Analysis of repeated measurements (KLMED8008)

Eirik Skogvoll, MD PhD

Professor and Consultant

Institute of Circulation and Medical Imaging

Dept. of Anaesthesiology and Intensive Care

Day 5

- Practical issues …?
  - Lectures
  - Textbook
  - Software
  - Exam
- Brief review of exercise 1
- Cluster randomized trials – sample size dtermination
- Linear mixed effects models: models with random intercept (Textbook chapter 3)
- Sample size

Repeated measurements

Ignoring dependency between observations may lead to…

- p-values becoming <u>too small</u> when doing between-patient comparisons (i.e. yield false positive results)

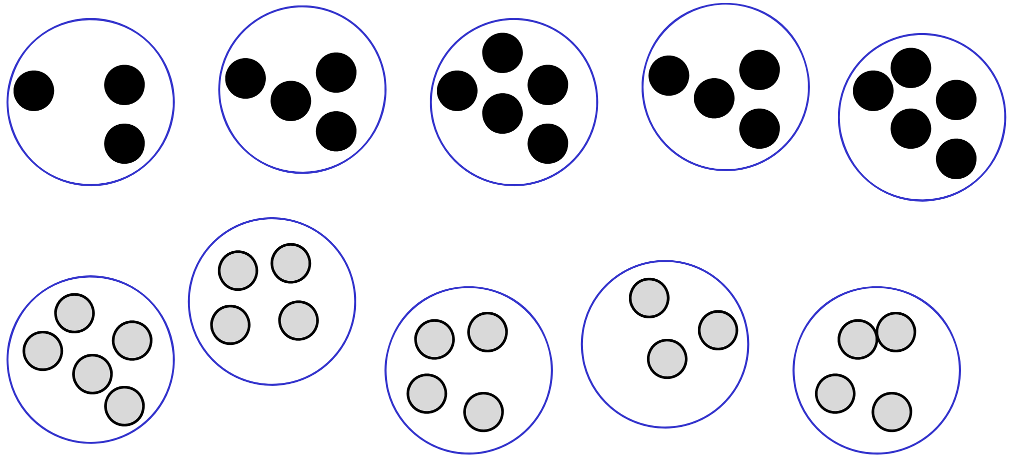
Textbook 3.10.2 (p. 167), *Veierød et al.* 7.1 (p. 231)

<u>Observations:</u>

● Treatment, n=20

○ Control, n=20

◯ Patients



Repeated measurements

Ignoring dependency between observations may lead to…

- p-values becoming too large when doing within-patient comparisons (i.e. yield false negative results)

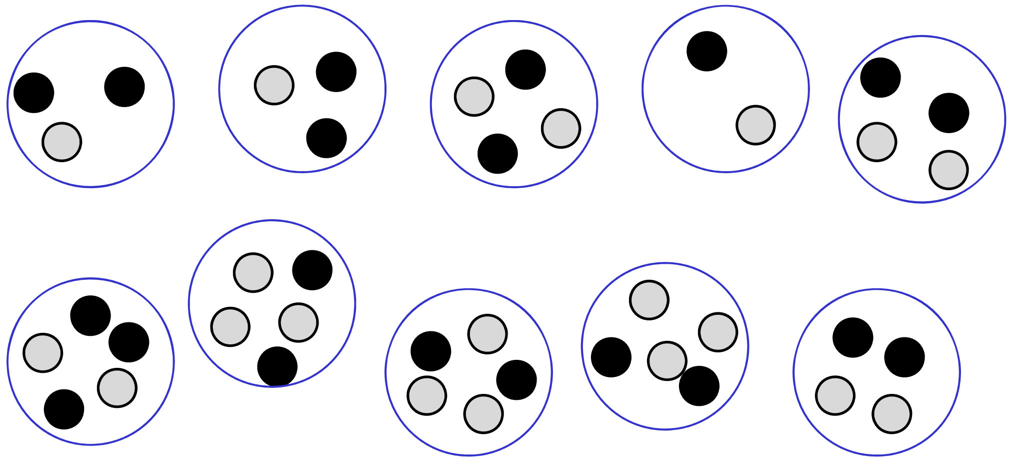
Textbook 3.10.2 (p. 167), *Veierød et al.* 7.1 (p. 231)

