4. Special designs
4.2.- Cross-over

Medical Statistics

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Outline

1. Introduction
2. Aliased effects
3. Estimating the direct effect
4. Estimating the period effect
5. Estimating the carry-over effect
6. Sample size and Final remarks

What is a *Crossover*?

Studies with repeated intervention.
Each case receives various treatments in different order (or place)
Enables examination of treatment differences "within" cases.
More than 1 sequence is required to make a 'cross-over'.
Cases are assigned to sequences, not treatments.

Favorable aspects

Very intuitive for controlling individual effect:
- Decreases the variability (removes between-units variance)
- Reduces the potential bias of non-comparable groups.

Allows estimation of the effect of A in subjects who previously received B:
- Effect of double dose if the current one is not effective?
- Interchangeability of alternative treatments?

Unfavorable aspects

What about the late or delayed effects of previous treatment?
- *Carry-over*: persistence of an effect beyond its period.

As treatments cannot be administered in the exact or identical way, the period (or place of administration) must be balanced

Losses, drop-outs and missing data:
- Unbalanced groups
- How to analyze spontaneous cross-over?

Benefit in efficiency depends on the intra-variance case:
- If it is low, little benefit for adjusting it
- If it is too high, it may not be as efficient as expected

Conditions for designing a cross-over

- Stable chronic disease
- Palliative care, not curative: patients return to baseline
- No carry-over effect: enough washing period
- Expected losses negligible

Examples: asthma, angina, or diabetes.

Clinician should assess whether these conditions met
Statistician should thoroughly analyze possible transgressions.
A carry-over effect would practically invalidate the study!

NOTE: Adverse effects (eg, vomiting) may be learned and inherited
Wash them with time and innocuous treatments

2x2 cross-over design

Effects: τ_t treatment with two levels: t = A, B
π_j period with two levels: j = 1,2
O_k order or sequence, with two levels: k = AB, BA

Sequence	Case	Period 1		Period 2	
		A	B	A	B
1: AB	1 2 .. n	1 2 .. n			1 2 .. n
2: BA	n+1 n+2 .. n+m		n+1 n+2 .. n+m	n+1 n+2 .. n+m	

Table 2x2x2

8 possible combinations or boxes, but only 4 observables (shaded combinations are impossible).

Med. Stat: Cross-over

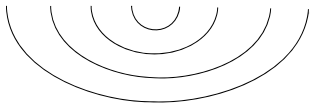
Effect contrast would be:

	μ	τ	π	O	ττ	τO	πO	τπO
m_{A11}	+	+	+	+	+	+	+	+
m_{B11}	+	-	+	+	-	-	+	-
m_{A21}	+	+	-	+	-	+	-	-
m_{B21}	+	-	-	+	+	-	-	+
m_{A12}	+	+	+	-	+	-	-	-
m_{B12}	+	-	+	-	-	+	-	+
m_{A22}	+	+	-	-	-	-	+	+
m_{B22}	+	-	-	-	+	+	+	-

$\mathbf{m_{tjk}}$: mean in treatment **t**, period **j** and sequence **k**

But means m_{B11}, m_{A21}, m_{A12}, m_{B22} cannot be observed

	μ	τ	π	O	ττ	τO	πO	τπO
m_{A11}	+	+	+	+	+	+	+	+
m_{B21}	+	-	-	+	+	-	-	+
m_{B12}	+	-	+	-	-	+	-	+
m_{A22}	+	+	-	-	-	-	+	+



some factors are aliased

Med. Stat: Cross-over

The 2x2 cross-over has some **aliased effects**: $\tau = \pi O$
$\pi = \tau O$
$O = \tau\pi$
$\tau\pi O = \mu$

Some have to be 0 in order to be able to estimate the others

Because of randomization of sequences, any effect implying sequence **O** is known to be 0, so that $0 = O = \pi O = \tau O = \tau\pi O$

Then, $\tau, \pi, \tau\pi$ and μ can be estimated.

But, there is a further effect: the residual, delayed treatment effect from one period to another ('carry-over'): if there are late effects, the differences in the second period also contain their carry-over effects from the first period.

