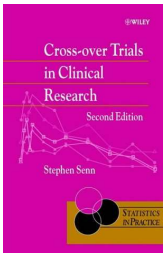
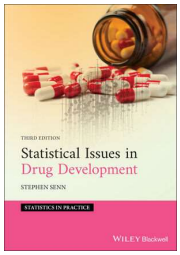
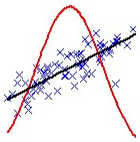
Design and Analysis of N of 1 Trials

Lecture 2





Stephen Senn
Consultant Statistician, Edinburgh



DIAMOND
Development of generalisable methodology for n-of-1 trials delivery for very low volume treatments

What I hope to do in this lecture

- Background/General
  - Brief reminder of some purposes of clinical trials generally
  - Randomisation theory, the Rothamsted School, ANOVA
- Specific to n-of-1 trials
  - Show how the combination of a particular purpose and the randomisation approach leads to justification of one type of analysis
  - Make a link to fixed effects meta-analysis
  - Give some practical advice regarding analysis

Background

This section is not specific to n-of-1 trials

3

Possible objectives of an analysis

- Is one of the treatments better?
  - Significance tests
- What can be said about the average effect in the patients that were studied?
  - Estimates, confidence intervals
- What can be said about the average effects in future patients?
- What can be said about the effect of a given patient in the trial?
- What can be said about a future patient not in the trial?

4

Two different philosophies

Randomisation philosophy

- The patients in a clinical trial are taken as fixed
- The population about which inference is made is all possible randomisations
- The patients don't change, only the pattern of assignments of treatments change

Sampling philosophy

- The patients are regarded as a sample from some possible population of patients
  - NB This is not the target population but some theoretical superpopulation
- This is usually handled by adding error terms corresponding to various components of variance
- This approach is much more common

5

In this lecture

- I will look at the first of these purposes
  - Discovering whether the treatment is capable of working at all
- For this purpose it is sufficient to demonstrate that the null hypothesis that the treatment worked in no patients at all is false
- The alternative that may then be asserted is that the treatment worked in at least one patient and this is enough to demonstrate that it is capable of working
- The further questions require mixed models and they will be dealt with in lecture 3

6

Is one of the treatments better? Significance tests

Rothamsted School

- Leading statisticians such as Fisher, Yates, Nelder, Bailey, Payne
- Developed analysis of variance not in terms of linear models but in terms of symmetry
- High point was John Nelder's theory of general balance (1965)

General Balance

1) Establish and define block structure
2) Establish and define treatment structure
3) Given randomisation, the analysis then follows automatically

7

Basic Idea

- Splits an experiment into two radically different components
  - The block structure, which describes the way that the experimental units are organised
    - The way that variation amongst units can be described prior to any treatment being allocated
  - The treatment structure, which reflects the way that treatments are combined for the scientific purpose of the experiment

8

Design Driven Modelling

- Together with a third piece of information, the design matrix, these determine the analysis of variance
  - Note that because both block and treatments structure can be hierarchical such a design matrix is not on its own sufficient to derive an ANOVA
    - But together with Nelder's block and treatment structure it is
      - For designs exhibiting *general balance*
- This approach is incorporated in GenStat®

Wilkinson and Rogers (1973) Notation

This is a common notation

A = main effect of factor A, B = main effect of factor B

A.B = interaction of A and B, A.B.C = three factor interaction of A, B and C

+ sign used to add effects - used to subtract them

A*B = A + B+ A.B = main effects of A and B and their interactions

A*B*C = A + B + C +A.B + A.C + B.C + A.B.C

A/B is nesting (B within A) and is equivalent to A + B.A

Wilkinson & Roger Notation in three packages

Term	GenStat®	R	SAS®
Main effects	A, A + B etc	A, A+ B etc	A, A B
Interactions	A.B, A.B.C etc	A:B, A:B:C etc	A*B, A*B*C
Nested	A/B, A/B/C etc	A/B, A/B/C etc	B(A), C(B(A)) ?
Main effect plus interactions	A*B=A+B+A.B A*B*C=A+B+C+A.B+A.C+B.C+A.B.C	A*B=A+B+A:B A*B*C=A+B+C+A:B+A:C+B:C+A:B:C	A|B = A B A*B A|B|C=A B C A*B A*C B*C A*B*C
Subtracting effects	A*B*C-A.B.C= A+B+C+A.B+A.C+B.C	A*B*C-A:B:C= A+B+C+A:B+A:C+B:C	A|B|C@2= A B C A*B A*C B*C