Observations:

● Treatment,n=20

○ Control, n=20

◯ Individual



Cluster-randomized design

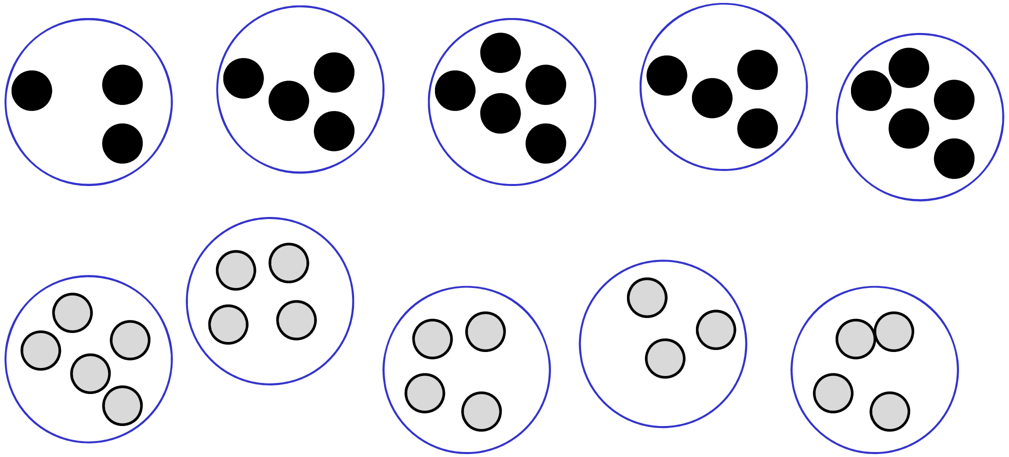
- Sometimes impossible to allocate treatment/ control to individual subjects:
  - ”contagion” within general practice, household, school
  - Practical limitations within geographical areal etc.
- Allocation of treatment must therefore be done to clusters of subjects:

