[ \hat{V}(\hat{\alpha}) = \frac{n(N - n)}{4N^2(N - 2n)} \]  

(4)

The log odds ratio is calculated as

\[ \beta = \frac{\beta^*}{(2\alpha - 1)} \]  

(5)

where \( \beta \) is the true log(OR) and \( \beta^* \) is the observed log(OR) from the misclassified data.

We use the following approximation for the variance of \( \beta \) (using the delta method)

\[ \hat{V}(\hat{\beta}) = \frac{1}{(2\hat{\alpha} - 1)^2} \left( \frac{1}{a^2} + \frac{1}{b^*} + \frac{1}{c} + \frac{1}{d'} \right) + \frac{4\beta^*_2}{(2\hat{\alpha} - 1)^2} \hat{V}(\hat{\alpha}) \]  

where \( a^* \), \( b^* \), \( c^* \) and \( d' \) are the observed misclassified cell numbers.
Binary Risk Factor (RF) and Confounding Factor (CF) both Subject to Misclassification

This problem is dealt with by Duffy et al.\(^\text{11}\) assuming an internal 2 \(\times\) 2 \(\times\) 2 table (case-control status by RF by CF) and external repeal measurement. The error probabilities are estimated for the risk factor and confounding factor independently using equation (3). Then the individual probabilities of being (RF positive, CF positive), (RF positive, CF negative), and so on are estimated by a matrix correction to the observed probabilities for cases and controls. Formal interval estimation is performed using the profile likelihood, but a similar approximation to that in equation (6) is available (details available from SAB). Again, if the repeat data are internal to the study, they can be separated from the main data set and the analysis performed as usual—this leads to the same expected odds ratio estimate but to a conservative confidence interval.

RESULTS

Table 1 gives a summary of the haemostatic variables. The figures are based on the means of the two measurements within individual blood samples that were taken for each variable. For each factor the mean (in international units), the standard deviation and the number of subjects is given for cases and controls with a single measurement and for controls with repeat measurements (i.e. an initial first measurement and a repeat measurement a year later).

Table 2 gives the results of the random effects analysis of variance. The mean used to calculate the coefficients of variation is the overall mean of all the measurements for the controls with repeat data.

The within-years \(cv\) represents the pure laboratory error within samples. This is relatively low overall, with the highest, \(7\%\), being for TPA and FVII. Temporal variation (between-years) is highest for PAI and vWF (41% and 30% respectively). It is clear that the major contribution to within-person variation is the temporal fluctuation of the true value. Hence, for further analysis the mean of the two within-blood-samples measures was taken as a single determination and formal correction for the misclassification was based on the between year changes.

Table 3 gives the corrected (crude) and uncorrected odds ratio using each of the haemostatic variables as a risk factor. It also gives the estimated value \(\hat{\alpha}\) (i.e. Pr [Risk factor is correctly classified]). The median value that is given is the cutoff point for the risk factor being present or absent.

Note that there is a considerable correction to the risk factor for vWF. This is because there was a substantial discrepancy between the first and the second measurement one year later (between-years) indicating a greater degree of misclassification. This increased uncertainty is reflected in a much wider confidence interval (Table 4).

Table 5 is a cross tabulation of all the haemostatic variables, as risk, and as confounding factors. The Table shows the odds ratio for each factor adjusted for each of the other factors in turn. Odds ratios are presented for the situation when both factors are corrected for misclassification (1), neither are corrected (2), only the confounding factor is corrected (3) and only the risk factor is corrected (4). The FVII and vWF have much the same effects on risk (approximately 0.9 and 3.8, respectively) when adjusted for any of the other variables. The TPA and PAI have their effects reversed when adjusting for vWF.

<table>
<thead>
<tr>
<th>von Willebrand</th>
<th>Plasminogen activator inhibitor-1</th>
<th>Tissue plasminogen activator</th>
<th>Factor VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD No. of subjects</td>
<td>SD No. of subjects</td>
<td>SD No. of subjects</td>
<td>SD No. of subjects</td>
</tr>
<tr>
<td>Cases</td>
<td>1.37 0.65 94</td>
<td>69.69 39.92 94</td>
<td>7.22 3.27 91</td>
</tr>
<tr>
<td>Controls (single)</td>
<td>1.13 0.54 158</td>
<td>74.90 44.29 160</td>
<td>7.06 3.44 158</td>
</tr>
<tr>
<td>Controls (double)</td>
<td>1.18 0.60 77</td>
<td>67.54 31.73 75</td>
<td>7.57 3.89 74</td>
</tr>
<tr>
<td>First</td>
<td>1.24 0.49 82</td>
<td>94.58 39.24 81</td>
<td>7.97 3.72 79</td>
</tr>
<tr>
<td>Repeat*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Repeat measurements were taken one year after the first measurement.
Results in Tables 3, 4 and 5 indicate that in terms of classification above and below the median, more misclassification occurs in von Willebrand factor than in the other three factors. As a result, the correction is greater for von Willebrand factor and the corrected confidence intervals are considerably wider. Table 5 also illustrates the fact that severe misclassification of a confounding factor can indicate dramatic changes in the odds ratio for the risk factor of interest. This can be seen from the differences between results correcting for misclassification and not correcting when plasminogen activator inhibitor-1 or tissue plasminogen activator is adjusted for von Willebrand factor.