NB I am never very confident about the SAS® form

11

Specific application

These ideas will now be applied to n of 1 trials

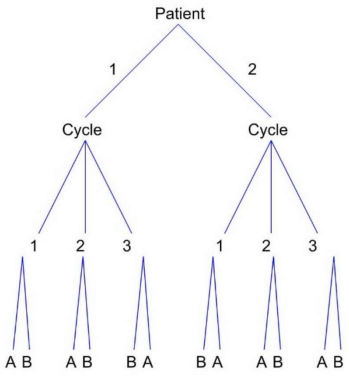
12

Chen and Chen (2014)

- Paper on n of 1 trials in *PLOS One*
- Considered appropriate analyses of n of 1 trials randomised in cycles
- Compared performance of various analyses and compared them via simulation
- Amongst the various approaches they investigated was the matched pairs design where 'pair' was defined by a cycle
  - Found this performed well
- My initial reaction on seeing this is that this is wrong but on closer examination there is a sense in which it can be justified
- We shall now look at this

Simulated example

- Twelve patients suffering from a chronic rare respiratory complaint
  - For example cystic fibrosis
- Each patient is randomised in three pairs of periods, comparing two treatments A and B
- Adequate washout is built in to the design
- Thus we have 12 x 3 x 2 = 72 observations altogether
- Efficacy is measured using forced expiratory volume in one second (FEV_1) in ml
- How should we analyse such an experiment?



Data generation model

The data themselves were generated from quite a complicated model that allowed for

1) Random main effects of patients
2) Random effects of cycles within patients
3) Pure within cycle occasion to occasion error
4) An overall effect of treatment
5) A treatment by patient interaction

However, this is not really relevant because once 1, 2 and 3 have been used to generate the values a random treatment allocator randomly assigns an additional value to each of the 72 block and plot results

One approach to analysis accepts the first part as given and irrelevant and then analyses using the randomisation framework

15

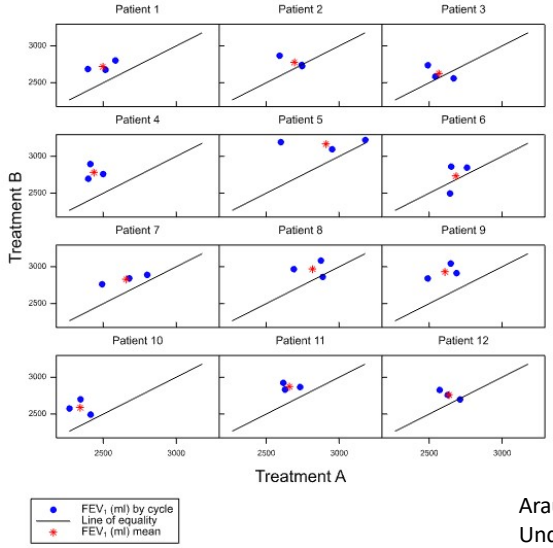
	Treatment					
Patient	A	B	A	B	A	B
1	1	2	3	4	6	5
	2394	2686	2515	2675	2583	2802
2	2	1	3	4	6	5
	2746	2726	2592	2867	2743	2742
3	1	2	3	4	6	5
	2668	2560	2542	2584	2491	2737
4	1	2	3	4	6	5
	2397	2696	2411	2895	2499	2760
5	2	1	3	4	5	6
	3179	3221	2952	3096	2600	3192
6	1	2	4	3	5	6
	2643	2496	2759	2847	2651	2860
7	1	2	3	4	5	6
	2678	2843	2492	2763	2801	2890
8	2	1	3	4	5	6
	2887	2862	2875	3083	2689	2967
9	2	1	3	4	6	5
	2490	2841	2648	3044	2688	2914
10	2	1	3	4	6	5
	2268	2576	2413	2493	2344	2699
11	2	1	4	3	6	5
	2617	2923	2629	2832	*2732*	*2866*
12	1	2	4	3	5	6
	2627	2759	*2712*	*2698*	*2572*	*2826*

Simulated FEV_1 values in mL from a series of n of 1 trials

The underlined values will be treated as missing in some subsequent analyses

Araujo A, Julious S, Senn S. Understanding Variation in Sets of N-of-1 Trials. PloS one. 2016;11(12):e016716

16



Trellis plot of results under treatment B against results under treatment A by cycle and patient.