<u>Observations:</u>

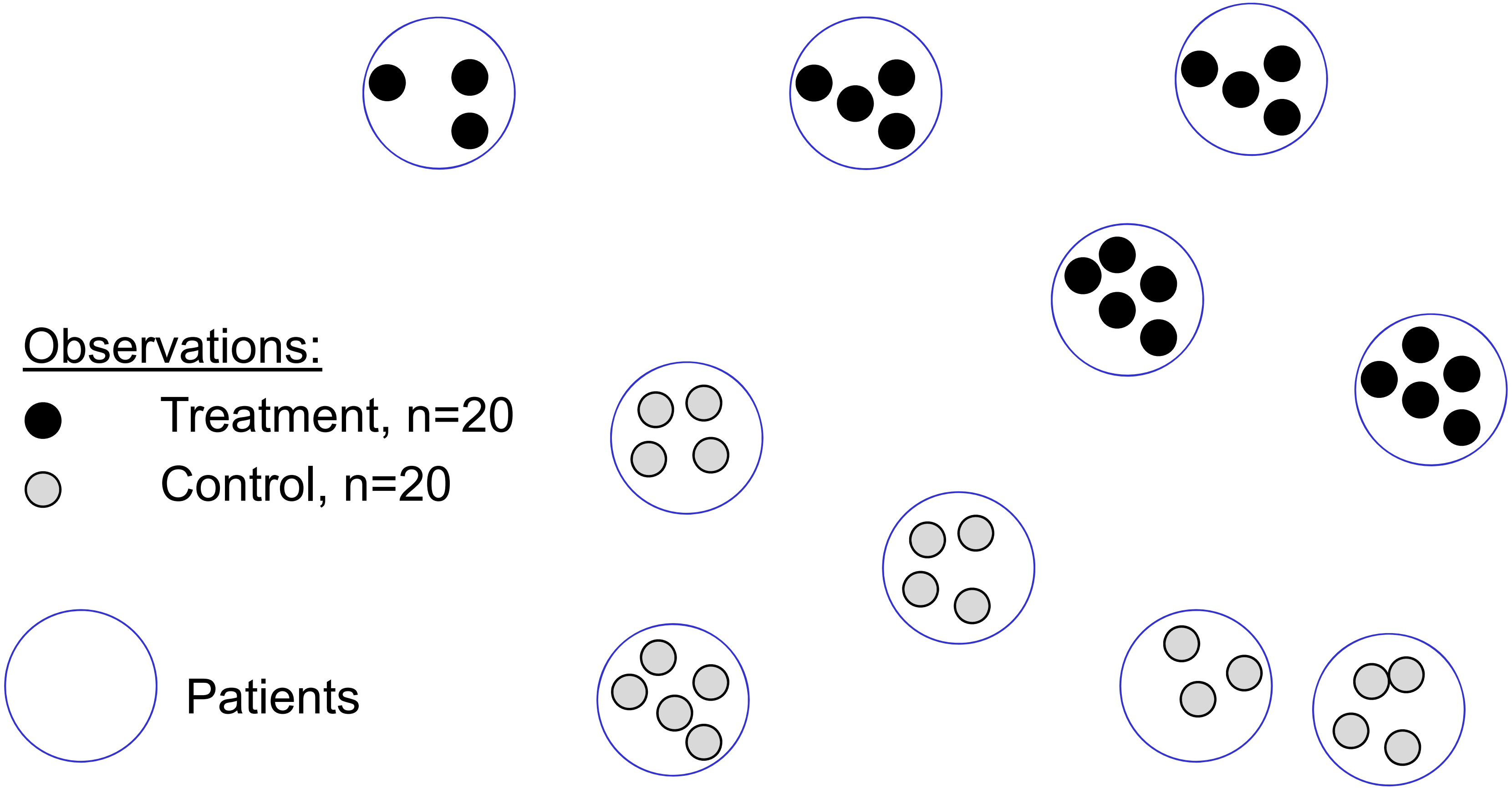
● Treatment, n=20

○ Control, n=20

◯ Patients



So… how large is ”n” in each group? 20?



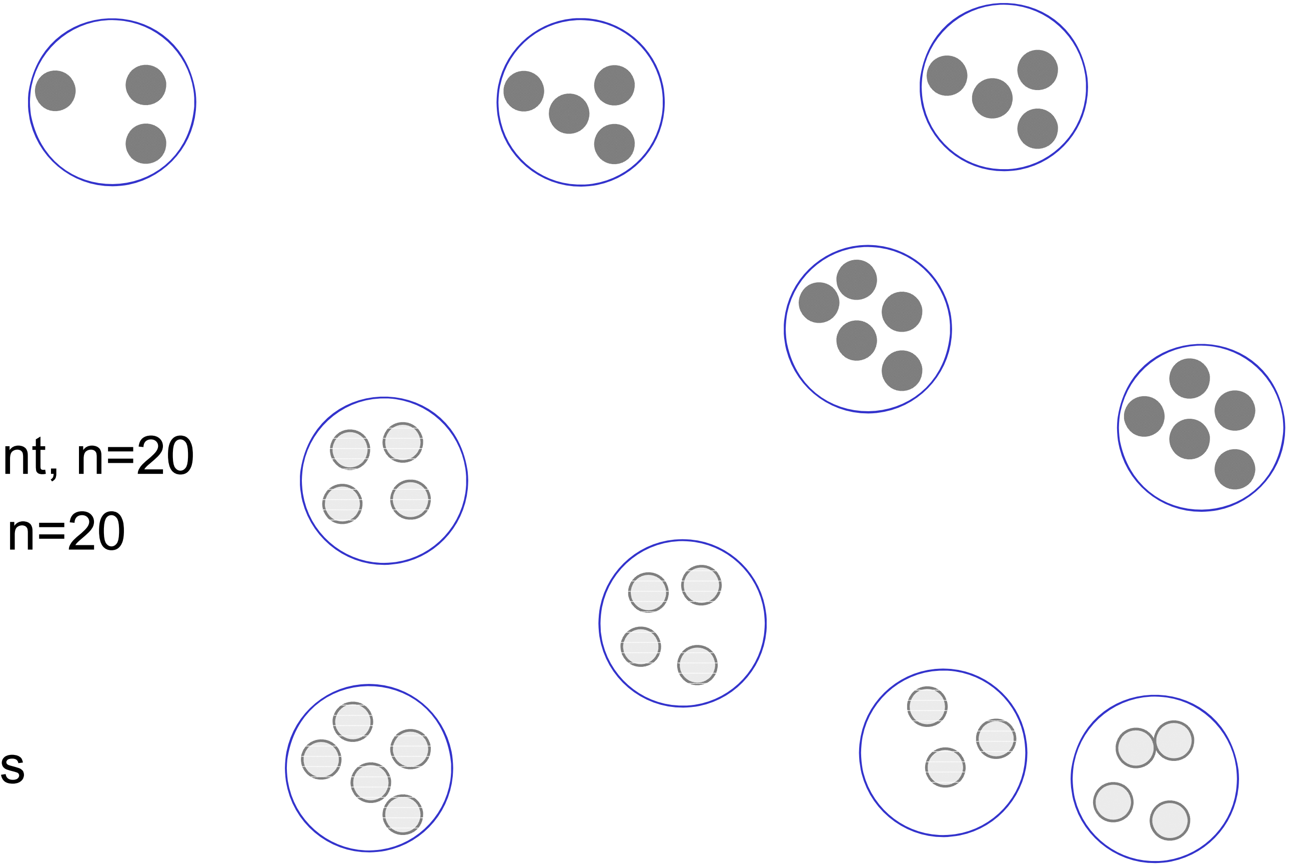
Observations:

