Univerza v Ljubljani
Fakulteta za farmacijo

Biološka ekvivalenca
Statistične metode

Iztok Grabnar

Definicije

EMEA: Note for guidance on the investigation of bioavailability and bioequivalence

- **Biološka uporabnost**

  Biovailability means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action.

- **Bioekvivalenca**

  Absence of significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

- **Terapevtska ekvivalenca**

  A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and, clinically, shows the same efficacy and safety as that product, whose efficacy and safety has been established.

Terapevtska ekvivalenca

- EMEA
  - A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and, clinically, shows the same efficacy and safety as that product, whose efficacy and safety has been established.

Dokaz bioekvivalence

- **Farmakokinetična raziskava**
- **Farmakodinamična raziskava**
- **Klinična raziskava**
- ***In Vitro* raziskava**

Generično zdravilo

Farmacevtska ekvivalenca	+	Biološka ekvivalenca	=	Terapevtska ekvivalenca
Enaka učinkovina Enaka jakost Enak način aplikacije/ farmacevtska oblika Primerljivo navodilo		Enaka biološka uporabnost		Enaka učinkovitost in varnost Medsebojna zamenljivost

Vloga raziskav biološke ekvivalence

- Nadomesti klinične raziskave učinkovitosti in varnosti – pridobitev dovoljenja za promet generičnega zdravila
- Potrditev enake učinkovitosti in varnosti po spremembi zdravila (SUPAC)

Testno in referenčno zdravilo

- Referenčno zdravilo
- Klinično dokazana učinkovitost in varnost

Protokol raziskave bioekvivalence

BIOEQUIVALENCE STUDY PROTOCOL

I. Title
- A. Principle investigator (study director)
- B. Project/protocol number and date

II. Study objective

III. Study design
- A. Design
- B. Drug products
  1. Test product(s)
  2. Reference product
- C. Dosage regimen
- D. Sample collection schedule
- E. Housing/confinement
- F. Fasting/meals schedule
- G. Analytical methods

IV. Study population
- A. Subjects
- B. Subject selection
  1. Medical history
  2. Physical examination
  3. Laboratory tests
- C. Inclusion/exclusion criteria
  1. Inclusion criteria
  2. Exclusion criteria
- D. Restrictions/prohibitions

V. Clinical procedures
- A. Dosage and drug administration
- B. Biological sampling schedule and handling procedures
- C. Activity of subjects

VI. Ethical considerations
- A. Basic principles
- B. Institutional review board
- C. Informed consent
- D. Indications for subject withdrawal
- E. Adverse reactions and emergency procedures