Outcome is forced expiratory volume in one second FEV_1(mL).

Araujo A, Julious S, Senn S. Understanding Variation in Sets of N-of-1 Trials. PloS one. 2016;11(12):e0167167.

17

R Code

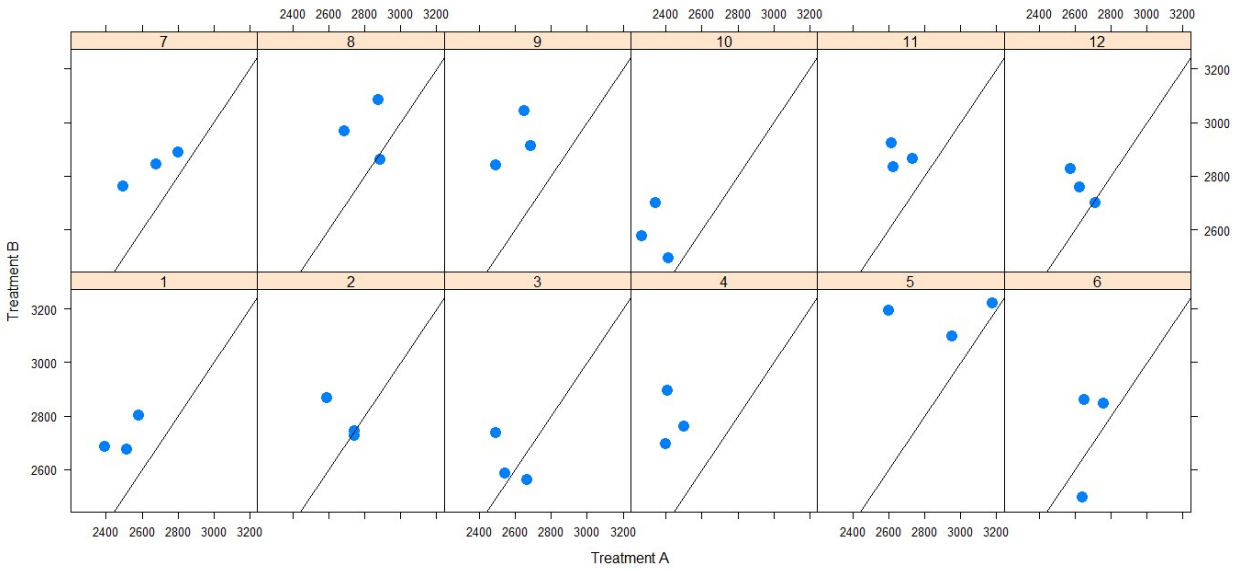
```
#Read data
FEV1.frame<read.table("Example_1.txt",header=T)

#_____________________________________________#
#Set and define variables and factors
FEV1<-FEV1.frame$Y
Cycle.F<-as.factor(FEV1.frame$Cycle)
Patient.F<-as.factor(FEV1.frame$Patient)
Period.F<-as.factor(FEV1.frame$Period)
Treat.F<-as.factor(FEV1.frame$Treat)

#_____________________________________________#
#Split FEV1
FEV1.A<-FEV1[Treat.F=='A']
FEV1.B<-FEV1[Treat.F=='B']
```

```
#Plot data
library(lattice)
xyplot(FEV1.B~FEV1.A|Pat.Red,xlab="Treatment
A",ylab="Treatment B",pch=19,
  cex=1.5,
  panel=function(x,y,...){
  panel.xyplot(x,y,...)
  panel.abline(a=0,b=1)
  })
```