● Treatment, n=20

○ Control, n=20

◯ Patients

Or 5 perhaps …?



Observations:

● Treatment, n=20

○ Control, n=20

○ Patients

It depends… on the ICC!

- ICC = intra-class (cluster) correlation
- ICC summarizes the extent that the *subjects within a cluster* are similar, relative to the clusters

$$\text{ICC} = \frac{\sigma_j^2}{\sigma_j^2 + \sigma_{ij}^2}$$

$$\text{Total variance} = \sigma_j^2 + \sigma_{ij}^2$$

$$\sigma_j^2 = \text{ cluster variance}$$

$$\sigma_{ij}^2 = \text{ residual variance (subject)}$$

- If subjects within a cluster are dissimilar, $\sigma^2_j = 0$ and $ICC = 0$
- If subjects within a cluster are identical, $\sigma^2_j = 1$, $\sigma_{ij}^2 = 0$ and $ICC = 1$

Some aspects …

- Ignoring clusters during trial planning may lead to increased type II error (i.e. lower power than required)
- Ignoring clusters during trial analysis may lead to increased type I error (”to sensitive”)

- Power does not increase substantially when the cluster size *k* exceeds $1/ICC$

  Some examples:

  $ICC = 0.01, \quad k = 1/0.01 = 100$ (i.e. max reasonable cluster size is 100)

  $ICC = 0.05, \quad k = 1/0.05 = \ \ 20$

  $ICC = \ \ 0.1, \quad k = 1/0.1 \ \ = \ \ 10$

Campbell, M. J., A. Donner, et al. (2007). *Stat Med* 26(1): 2-19.
Machin D, Campbell M, Tan SB, Tan SH. Sample Size Tables for Clinical Studies. 3 ed. Oxford: Wiley-Blackwell; 2009.

Cluster randomization – sample size

Starting point:

$m_{cluster} = c \cdot k$ = total number of subjects in each group (treatment/ control)

c = number of clusters

k = number of subjects in each cluster (i.e. cluster size)

Three problems:

1. Given n in each group [from usual calculations], how large is $m_{cluster}$?
2. Given $m_{cluster}$ and k, how many clusters (c) are required?
3. Given n [from usual calculations] and c, what is the cluster size (k)?