VII. Facilities

VIII. Data analysis
- A. Analytical validation procedure
- **B. Statistical treatment of data**

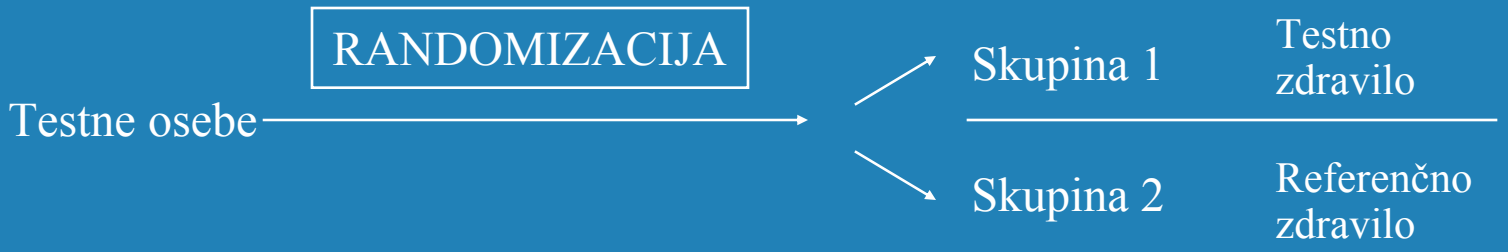
IX. Drug accountability

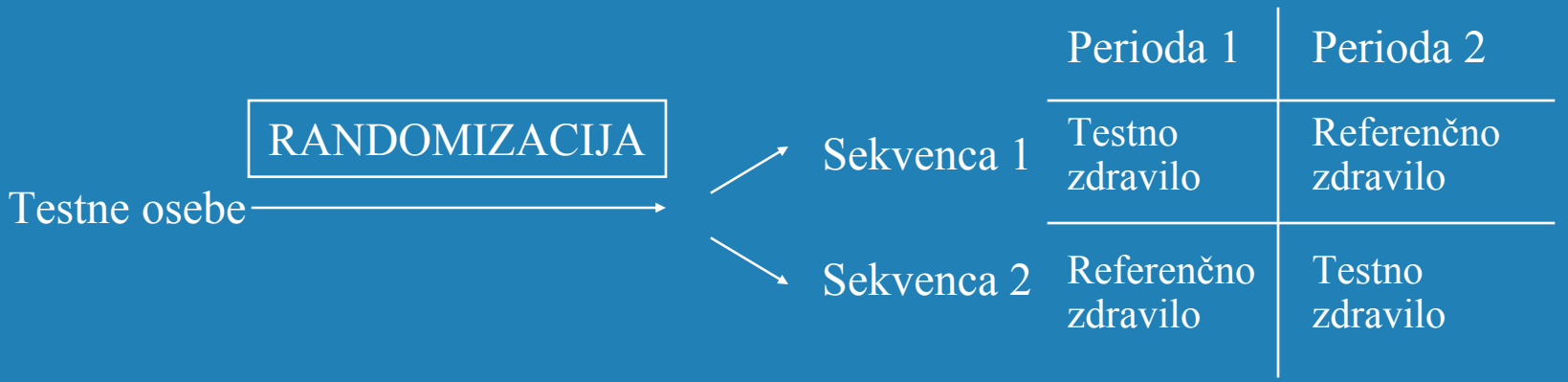
X. Appendix

Načrt raziskave

- Vrsta raziskave
  - eno ali več testnih zdravil
  - en odmerek ali več - stacionarno stanje
  - na tešče ali s hrano
- Eksperimentalni načrt
  - Vzporedni (parallel) ali navzkrižni (crossover)
  - Ponovljena navzkrižna raziskava

Vzporedni in navzkrižni eksperimentalni načrt





Navzkrižni eksperimentalni načrt

- Vsak osebek dobi testno in referenčno zdravilo
- Vsak osebek je sam sebi kontrola - odstranimo vpliv interindividualne variabilnosti
- Najboljša ocena za razliko v biološki uporabosti

Ponovljeni navzkrižni eksperimentalni načrt

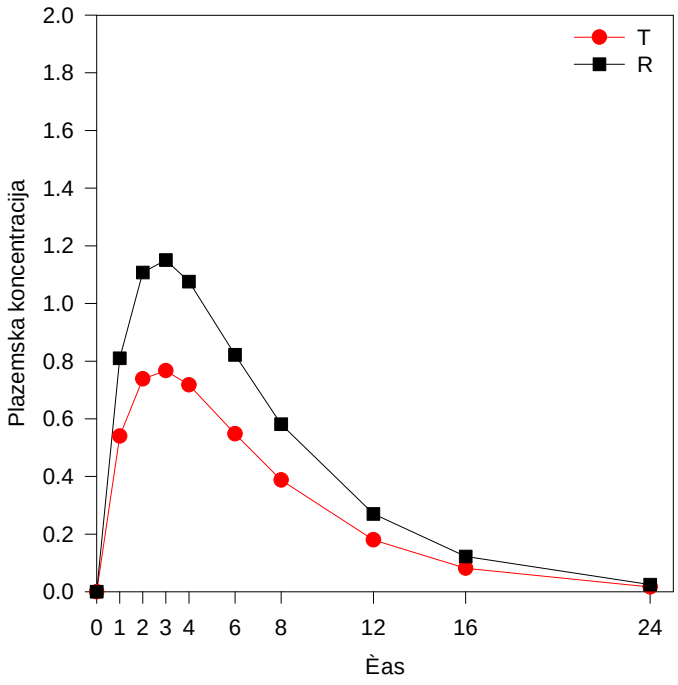
Sekvenca	Perioda	
	I	II
1	T	T
2	R	R
3	T	R
4	R	T

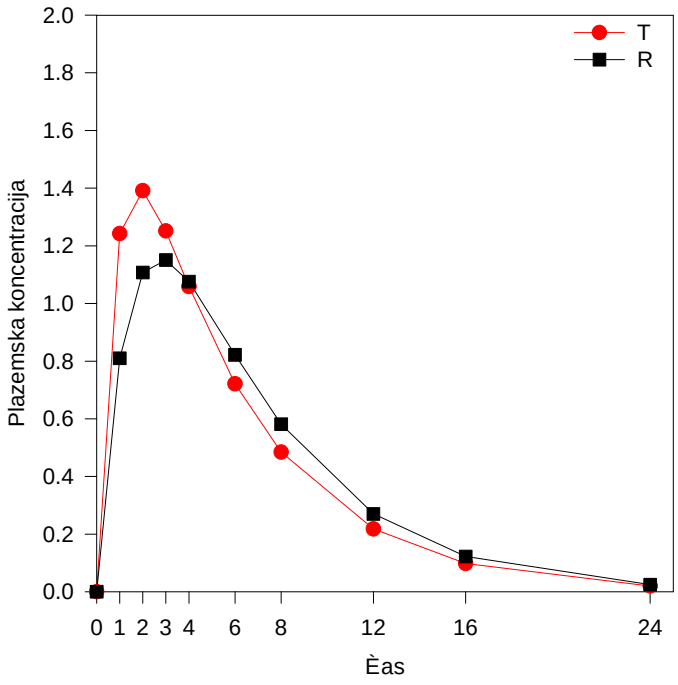
Sekvenca	Perioda		
	I	II	III
1	T	R	R
2	R	T	T

