

## Meta-analysis of experimental stroke data -User's Guide

The meta-analysis is performed in Microsoft Excel and requires a substantial user input.

Worksheets:

<Raw Data>	in which raw data is input
<Multiple Outcomes>	for the calculation of effect size where more than one outcome has been measured
<Fixed Raw Data>	Allows sorting of comparisons by characteristics; formed by "Paste Special" command of <Raw Data>
<Meta-analysis>	the calculation engine: by sorting <Fixed Base Data> according to criteria of interest these come through to <Meta-analysis>, and by deleting other rows, a stratified meta-analysis on that subgroup of studies is performed. Be careful – if you delete all comparisons, you will have to re-enter the formulae
<Result>	Sheet for recording results of stratified meta-analysis, insert by "copy" and "paste special" from <Meta-analysis>.

In the following descriptions, only data highlighted in grey require user input – the rest is calculated automatically by the spreadsheet

<Raw Data>

A	Author
B	Year of publication – if 2 by same author in same year, use 2000a, 2000b, etc.
C	species of animal used
D	Publication ID – unique ref for each publication – I use RefMan I.D.
E	Drug – although this is clearly the same throughout, keeping it in will allow data for other drugs to be aggregated for regression analysis by cut and paste
F	Drug dose – include units
G	Whether drug administered as single dose or multiple doses
H	Time of administration – if multiple doses, time of first dose
I	Number in Control Group
J	Number in treatment group
K	Calculated field: number in control group/ number of experimental groups
L	Outcome measure – infarct volume, neurobehavioural score, or combined
M	Infarct volume or infarct area
N	Outcome in controls – score, volume, etc.
O	Standard error in controls
P	Calculated or entered field: standard deviation in controls
Q, R, S	as N, O, P, but for treatment group
T	Time to sacrifice/ outcome measure: where multiple times, use data from last
U	Number of treatment groups served by control group
V, W, X, Y	Calculated fields: Control, SD, Treatment, SD as percentage of outcome in controls
Z	Calculated Field: Effect size
AA	Calculated Field: Standard Error
AB to AK	components of quality score
AL	animal (mouse/ rat/ gerbil/ monkey etc.)
AM	route of drug admin (ip, iv, oral, scut, icarotid)
AN	permanent, temporary or thrombotic
AO	sex (male/ female/ mixed)
AP	comorbidity (blank or state)
AQ	anaesthetic used
AR	means of occlusion
AS	co-treatments (blank or state)
AT	Method of quantification of infarct volume (TTC, histology, etc)
AU	Neurobehavioural scales used (rotorod, foot fault etc)
AV	Funding source (academic/ charity/ industry)
AW to AZ	User Defined attributes
BA	Calculated field - Quality score
BB	Calculated field; Summary data; helps sorting comparisons in <Fixed Raw Data> worksheet.

### <Multiple outcomes>

This worksheet performs a mini-metaanalysis to aggregate data within a single comparison. It is required where outcome is measured in the same animals on different neurobehavioural scores or on neurobehaviour and infarct volume. The guiding principle is that no animal can contribute to more than one comparison in the meta-analysis. Where infarct volume and more than one neurobehavioural tests are used, I take a nested approach ... combine neurobehaviour in one cycle, then combine aggregate neurobehaviour with infarct volume in the second, then use the results from this to enter into <Raw Data>.

For each of treatment and control enter the number of animals, mean and standard deviation. Two techniques are used, fixed effects and random effects. For a full discussion see <Meta-analysis>. Suffice it to say that cell AA4 gives the overall estimate of effect, and AB4 the standard error of this estimate. Enter this data into <Raw data>: Control = 100, Control SD = 0, Treatment =  $(100-AA4)$  [given in AAI1] and Treatment standard error (not deviation) = AB4. Where the numbers differ between tests (e.g. infarct volume not available in all animals with a neurological score), the number entered in to <Raw Data> should be the highest number in each group.