18



19

How the Rothamsted School approach works

- The block structure of the experiment is established
- This is cycles within patients
  - Patient/Cycle = Patient+Patient.Cycle for GenStat®
  - Patient/Cycle = Patient+Patient:Cycle for R
  - Patient(Cycle) = Patient Patient(Cycle) for SAS®
- The treatment structure is established
  - For all three packages this is simply Treatment
- The outcome variable is declared
- Given the design matrix, the analysis now follows
  - This is fully implemented in GenStat® In the other packages it has to be programmed

20

The general balance approach in GenStat®

```
BLOCKSTRUCTURE Patient/Cycle
TREATMENTSTRUCTURE Treatment
ANOVA[FPROBABILITY=YES;NOMESSAGE=residual] Y
.
```

Analysis of variance

Variate: FEV_1 (mL)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Patient stratum	11	1458791.	132617.	10.04	
Patient.Cycle stratum	24	316885.	13204.	1.04	
Patient.Cycle.*Units* stratum					
Treatment	1	641089.	641089.	50.57	<.001
Residual	35	443736.	12678.		
Total	71	2860501.			

Estimates of parameters

Parameter	estimate	s.e.	t(35)
Constant	2445.6	80.7	30.30
Patient 2	196.	113.	1.74
……..			
Patient 12	153.	113.	1.36
……..			
Patient 1 .Cycle 2	55.	113.	0.49
……			
Patient 12 .Cycle 3	6.	113.	0.05
Treatment B	188.7	26.5	7.11

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Patient	1
Treatment	A

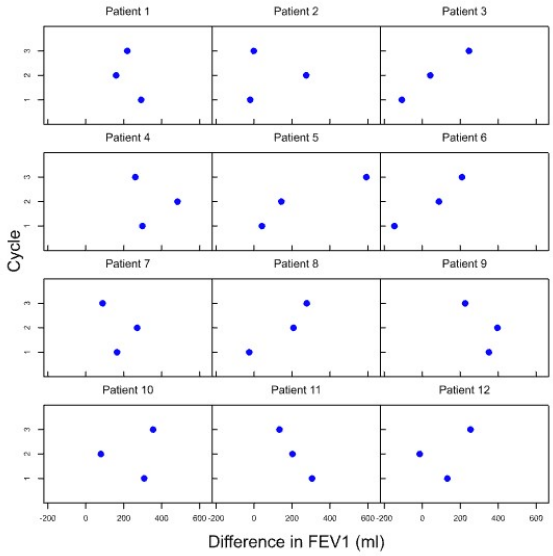
Analysis in R

```
> #Linear model analysis one
> fit1<-lm(FEV1~Treat.F+Patient.F/Cycle.F)
> #extract terms of interest
> estimates1<-coefficients(fit1)
> est1.treat<-estimates1[2]
> SE1.treat<-sqrt(diag(vcov(fit1)))[2]
> t1.treat<-est1.treat/SE1.treat
> fit1.frame<-data.frame(est1.treat,SE1.treat,t1.treat)
➢ fit1.frame

       est1.treat SE1.treat t1.treat
Treat.FB   188.7222  26.53945 7.111008
```


What about the matched pairs approach?

- We don't analyse the original data
- Instead we reduce everything to a difference between pairs
  - In this case 'pair' is defined by patient and cycle within patient
  - There are thus 12 x 3 = 36 pairs in total
- The trellis plot corresponding to this follows
  - Instead of plotting B against A it shows B-A per cycle per patient



Plot of the 36 differences per cycle

25

Matched pairs t using cycles to define pair

One-sample t-test

Variate: Diff.

Summary

	Sample Size	Mean	Variance	Standard deviation	Standard error of mean
Paired difference	36	188.7	25356	159.2	26.54

95% confidence interval for mean: (134.8, 242.6)

Test of null hypothesis that mean of Paired difference is equal to 0

Test statistic t = 7.11 on 35 d.f.

Same DF as for ANOVA & is one fewer than total number of cycles

Probability < 0.001

$7.11^2 = 50.57$ This analysis is equivalent to the previous ANOVA

26

Analysis in R

```
> #Paired t-test analysis
> #Calculate differences per cycle
> FEV1diff<-FEV1.B-FEV1.A
> fit2<-t.test(FEV1diff)
> fit2

        One Sample t-test

data:  FEV1diff
t = 7.111, df = 35, p-value = 2.749e-08
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 134.8443 242.6002
sample estimates:
mean of x
 188.7222
```


27

Consequences

- The matched pairs t-test examined by Chen and Chen (2014) *is* a valid analysis
- It is justified by the randomisation theory of the Rothamsted School and by John Nelder's approach
- However, one must be careful
  - It is a valid analysis for testing a specific null
    - That the two treatments are identical
  - In this case ***under the null hypothesis, the interaction is zero***
- This raises the question can we do better?