Med. Stat: Cross-over

Let be $Y_{tji} = \mu + \tau_t + \pi_j + O_k + \alpha_i + \varepsilon_{ij}$

where:

μ	is the overall mean	
τ_t	is the fixed direct (instant) treatment effect **t=A,B**	
π_j	is the fixed period effect **j=1,2**	
O_k	is the fixed delayed (carry-over) previous treatment effect **k=A,B**	
α_i	is the random effect of individual or case **i=1,2,...,.n+m**	$\alpha \rightarrow N(0,\sigma^2_\alpha)$
ε_{ij}	is the random effect of individual **i** in period **j**	$\varepsilon \rightarrow N(0,\sigma^2_\varepsilon)$

Notes

1) It is expected that $\sigma^2_\alpha > \sigma^2_\varepsilon$
2) No carry-over effect in the first period
3) Carry-over effect is indexed with sequence **k**

Med. Stat: Cross-over

Inference for the direct treatment effect τ_t

Let d_i be the difference between the first and the second period:

In group 1, with AB order: $d_{i1} = Y_{iA1k} - Y_{iB2k}$

In group 2, with BA order: $d_{i2} = Y_{iB1k} - Y_{iA2k}$

With expected values:

$$E(d_{i1}) = E(Y_{iA1k} - Y_{iB2k}) = E(Y_{iA1k}) - E(Y_{iB2k}) = [\mu + \tau_A + \pi_1] - [\mu + \tau_B + \pi_2 + O_A]$$
$$= (\tau_A - \tau_B) + (\pi_1 - \pi_2) - O_A$$

$$E(d_{i2}) = E(Y_{iB1k} - Y_{iA2k}) = E(Y_{iB1k}) - E(Y_{iA2k}) = [\mu + \tau_B + \pi_1] - [\mu + \tau_A + \pi_2 + O_B]$$
$$= (\tau_B - \tau_A) + (\pi_1 - \pi_2) - O_B$$

$$E(d_{i1} - d_{i2}) = E(d_{i1}) - E(d_{i2}) = 2(\tau_A - \tau_B) - (O_A - O_B)$$

Med. Stat: Cross-over

We test **H:** $\tau_A = \tau_B$

with difference $\mathbf{d_i}$

Since $\mathbf{d_i}$ already accounts for matching, we may test **H:** $\tau_A = \tau_B$ with and **independent** t-test to compare $\mathbf{d_i}$ in both sequences formed by different individuals.

To estimate $\tau_A = \tau_B$ effect, results are **divided by 2**:

Since each case provides information on both treatments, $\mathbf{d_i}$ expectancy doubles

We must assume that $\mathbf{O_A = O_B}$ (or both are null)

This is a prior premise: null or equal residual effect. Wash-out periods between treatments would improve assumption credibility.

Med. Stat: Cross-over

Example: Effect of two anti-inflammatory drugs (A, B) in gastric bleeding, quantified by radioactive methods. Two groups of 8 patients received both in different sequences (AB or BA) with an intermediate wash-out period.

The table shows the means and standard deviations of the variable $d_{i.} = Y_{i:1k} - Y_{i:2k}$

Order	n	m_d	S_d
AB	8	1.4875	1.4904
BA	8	-0.3625	1.3575

Assuming normality and homoscedasticity of the differences $\mathbf{d_{i.}}$, test the direct treatment effect by the Student (independent) t-test:

$S^2 = (7\times1.4904^2+7\times1.3575^2)/14] = 1.4255^2$ Pooled variance estimation

$t = [1.4875-(-0.3625)] / [1.4255\sqrt{(1/8+1/8)}] = 2.6 > t_{14,0'975}=2.145$ and H is rejected

And the treatment effect 95% confidence interval $_{95\%}$CI is:

$$½[1.4875-(-0.3625)] \pm ½\cdot2.145[1.4255\sqrt{(1/8+1/8)}] \rightarrow 0.161 , 1.689$$

$$\Rightarrow IC_{0.95}\ (\tau_A - \tau_B) = [\ 0.161 , 1.689]$$

Inference for the period effect π_j

Sum of both d_i has expectation:

$$E(d_{i1} + d_{i2}) = E(d_{i1}) + E(d_{i2}) = 2(\pi_1 - \pi_2) - (O_A + O_B)$$

So, assuming no (or equal) carry-over effects [$O_A = O_B = 0$],
equality of period effects $\pi_1 = \pi_2$ can again be tested
by Student's (independent) t-test,
but only by previously changing the sign of one of the two sequences
[Or adding means instead of subtracting.]