Sekvenca	Perioda			
	I	II	III	IV
1	T	T	R	R
2	R	R	T	T
3	T	R	R	T
4	R	T	T	R

Farmakokinetična raziskava

- Zdravi prostovoljci
- Navzkrižni (crossover) eksperimentalni načrt
- Doba izpiranja (vsaj 5 $t_{1/2}$)
- 12 – 18 vzorcev krvi (vsaj 3 $t_{1/2}$)
- Analiza vzorcev (validirana metoda)
- Farmakokinetična analiza (določitev parametrov biološke uporabnosti)


Plazemska koncentracija
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
T
R
0 1 2 3 4 6 8 12 16 24
Èas


Plazemska koncentracija
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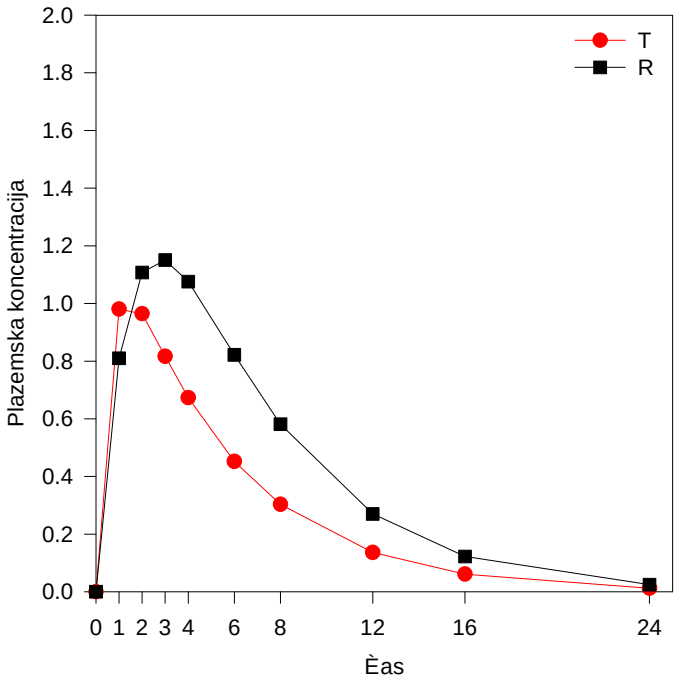
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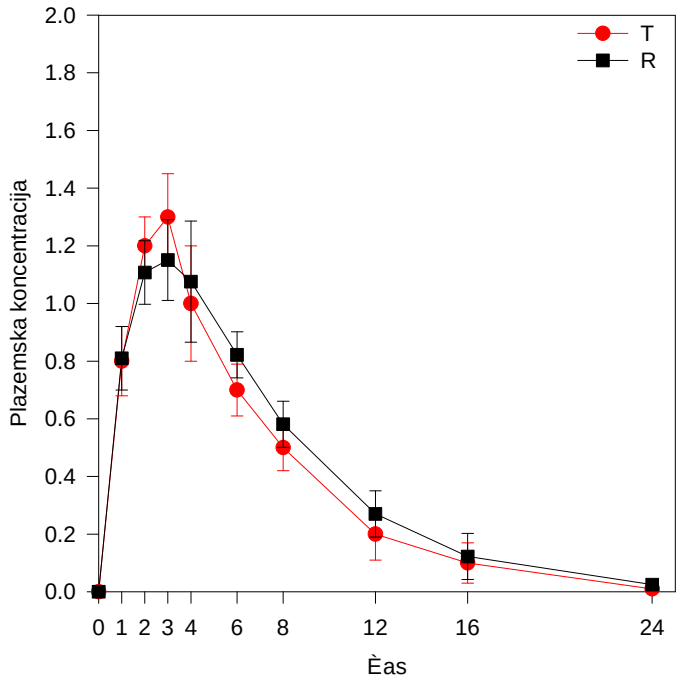
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Plazemska koncentracija
T
R
0 1 2 3 4 6 8 12 16 24
Èas

Pilotna raziskava

- Manjše število subjektov
- Preverjanje ustreznosti analizne metode
- Ocena variabilnosti – velikost vzorca
- Optimizacija časov odvzema vzorcev