Cluster randomized trial – ICC adjustment

Principle:

- Low ICC: every subject within cluster adds information
- High ICC: each subject adds little information

In practice, guess the ICC, and calculate the ”Design Effect” (DE).
(DE is a multiplication factor for increasing the sample size)

$$\boxed{\begin{array}{l} DE = 1 + (k-1)\cdot ICC \\ k = \text{number of subjects within each cluster} \end{array}}$$

Examples:

$ICC = 0,\ k = 5 \quad DE = 1+(5-1)\cdot 0 = 1+0 \qquad = 1$ *(reference)*

$ICC = 0.2,\ k = 5 \quad DE = 1+(5-1)\cdot 0.2 = 1+4\cdot 0.2 = 1+0.8 = 1.8$ *(80 % increase)*

$ICC = 0.5,\ k = 5 \quad DE = 1+(5-1)\cdot 0.5 = 1+4\cdot 0.5 = 1+\ 2 \ = \ 3$ *(300 % increase)*

Cluster randomization – sample size

1. If n is the required sample size in each group, how many subjects do you need in each cluster $m_{cluster}$:

$$m_{cluster} = c \cdot k = DE \cdot n$$

2. Given $m_{cluster}$ and k, how many clusters (c) are required?

$$\boxed{c = \frac{DE \cdot n}{k} = \frac{m_{cluster}}{k}}$$

3. Given n and c, how large do the clusters become (k)?

$$\boxed{k = \frac{n \cdot (1 - ICC)}{(c - ICC \cdot n)}}$$

”Design Effect” for different values of *k* and *ICC*

```
   ICC
k  0.01 0.05  0.1 0.15  0.2 0.25  0.3 0.35  0.4
1   1.0  1.0  1.0  1.0  1.0  1.0  1.0  1.0  1.0
2   1.0  1.1  1.1  1.1  1.2  1.2  1.3  1.4  1.4
3   1.0  1.1  1.2  1.3  1.4  1.5  1.6  1.7  1.8
4   1.0  1.1  1.3  1.5  1.6  1.8  1.9  2.0  2.2
5   1.0  1.2  1.4  1.6  1.8  2.0  2.2  2.4  2.6
15  1.1  1.7  2.4  3.1  3.8  4.5  5.2  5.9  6.6
25  1.2  2.2  3.4  4.6  5.8  7.0  8.2  9.4 10.6
35  1.3  2.7  4.4  6.1  7.8  9.5 11.2 12.9 14.6
45  1.4  3.2  5.4  7.6  9.8 12.0 14.2 16.4 18.6
55  1.5  3.7  6.4  9.1 11.8 14.5 17.2 19.9 22.6
65  1.6  4.2  7.4 10.6 13.8 17.0 20.2 23.4 26.6
75  1.7  4.7  8.4 12.1 15.8 19.5 23.2 26.9 30.6
85  1.8  5.2  9.4 13.6 17.8 22.0 26.2 30.4 34.6
95  1.9  5.7 10.4 15.1 19.8 24.5 29.2 33.9 38.6
```

Example

$$k = \frac{n \cdot (1 - ICC)}{(c - ICC \cdot n)}$$

$k =$ number of individuals in each cluster

- $n = 25, ICC = 0.05, c = 10$ $k = 25\cdot(1 - 0.05)/(10 - 0.05\cdot25) = 2.7$
- $n = 25, ICC = 0.05, k = 3$ $m_{cluster} = DE\cdot n = (1+(4-1) \cdot0.05) \cdot25 =28$
- $m_{cluster} =28, k = 3$ $c =28/3 = 9$

Linear mixed effects model

Example

```
. regress post pre

      Source |       SS       df       MS              Number of obs =      52
-------------+------------------------------           F(  1,     50) =   24.37
       Model |  6323.33822     1  6323.33822           Prob > F      =  0.0000
    Residual |  12970.9695    50  259.419389 $\hat{\sigma}^2$   R-squared     =  0.3277
-------------+------------------------------           Adj R-squared =  0.3143
       Total |  19294.3077    51  378.319759           Root MSE  $\hat{\sigma}$ =  16.107

------------------------------------------------------------------------------
        post |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         pre | $\hat{\beta}_1$ .8252027   .1671432     4.94   0.000     .4894858    1.16092
       _cons | $\hat{\beta}_0$ 23.54709    9.79174     2.40   0.020       3.8798   43.21438
------------------------------------------------------------------------------
```

Linear model

$$y_{ij} = \beta_0 + \beta_1 \cdot x_{1ij} + \ldots + \beta_p \cdot x_{pij} + \xi_{ij}$$

$x_{1ij} \ldots x_{pij}$ covariates (predictor variables, possibly categorical)