28

The answer is 'Yes'

- We can go one step further and remove the treatment by patient interaction from the residual sum of squares
- Under the null hypothesis the expected value of the interaction is no different from the residual
- However if the alternative is true we can use a smaller residual sum of squares
- This is analogous to the following idea when carrying out a two-sample t-test
  - Despite the fact that under the *null hypothesis* a variance estimate using $n_1 + n_2$ - 1 DF is more accurate than one using $(n_1 - 1) + (n_2 - 1)$, we use the latter because it is better under the *alternative hypothesis*

29

Analysis of variance

Variate: FEV_1 (mL)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Patient stratum	11	1458791.	132617.	10.04	
Patient.Cycle stratum	24	316885.	13204.	1.11	
Patient.Cycle.*Units* stratum					
Treatment	1	641089.	641089.	54.13	<.001
Patient.Treatment	11	159516.	14501.	1.22	0.324
Residual	24	284219.	11842.		
Total	71	2860501.			

30

The analogy to fixed effects meta-analysis

- The total degrees of freedom that we have for error are as given in the table on the right
- However if we treat each patient as a trial with *k* pairs we have *k*-1 DF for each patient
- This gives us exactly the same total
- Hence the analysis is equivalent to a fixed effects meta-analysis provided we pool the variance estimate

Source		
Patient	n-1	11
Cycle by Patient	n(k-1)	24
Treatment	1	1
Treatment by Patient	n-1	11
Residual	n(k-1)	24
Total	2nk-1	71

Fixed effects meta-analysis recipe

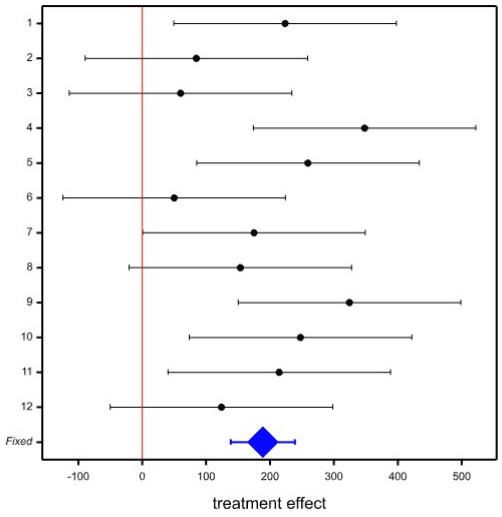
1. Calculate the differences B-A for each patient
2. Calculate the mean difference for each patient, *i*, as an estimate of the treatment effect $\hat{\delta}_i$
3. Calculate the DF for each patient. (In the balanced case these equal $k-1$. More generally we have k_i-1.)
4. Calculate the corrected sum of squares for the differences for each patient
5. Sum the corrected sum of squares over all patients
6. Divide this sum by the total degrees of freedom to obtain an estimate of the variance, $\hat{\sigma}^2$
7. For each patient produce an estimate of the variance of the treatment effect as $V(\hat{d}_i) = \hat{\sigma}^2/k_i$
8. Use the estimates of the treatment effects and their variances as input to a fixed meta-analysis routine

Estimates

	θ_i	se(θ_i)	95% CI
1	223.7	88.85	(49.52, 397.8)
2	84.7	88.85	(-89.48, 258.8)
3	60.0	88.85	(-114.15, 234.2)
4	348.0	88.85	(173.85, 522.2)
5	259.3	88.85	(85.18, 433.5)
6	50.0	88.85	(-124.15, 224.2)
7	175.0	88.85	(0.85, 349.2)
8	153.7	88.85	(-20.48, 327.8)
9	324.3	88.85	(150.18, 498.5)
10	247.7	88.85	(73.52, 421.8)
11	214.3	88.85	(40.18, 388.5)
12	124.0	88.85	(-50.15, 298.2)
Fixed	188.7	25.65	(138.45, 239.0)

Note that the standard errors of the estimates are all the same. This is because the same amount of information is available for each patient and we have used a pooled estimate of variance.