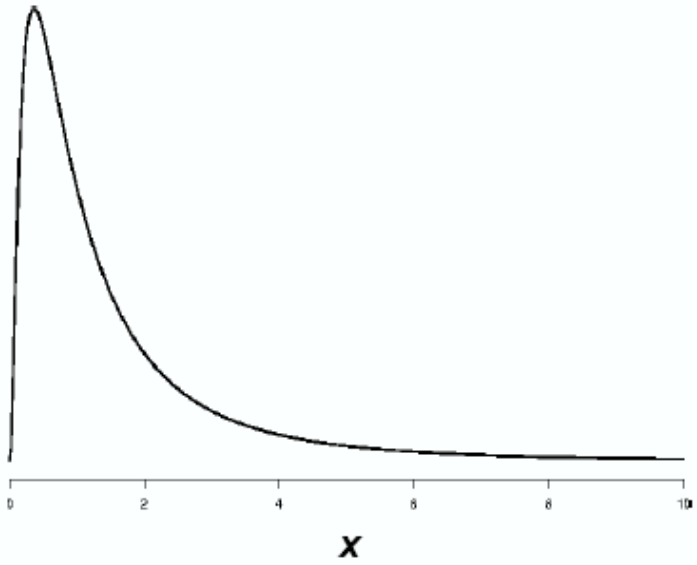
Pristop dokazovanja bioekvivalence

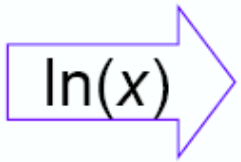
- Bioekvivalenčni kriterij
- Logaritemska transformacija
- statistično sklepanje - Interval zaupanja (90 %) za razmerje parametrov BU
- Limita bioekvivalence

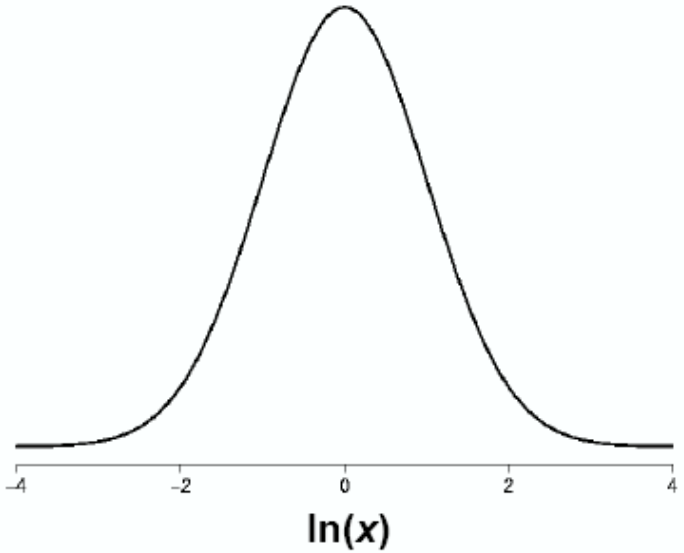
Logaritemska transformacija

Distributions: Normal vs. Lognormal

- If the natural log of a random variable, *X*, is distributed normally ($\ln(X) \sim N(\mu, \sigma^2)$), then *X* has a *lognormal distribution*