ξ_{ij} error term, $\xi_{ij} \sim N(0, \sigma^2)$

Linear mixed effects model

But unrealistic that ξ_{ij} is independent of x_{ij}

Define $\xi_{ij} = \varsigma_j + \varepsilon_{ij} \quad \varsigma_j \sim \mathrm{N}(0, \psi) \quad \varepsilon_{ij} \sim \mathrm{N}(0, \theta)$

$$y_{ij} = \beta_0 + \beta_1 \cdot x_{2ij} + \ldots + \beta_p \cdot x_{pij} + \varsigma_j + \varepsilon_{ij}$$

$$y_{ij} = (\beta_0 + \varsigma_j) + \beta_2 \cdot x_{2ij} + \ldots + \beta_p \cdot x_{pij} + \varepsilon_{ij}$$

Compare variance components

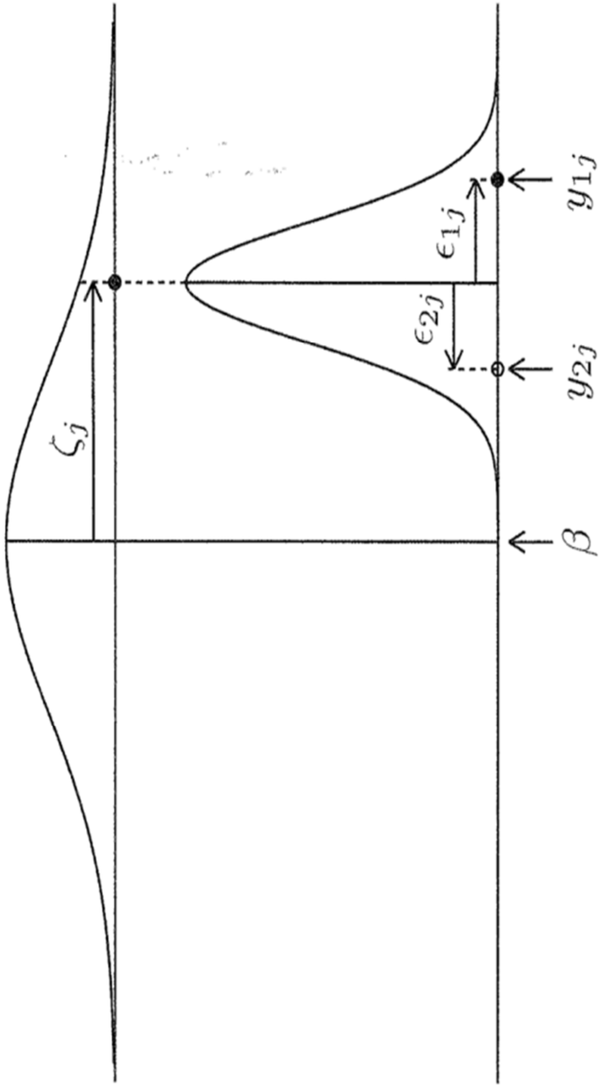
$$y_{ij} = \beta + \varsigma_j + \varepsilon_{ij}$$