Example (cont):

To test the period effect:

$t = [1.4875+(-0.3625)]/1.4255\sqrt{(1/8+1/8)} =$
$= 1.58 < t_{14,0'975}=2.145$
No significant

Inference for previous treatment carry-over effect O_k

Let s_i be the sum of first and second period outcome :
In group 1, with AB order: $s_{i1} = Y_{iA1k} + Y_{iB2k}$
In group 2, with BA order: $s_{i2} = Y_{iB1k} + Y_{iA2k}$

And expectations:

$$E(s_{i1}) = E(Y_{iA1k} + Y_{iB2k}) = E(Y_{iA1k}) + E(Y_{iB2k}) = [\mu + \tau_A + \pi_1] + [\mu + \tau_B + \pi_2 + O_A]$$
$$= 2\mu + \tau_A + \tau_B + \pi_1 + \pi_2 + O_A$$

$$E(s_{i2}) = E(Y_{iB1k} + Y_{iA2k}) = E(Y_{iB1k}) + E(Y_{iA2k}) = [\mu + \tau_B + \pi_1] + [\mu + \tau_A + \pi_2 + O_B]$$
$$= 2\mu + \tau_A + \tau_B + \pi_1 + \pi_2 + O_B$$

$$E(s_{i1} - s_{i2}) = E(s_{i1}) - E(s_{i2}) = (O_A - O_B)$$

The equality of both carry-over effects [$O_A = O_B$] can be tested with the (independent Student) t-test of s_i in both sequences

Example (cont)

The table shows means and standard deviations of s_i in both sequences :

Order	n	m_S	S_S
AB	8	7.2625	3.1645
BA	8	6.1375	2.5309

The t-test is: $t = (7.2625-6.1375)]/2.8653\sqrt{(1/8+1/8)} = 0.79$

[being $2.8653^2=(3.1645^2+2.5309^2)/2$]

Non significant, since $0.79 < < t_{14,0'975}=2.145$

Note the variance values for **d** (1.4255^2) and **s** (2.8653^2).
Standard deviation of **s** doubles SD of **d**:

less power to test the assumption $\mathbf{O_A = O_B}$

than **H:** $\boldsymbol{\tau_A = \tau_B}$

Comments on the carry-over test:

Let's compute variances of $\mathbf{d_i}$ and $\mathbf{s_i}$:

$d_i=Y_{t1i} - Y_{t'2i}=[\mu + \tau_t + \pi_1 + \alpha_i + \varepsilon_{i1}]-[\mu + \tau_{t'} + \pi_2 + O_k + \alpha_i + \varepsilon_{i2}]$

$=[\tau_t - \tau_{t'}]+[\pi_1-\pi_2]+O_k+[\varepsilon_{i1}-\varepsilon_{i2}]$ where τ_t , $\tau_{t'}$, π_1 , π_2 and O_k are fixed effects: var=0, but ε_{i1} and ε_{i2} are random variables

$\mathbf{V(d_i) =} V([\tau_t - \tau_{t'}] + [\pi_1 - \pi_2] + O_k + [\varepsilon_{i1} - \varepsilon_{i2}]) = V[\varepsilon_{i1} - \varepsilon_{i2}] \mathbf{= 2\,\sigma^2_\varepsilon}$

$S_i = Y_{t1i} + Y_{t'2i} = [\mu + \tau_t + \pi_1 + \alpha_i + \varepsilon_{i1}]+[\mu + \tau_{t'} + \pi_2 + O_k + \alpha_i + \varepsilon_{i2}]$

$= 2\mu + [\tau_t + \tau_t'] + [\pi_1 + \pi_2] + O_k + 2\alpha_i + [\varepsilon_{i1} + \varepsilon_{i2}]$

$\mathbf{V(s_i) =} V(2\mu + [\tau_t + \tau_{t'}] + [\pi_1 + \pi_2] + O_k + 2\alpha_i + [\varepsilon_{i1} + \varepsilon_{i2}]) =$

$= V[2_{\alpha i} + \varepsilon_{i1} + \varepsilon_{i2}] \mathbf{= 4\,\sigma^2_\alpha + 2\sigma^2_\varepsilon}$

$\mathbf{V(s_i) = 4\,\sigma^2_\alpha + 2\sigma^2_\varepsilon >> 2\,\sigma^2_\varepsilon = V(d_i)}$

Higher variance and less power to test the assumption $\mathbf{P: O_A = O_B}$

than the hypothesis $\mathbf{H: \tau_A = \tau_B}$

Wrong strategy.