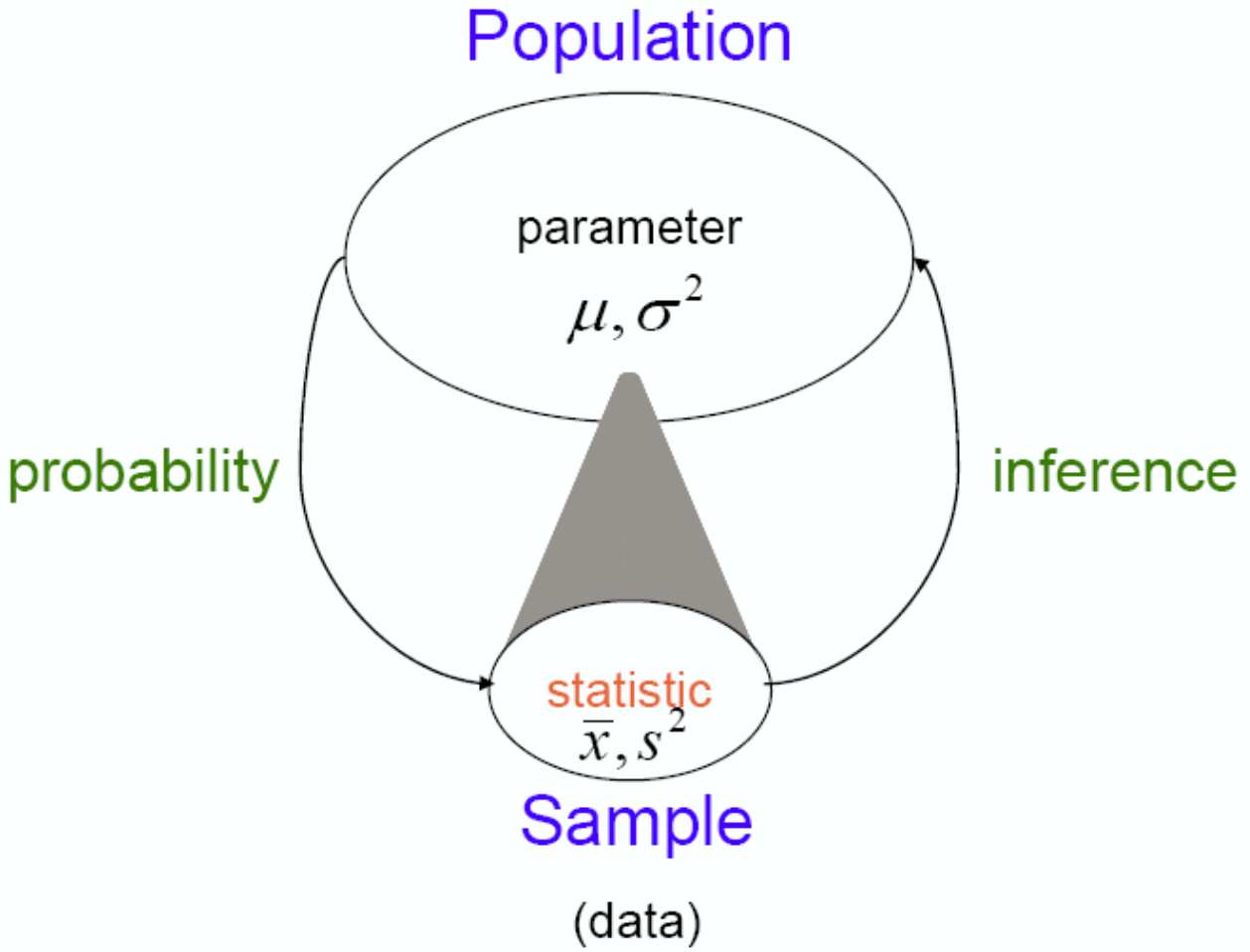


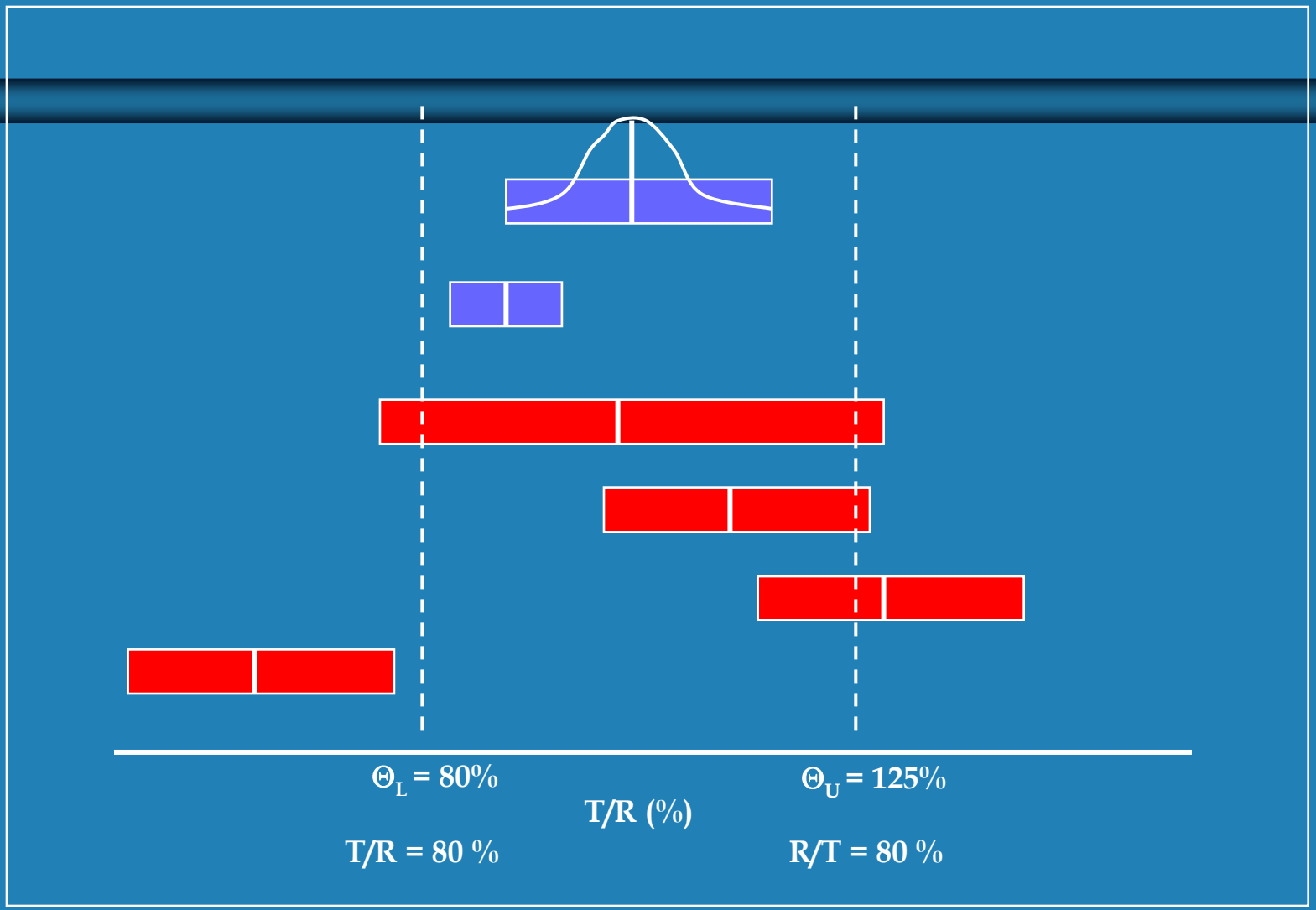


Vzroki log transformacije

- FK parametri so običajno porazdeljeni log-normalno
- FK parametre običajno opisuje multiplikativni model: produkt pretvorimo v vsoto
- Test za razmerje parametrov T in R pretvorimo v test razlike med T in R