33

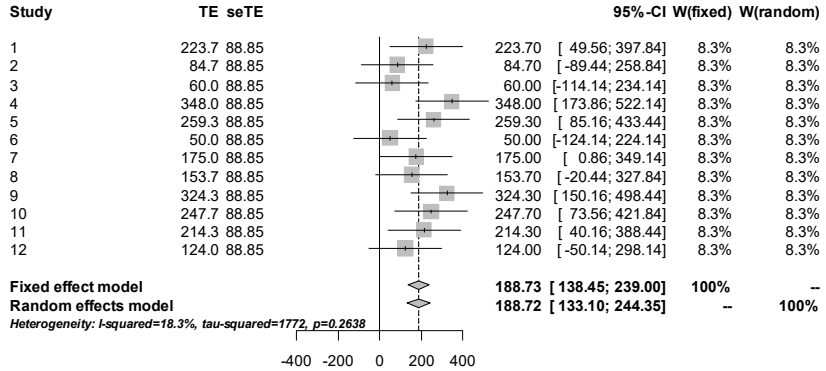


34

Consequences

- Practical
  - This gives us an approach to exploiting the very many meta-analysis routines that there are in various packages to analysing and presenting sets of n-of-1 trials
  - We need to make sure, however, to use pooled variance estimates to do this
- Theoretical
  - Shows a justification more generally for fixed-effects meta-analysis as a valid approach to testing whether a treatment can be effective
  - Suggests also exploring the random-effects meta-analysis approach as a means of answering some of the other questions that might arise with analysis of n-of-1 trials

35



Analysis using Guido Schwartzer's package 'meta' in R

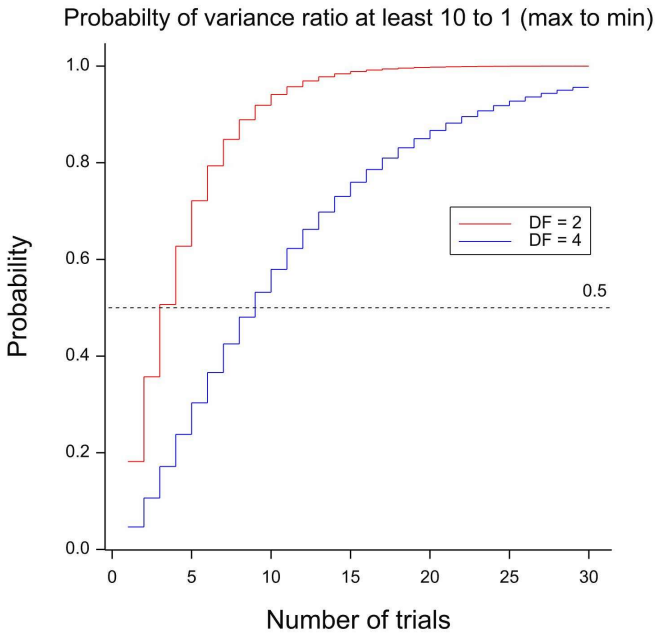
36

One important difference to conventional meta-analysis

- In a conventional meta-analysis the variance would be estimated independently within each trial
- Here a pooled variance has been used
- Because the degrees of freedom are so few, independent variance estimation would be a bad idea
- Even when true variances are identical they can easily vary randomly very greatly as the next slide shows
- This shows the probability that the highest to lowest will vary by a ratio of at least 10 to 1 as a function of the number of patients and for two cases
  - Degrees of freedom = 2 and Degrees of freedom =4

Senn SJ. Letter to the Editor: in defence of the linear model. Controlled clinical trials. 2000;21:589 - 592.

37



38

Practical advice

- This general fixed effects approach is appropriate if either of these is the case
  - You are trying to prove the treatment can work
  - You are interested in the mean effect in the patients actually studied
- You can exploit standard meta-analysis software to do the necessary work
  - However, you should use a pooled variance estimate to obtain the standard errors for individual patients