The results in Table 2 show that risk estimates with respect to plasminogen activator inhibitor-1 are very difficult to interpret due to the variation attributable to between-subject, between-year variance. An alternative

### Table 2: Results from the Random Effects ANOVA for the haemostatic variables

<table>
<thead>
<tr>
<th></th>
<th>von Willebrand</th>
<th>Plasminogen activator inhibitor-1</th>
<th>Tissue plasminogen activator</th>
<th>Factor VII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>0.1767</td>
<td>369.8</td>
<td>11.541</td>
<td>367.79</td>
</tr>
<tr>
<td>$cv$</td>
<td>35%</td>
<td>24%</td>
<td>44%</td>
<td>17%</td>
</tr>
<tr>
<td>% tv</td>
<td>57%</td>
<td>25%</td>
<td>77%</td>
<td>62%</td>
</tr>
<tr>
<td>d.f.</td>
<td>76</td>
<td>74</td>
<td>73</td>
<td>76</td>
</tr>
<tr>
<td><strong>Between years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{xy}$</td>
<td>0.1282</td>
<td>1127.9</td>
<td>3.039</td>
<td>155.67</td>
</tr>
<tr>
<td>$cv_{xy}$</td>
<td>30%</td>
<td>41%</td>
<td>22%</td>
<td>11%</td>
</tr>
<tr>
<td>% tv</td>
<td>42%</td>
<td>75%</td>
<td>20%</td>
<td>27%</td>
</tr>
<tr>
<td>d.f.</td>
<td>77</td>
<td>75</td>
<td>74</td>
<td>77</td>
</tr>
<tr>
<td><strong>Within years (duplicate assays)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_g$</td>
<td>0.0033</td>
<td>8.4</td>
<td>0.314</td>
<td>65.1</td>
</tr>
<tr>
<td>$cv_g$</td>
<td>5%</td>
<td>4%</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td>% tv</td>
<td>1%</td>
<td>0%</td>
<td>3%</td>
<td>11%</td>
</tr>
<tr>
<td>d.f.</td>
<td>154</td>
<td>150</td>
<td>148</td>
<td>154</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>1.207</td>
<td>81.22</td>
<td>7.803</td>
<td>111.04</td>
</tr>
</tbody>
</table>

$\sigma^2$ – variation, $cv$ – coefficient of variation, % tv – percentage of the overall variability of a single measurement, d.f. – degrees of freedom

### Table 3: Risk estimates for the four factors (above median relative to below where $\alpha = Pr$ (Risk factor correctly classified), corrected and uncorrected for misclassification

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Median</th>
<th>$\alpha$</th>
<th>Corrected</th>
<th>Uncorrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand</td>
<td>1.13</td>
<td>0.76</td>
<td>3.56</td>
<td>1.88</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>62.15</td>
<td>0.81</td>
<td>0.80</td>
<td>0.87</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>6.7</td>
<td>0.89</td>
<td>1.41</td>
<td>1.30</td>
</tr>
<tr>
<td>Factor VII</td>
<td>106</td>
<td>0.88</td>
<td>0.91</td>
<td>0.93</td>
</tr>
</tbody>
</table>

DISCUSSION

Results in Tables 3, 4 and 5 indicate that in terms of classification above and below the median, more misclassification occurs in von Willebrand factor than in the other three factors. As a result, the correction is greater for von Willebrand factor and the corrected confidence intervals are considerably wider. Table 5 also illustrates the fact that severe misclassification of a confounding factor can indicate dramatic changes in the odds ratio for the risk factor of interest. This can be seen from the differences between results correcting for
The interpretation of this is that there is a large systematic increase from year 1 to year 2 (Table 1). In any case, we would not give much credibility to the related risk estimates. It is likely that the problem with this variable arises from the way the blood was processed. For the repeat measurements (i.e. the measurements taken a year later, controls only) the blood was not centrifuged immediately after venepuncture thus allowing released PAI from platelets to enter the plasma, resulting in much higher assayed levels than normally found in the plasma. The major correction to the odds ratio for von Willebrand factor indicates the need to consider misclassification bias in design and analysis. The corrected odds ratio is considerably different from the uncorrected (from 1.88 to 3.56). Another point to note is that the confidence intervals for the odds ratios for von Willebrand factor are very wide. This is because the confidence interval takes into account the variability of our estimated misclassification probability, \( \hat{\alpha} \). This is also desirable and fits with the intuitive idea that if misclassification is bad enough to produce a major change in the estimated odds ratio, it is bad enough to introduce considerable uncertainty in our corrected estimate.

Implicit in this analysis are certain assumptions, notably independence of errors conditional on true status, non-differential error (i.e. error probabilities are the same for cases and controls) and the independence of measurement error between variables. There are various methods for correction for measurement and all make some of these assumptions. A full multivariate correction would dispense with the last assumption but for this we would need either a validation study or a larger repeat measures study.

This paper highlights an explicit maximum likelihood approach for the correction of measurement errors. Measurement errors introduce many complications into most statistical analyses which are beyond the scope of this paper. For example, to look into the effect of differential measurement one would require repeat data on cases (which may not be useful if cases are undergoing treatment). Some of the other methods mentioned above for the correction of measurement errors have been discussed elsewhere.

**REFERENCES**


(Revised version received April 1996)