Statistično sklepanje




Θ_L = 80%
Θ_U = 125%
T/R (%)
T/R = 80 %
R/T = 80 %

Variabilnost FK parametrov

Vplivi:

- Oseba
- Sekvenca
- Perioda
- Zdravilo
- Preneseni učinek

Statistični model

$$Y_{ijk} = \mu + G_k + S_{ik} + P_j + F_{(j,k)} + C_{(j-1,k)} + e_{ijk}$$

Y_{ijk}	farmakokinetični parameter pri osebku i, v sekvenci k in periodi j
μ	celokupna aritmetična sredina
G_k	vpliv sekvence k (k=1,…,g) $\Sigma G_j = 0$
S_{ik}	vpliv osebka i v sekvenci k (k=1,…,g) - interindividualna variabilnost: $N(0,\sigma_s^2)$
P_j	vpliv periode j (j=1,…,p) $\Sigma P_j = 0$
$F_{(j,k)}$	vpliv zdravila v periodi j in sekvenci k $\Sigma F_{(j,k)} = 0$
$C_{(j-1,k)}$	vpliv "carryover" učinka prvega reda za zdravilo v sekvenci k s periodo j-1 $C_{(0,k)} = 0$ in $\Sigma C_{(j-1,k)} = 0$
e_{ijk}	nepojasnjena varianca - intraindividualna variabilnost: $N(0,\sigma_t^2)$ t = 1,…,l

ANOVA

Table 3.5.1 Analysis of Variance Table for a Standard 2×2 Crossover Design

Source of variation	df	SS	MS = SS/df	E(MS)	F
Intersubjects					
Carryover	1	SS_{carry}	SS_{carry}	$\frac{2n_1n_2}{n_1+n_2}(C_T-C_R)^2+2\sigma_s^2+\sigma_e^2$	$F_C = MS_{carry}/MS_{inter}$
Residuals	n_1+n_2-2	SS_{inter}	SS_{inter}/n_1+n_2-2	$2\sigma_s^2+\sigma_e^2$	$F_V = MS_{inter}/MS_{intra}$
Intrasubjects					
Direct drug	1	SS_{drug}	SS_{drug}	$\frac{2n_1n_2}{n_1+n_2}\left[(F_T-F_R)+\frac{C_R-C_T}{2}\right]^2+\sigma_e^2$	$F_d^* = MS_{drug}/MS_{intra}^a$
Period	1	SS_{period}	SS_{period}	$\frac{2n_1n_2}{n_1+n_2}(P_2-P_1)^2+\sigma_e^2$	$F_p = MS_{period}/MS_{intra}$
Residuals	n_1+n_2-2	SS_{intra}	SS_{intra}/n_1+n_2-2	σ_e^2	
Total	$2(n_1+n_2)-1$	SS_{total}			

[a] F_d^* is valid only if $C_R = C_T$.

Sekvenca	Perioda I	Perioda II
1 (RT)	$\mu_{11} = \mu + P_1 + F_R$	$\mu_{21} = \mu + P_2 + F_T + C_R$
2 (TR)	$\mu_{12} = \mu + P_1 + F_T$	$\mu_{22} = \mu + P_2 + F_R + C_T$

$$\frac{(\mu_{11} - \mu_{21}) - (\mu_{12} - \mu_{22})}{2(F_R - F_T) - (C_R - C_T)}$$

Obrnjeni ničelna in alternativna statistična hipoteza

- Dokaz enakosti
- Običajno gre v kliničnih raziskavah za dokaz različnosti (npr. zdravilo – placebo)

Dvojni Schuirmannov enostranski t-test

$H_{01} : \mu_{\ln T} - \mu_{\ln R} \leq \ln 0.8$ $\quad$ $H_{a1} : \mu_{\ln T} - \mu_{\ln R} > \ln 0.8$

BU_T ni značilno manjša kot BU_R (α=0.05)

$H_{02} : \mu_{\ln T} - \mu_{\ln R} \geq \ln 1.25$ $\quad$ $H_{a2} : \mu_{\ln T} - \mu_{\ln R} < \ln 1.25$

BU_T ni značilno večja kot BU_R (α=0.05)