First test the carry-over effect and
- a) If not significant: test direct effect with d_i
- b) If significant: test direct effect with first period data
  (and discard second period 'contaminated' data by carry-over)

Wrong because:
1) you should then design C-O with power to test with unpaired first period data (loosing mean C-O advantage)

2) The sum $S_i = Y_{t1i} + Y_{t'2i}$ and the first period data Y_{t1i} are correlated: a significant carry-over effect increases the likelihood of a significant direct effect

See section 3.14 of Senn S., 1993, Cross-over Trials in Clinical Research, Wiley.

Sample size to compare 2 means (independent samples)

Reminder

$$\begin{cases} H0: \mu A - \mu B = 0 \\ H1: \mu A - \mu B = \Delta \end{cases}$$

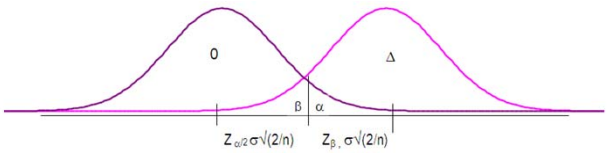
Let be: alpha risk, one or two-sided (usually $\alpha = 0'05$, two-sided),

some desired power (usually $1-\beta = 0'8$),

some given standard deviation (σ), and

some ~~expected~~ effect size Δ to be tested

$V(YA - YB) = V(YA) + V(YB) = \sigma^2/n1 + \sigma^2/n2 = 2\sigma^2/n$ (given n1 = n2)



$\Delta = Z\alpha/2\ \sigma\sqrt{(2/n)} + Z1\text{-}\beta\ \sigma\sqrt{(2/n)}$

And so, each sample size should be: $n = [\ 2\ \sigma^2\ (Z\alpha/2 + Z1\text{-}\beta)^2\ ]\ /\ \Delta^2$

Med. Stat: Cross-over

Now, same α, β and effect Δ. If, for simplicity, $n_1 = n_2 = N/2$ for each sequence, then:

$d_{ik} = Y_{it1k} - Y_{it'2k} \Rightarrow V(d_{ik}) = 2\sigma^2_\varepsilon$

$E[\underline{d}_{i1} - \underline{d}_{i2}] = 2(\tau_A - \tau_B) \Rightarrow (\underline{\tau}_A - \underline{\tau}_B) = ½ (\underline{d}_{i1} - \underline{d}_{i2})$

$V[½ (\underline{d}_{i1} - \underline{d}_{i2})] = ¼ [V(\underline{d}_{i1}) + V(\underline{d}_{i2})] = ¼ \cdot 2 [2\sigma^2_\varepsilon / (N/2)] = ¼ [8\sigma^2_\varepsilon / N] = \mathbf{2\sigma^2_\varepsilon / N}$

$\Delta = Z_{\alpha/2}\, \sigma_\varepsilon \sqrt{(2/N)} + Z_\beta\, \sigma_\varepsilon \sqrt{(2/N)} \Rightarrow N = [2\, \sigma^2_\varepsilon\, (Z_{\alpha/2} + Z_\beta)^2] / \Delta^2$

Note that the formula is identical to the previous, but:
- The error refers to the within-subject variability
- 'N' is now the total 'N' (each observation brings the two values)

Example: How many cases need a parallel and a cross-over design if

$\Delta = 5u$, $\sigma^2_\alpha = (9u)^2$, $\sigma^2_\varepsilon = (4u)^2$, $\alpha = 0'05$ two-sided and $\beta = 0'2$?

$n = [2 \cdot (9^2 + 4^2)(1'96 + 0'84)^2] / 5^2 \approx 60'84 \rightarrow 61$ cases per group

$N = [2 \cdot (4^2)(1'96 + 0'84)^2] / 5^2 \approx 10'04 \rightarrow 11$ total cases $\rightarrow 6$ per sequence