$i =$ children nested within mother

$j =$ mother

$\varsigma_j \sim N(0,\psi)$ $\psi:$ between-subject variance

$\varepsilon_i \sim N(0,\theta)$ $\theta:$ within-subject variance



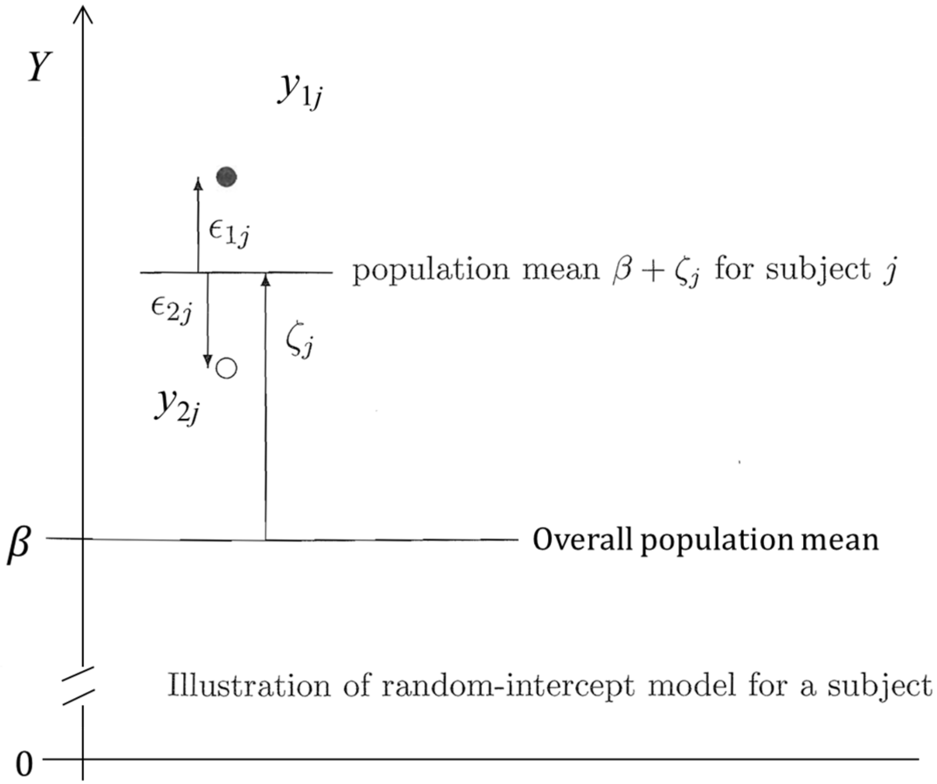


Illustration of random-intercept model for a subject j

Textbook ; figures 2.3 and 2.7 combined

Linear mixed effects model

- Involves both fixed and random factors/ effects; is thus *mixed*
- A starting point: grouped or clustered observations of a continuous outcome variable
- The groups are ”internally similar”: observations within the group are correlated
- Groups are ”drawn” at random from the population of similar groups or clusters
- It is the intention to generalize to this population
- Examples:
  - Repeated observations on the same subject
  - Observations on different schools, hospitals, cities, countries…

Winer revisited

- Linear model
- Robust linear model (but this *really* requires large «n»)
- Linear *mixed* model

Winer revisited

- Reaction time (score) with four drugs was measured repeatedly in the same 5 persons:

```
     +------------------------------------------------+
     | person   score_1   score_2   score_3   score_4 |
     |------------------------------------------------|
  1. |      1        30        28        16        34 |
  2. |      2        14        18        10        22 |
  3. |      3        24        20        18        30 |
  4. |      4        38        34        20        44 |
  5. |      5        26        28        14        30 |
     +------------------------------------------------+
```