Interval zaupanja

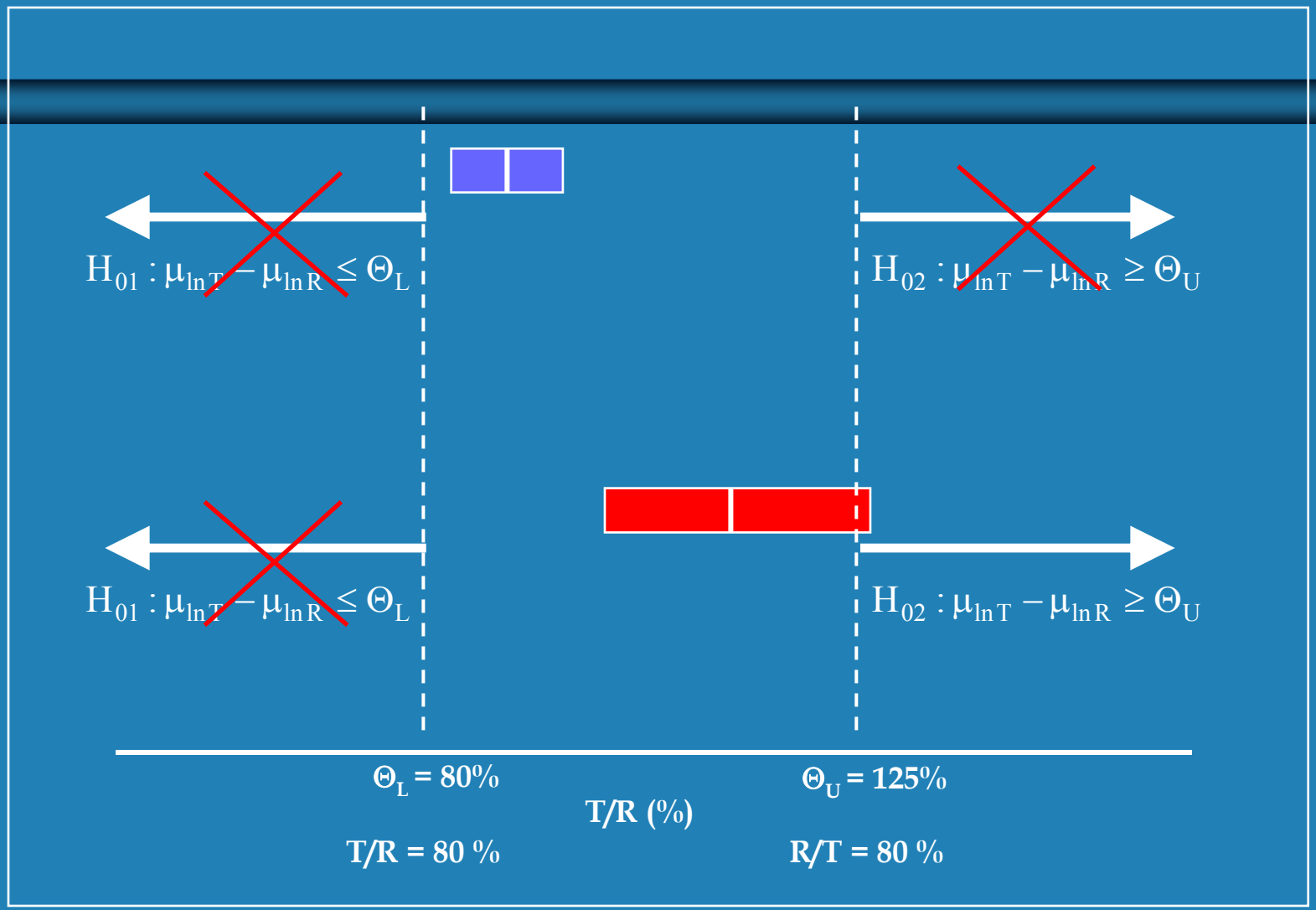
90 % interval zaupanja za razmerje aritmetičnih sredin:

$$(\mu_{\ln T} - \mu_{\ln R}) - t_{0.90,n_1+n_2-2}\sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} < \overline{\Delta} < (\mu_{\ln T} - \mu_{\ln R}) + t_{0.90,n_1+n_2-2}\sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}$$

Dvojni enostranski t-test (α=5%)

$$(\mu_{\ln T} - \mu_{\ln R}) - t_{0.90,n_1+n_2-2}\sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} > \ln 0.8$$

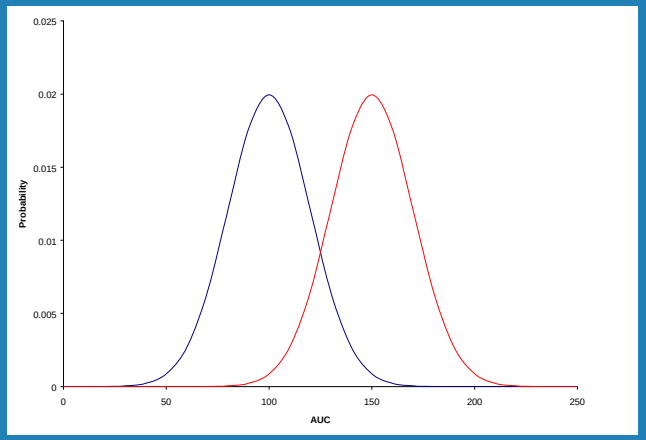
$$(\mu_{\ln T} - \mu_{\ln R}) + t_{0.90,n_1+n_2-2}\sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} < \ln 1.25$$