### <Fixed Raw Data>

This worksheet is entirely derived from <Raw Data>: In <Raw Data> select all (Ctrl+A), move into <Fixed Raw Data>, select cell A1, Paste Values (Alt+E, S, Alt+V). This allows you to remove data from the stratified meta-analysis without deleting it from the database.

<Meta-analysis >

A	Treatment (n) - from <Fixed Raw Data>
B	Control (n) - from <Fixed Raw Data>
C	Effect size - from <Fixed Raw Data>
D	Standard error: - from <Fixed Raw Data>
E	Weight by inverse variance: $1/D^2$
F	Effect size * weight, $C * E$
G	Weight * Square of difference between this effect size and fixed effect estimate of effect size ( $W3$ ), $= E * (C - W3)^2$ : used in calculation of heterogeneity statistic (AA4)
H	Square of weights, $= E^2$
I	Weight by inverse variance and $\tau$ (DerSimonian and Laird, DSL); Weight is $1/(\text{variance plus } \tau^2)$ ; $\tau^2$ is given in cell X7 and is given by the formula (Heterogeneity – (n-1))/(sum of weights-(sum of square of weights/sum of weights)) where heterogeneity is the sum of the squares of the deviations from the fixed effect estimate, n is the number of comparisons, and weights are the weights given under inverse variance. Where $\tau^2$ is negative it is set to zero and weighting is given on inverse variance alone. $\tau^2$ is calculated in cell X7 and the value used for calculations is given in X8 – the spreadsheet confines this to zero if $\tau^2$ is negative.
J	Effect size * DSL weight, $I * E$
M	Effect size as proportion, to 3 significant figures
N	95% CI distance from mean to 3 significant figures
O	Lower 95% CI of comparison
P	Upper 95% CI of comparison
Q	Total animals to nearest whole number (accounting for one control group serving many treatment groups)
R	Summary data
S	Summary of estimate, 95% CI and n for each comparison

Row 131 gives some aggregate data: if there are more than 129 comparisons you will need to insert further rows:

A	animals in treatment groups
B	animals in control groups
C	number of comparisons
E	sum of inverse variance weights
F	sum of inverse variance weights*effect size
G	sum of squares of deviation of effect size from fixed effects estimate
H	sum of squares of inverse variance weights
I	sum of DSL weights
J	sum of DSL weights*effect size

W3 gives the effect size under fixed effects metaanalysis, as F131/E131: The standard error is given by  $1/\text{SQRT}(E131)$ . W4 gives effect size under random effects meta-

analysis, as J131/I131; The standard error is given by  $1/\text{SQRT}(I131)$ . The worksheet then reports the upper and lower 95% confidence estimates of both fixed and random effects models (Y3, Z4, Y5, Z5), the heterogeneity statistic (AA4), the significance of the heterogeneity statistic (AB4), the number of comparisons (AC4) and the total number of animals (AD4). These may be conveniently copied and pasted (use paste values again, or when you do another calculation you will change the result recorded for this one) into an appropriate place in the <Result> worksheet.

To do each comparison, first sort the data (Rows 2 onwards) in <Fixed Raw Data> according to the criterion in question. Then, delete all rows in <meta-analysis> except for the block that contains comparisons with the characteristic of interest, and read off the result in W4 to AD4. Then copy an entire data containing row of <Meta-analysis> and paste it into Row 3 of <meta-analysis>, and re-populate the worksheet by highlighting the row and dragging it to re-fill all rows, then repeat for the next block. Be careful not to delete all the data, or you will have to re-do the calculations. Be careful not to drag-and-fill over the summary data in Row 131, or again you will need to re-enter the formulae. If need be, insert rows above Row 131 to make space – the worksheet should adjust formulae accordingly.

The <Result> worksheet is the place to record results of the overall and stratified meta-analyses. The overall results should go into rows 10, 19 and 29: Paste values from cells W4 to AD4 of <meta-analysis> to B10 to I10 of <Results>. Stratify data according to appropriate (prespecified) categories of dose and time based on the biological properties of the drug in question and the distribution of these values in the dataset. Similarly, carry out meta-analyses with data stratified according to study quality score or other design features.

Questions to  
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