`Winer 1991 in Stata manual: [R] anova`

```
. use winer, clear
(T4.3 -- Winer, Brown, Michels)

. list

     +------------------------+
     | person   drug    score |
     |------------------------|
  1. |      1      1       30 |
  2. |      1      2       28 |
  3. |      1      3       16 |
  4. |      1      4       34 |
  5. |      2      1       14 |
     |------------------------|
  6. |      2      2       18 |
  7. |      2      3       10 |
  8. |      2      4       22 |
  9. |      3      1       24 |
 10. |      3      2       20 |
     |------------------------|
 11. |      3      3       18 |
 12. |      3      4       30 |
 13. |      4      1       38 |
 14. |      4      2       34 |
 15. |      4      3       20 |
     |------------------------|
 16. |      4      4       44 |
 17. |      5      1       26 |
 18. |      5      2       28 |
 19. |      5      3       14 |
 20. |      5      4       30 |
     +------------------------+
```

```
. regress score i.drug

      Source |       SS       df       MS              Number of obs =      20
-------------+------------------------------           F(  3,    16) =    4.69
       Model |       698.2     3  232.733333           Prob > F      =  0.0155
    Residual |       793.6    16        49.6           R-squared     =  0.4680
-------------+------------------------------           Adj R-squared =  0.3683
       Total |      1491.8    19  78.5157895           Root MSE      =  7.0427

------------------------------------------------------------------------------
       score |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        drug |
          2  |        -.8   4.454211    -0.18   0.860    -10.24251    8.642507
          3  |      -10.8   4.454211    -2.42   0.028    -20.24251   -1.357493
          4  |        5.6   4.454211     1.26   0.227    -3.842507    15.04251
             |
       _cons |       26.4   3.149603     8.38   0.000     19.72314    33.07686
------------------------------------------------------------------------------

. margins i.drug

Adjusted predictions                              Number of obs   =         20
Model VCE    : OLS

Expression   : Linear prediction, predict()

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        drug |
          1  |       26.4   3.149603     8.38   0.000     20.22689    32.57311
          2  |       25.6   3.149603     8.13   0.000     19.42689    31.77311
          3  |       15.6   3.149603     4.95   0.000     9.426891    21.77311
          4  |         32   3.149603    10.16   0.000     25.82689    38.17311
------------------------------------------------------------------------------
```

```
. regress score i.drug, robust cluster(person)

Linear regression                                      Number of obs =      20
                                                       F(  3,     4) =   47.85
                                                       Prob > F      =  0.0014
                                                       R-squared     =  0.4680
                                                       Root MSE      =  7.0427

                                (Std. Err. adjusted for 5 clusters in person)
------------------------------------------------------------------------------
             |               Robust
       score |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        drug |
          2  |        -.8   1.770593    -0.45   0.675    -5.715955    4.115955
          3  |      -10.8   2.808024    -3.85   0.018    -18.59633   -3.003675
          4  |        5.6   .8154753     6.87   0.002     3.335878    7.864122
             |
       _cons |       26.4   4.270831     6.18   0.003     14.54227    38.25773
------------------------------------------------------------------------------

. margins i.drug

Adjusted predictions                              Number of obs   =         20
Model VCE    : Robust

Expression   : Linear prediction, predict()

------------------------------------------------------------------------------
             |            Delta-method
             |     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        drug |
          1  |       26.4   4.270831     6.18   0.000     18.02932    34.77068
          2  |       25.6    3.18826     8.03   0.000     19.35113    31.84887
          3  |       15.6   1.874833     8.32   0.000     11.92539    19.27461
          4  |         32   3.898718     8.21   0.000     24.35865    39.64135
------------------------------------------------------------------------------
```

```
. xtmixed score i.drug || person:

Performing EM optimization:

Performing gradient-based optimization:

Iteration 0:   log likelihood = -55.795093
Iteration 1:   log likelihood = -55.795093

Computing standard errors:

Mixed-effects ML regression                     Number of obs      =        20
Group variable: person                          Number of groups   =         5

                                                Obs per group: min =         4
                                                               avg =       4.0
                                                               max =         4


                                                Wald chi2(3)       =     92.85
Log likelihood = -55.795093                     Prob > chi2        =    0.0000

------------------------------------------------------------------------------
       score |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
        drug |
          2  |        -.8   1.734358    -0.46   0.645     -4.19928     2.59928
          3  |      -10.8   1.734358    -6.23   0.000    -14.19928    -7.40072
          4  |        5.6   1.734358     3.23   0.001      2.20072     8.99928
             |
       _cons |       26.4   2.817092     9.37   0.000      20.8786     31.9214
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
person: Identity             |
                   sd(_cons) |   5.670981   1.899121      2.941741    10.93231
-----------------------------+------------------------------------------------
                sd(Residual) |   2.742261   .5006661       1.91735    3.922078
------------------------------------------------------------------------------
LR test vs. linear regression: chibar2(01) =    18.78 Prob >= chibar2 = 0.0000
```

```
. margins i.drug

Adjusted predictions                              Number of obs   =         20

Expression   : Linear prediction, fixed portion, predict()

─────────────┬────────────────────────────────────────────────────────────────
             │            Delta-method
             │     Margin   Std. Err.      z    P>|z|     [95% Conf. Interval]
─────────────┼────────────────────────────────────────────────────────────────
        drug │
          1  │       26.4   2.817092     9.37   0.000      20.8786     31.9214
          2  │       25.6   2.817092     9.09   0.000      20.0786     31.1214
          3  │       15.6   2.817092     5.54   0.000      10.0786     21.1214
          4  │         32   2.817092    11.36   0.000      26.4786     37.5214
─────────────┴────────────────────────────────────────────────────────────────
```

Birthweight and smoking

Dataset «smoking»,Textbook pp. 91 →