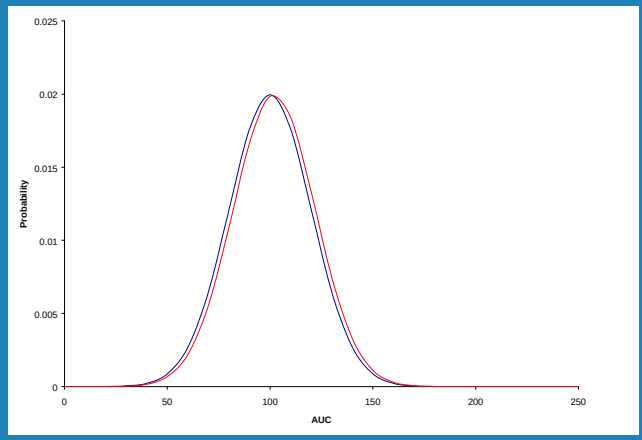

$H_{01} : \mu_{lnT} - \mu_{lnR} \leq \Theta_L$
$H_{02} : \mu_{lnT} - \mu_{lnR} \geq \Theta_U$
$H_{01} : \mu_{lnT} - \mu_{lnR} \leq \Theta_L$
$H_{02} : \mu_{lnT} - \mu_{lnR} \geq \Theta_U$
$\Theta_L = 80\%$
$\Theta_U = 125\%$
T/R (%)
T/R = 80 %
R/T = 80 %

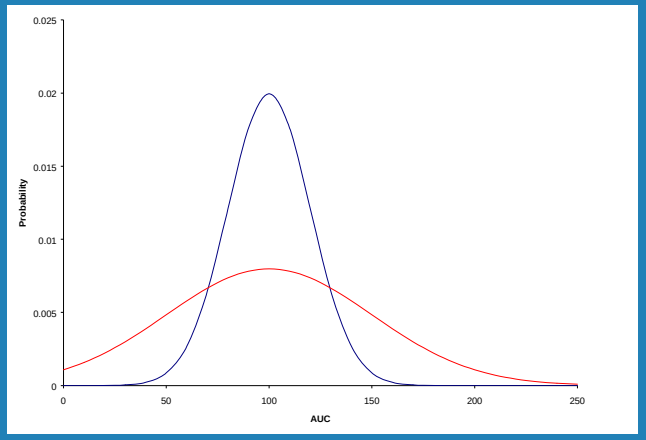
Razvoj raziskav bioekvivalence

- Začetek 20. stoletja - prve raziskave biološke uporabnosti
- Bioekvivalenca
  - 1970-1984 — Drugs price competition and patent term restoration act
  - 1984-1992 — Guidance on statistical procedures for bioequivalence studies using a standard two-treatment crossover design
  - 1992- — Koncept populacijske in individualne bioekvivalence (generično predpisovanje, generična zamenljivost)

Populacijska in individualna bioekvivalenca
Generično predpisovanje in generična zamenjava







?

Primer

Subjekt	Sekvenca	AUC		ln(AUC)	
		Form. T	Form. R	Form. T	Form. R
1	TR	290	210	5,670	5,347
2	RT	201	163	5,303	5,094
3	TR	187	116	5,231	4,754
4	TR	168	77	5,124	4,344
5	RT	200	220	5,298	5,394
6	RT	151	133	5,017	4,890
7	TR	294	140	5,684	4,942
8	RT	97	190	4,575	5,247
9	RT	228	168	5,429	5,124
10	TR	250	161	5,521	5,081
11	TR	293	240	5,680	5,481
12	RT	154	188	5,037	5,236
	Mean	209,42	167,17	5,298	5,078
	sd	63,34	46,31	0,331	0,315

ANOVA

Tests of Between-Subjects Effects

Dependent Variable: LNAUC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SEQENCE	6.130E-02	1	6.130E-02	.46	.513
SUBJECT(SEQUENCE)	1.332	10	.133	2.96	.051
PERIOD	.450	1	.450	10.02	.010
TREATMEN	.290	1	.290	6.44	.029
Error	.450	10	4.496E-02		
Total	2.583	23			

preneseni učinek

$$P\left\{F_{1,10} > \frac{0.0613}{0.1332}\right\} = 0.513$$

ANOVA

Tests of Between-Subjects Effects

Dependent Variable: LNAUC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
SEQENCE	6.130E-02	1	6.130E-02	.46	.513
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TREATMEN	.290	1	.290	6.44	.029
Error	.450	10	4.496E-02		
Total	2.583	23			

$\overline{T} = 5.298$

$\overline{R} = 5.078$

$$(\overline{T} - \overline{R}) \pm t_{0.90,10} \sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

$$(0.22) \pm 1.81 \sqrt{\frac{\sigma_e^{\,2}}{2}\left(\frac{1}{6} + \frac{1}{6}\right)} = (0.063, 0.376)$$

interval zaupanja

(1.065,1.457)