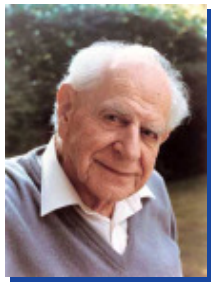
Basic Statistics for BE

Helmut Schütz

Keep in memory…

Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which it was intended to solve.



Karl R. Popper

Even though it's *applied* science we're dealin' with, it still is – *science*!

Leslie Z. Benet

Why logarithmic transformation of the data?

Like most biologic variables PK metrics (*e.g.*, *AUC*, C_{max}) follow a *log-normal* distribution

- If they would follow a *normal distribution* ('bell curve') the range of possible values *by definition* would be [–∞, +∞]
  - However, negative concentrations are not possible
- The *log-normal* distribution covers a range of [>0, +∞]
  - In statistical methods we apply in bioequivalence (*e.g.*, the ANOVA) we need *normal* distributed data
  - If we log-transform the orginal data we get exactly what we need
    - *Always* use the natural logarithm (base e) – not the decadic logarithm (base 10)
  - At the end of the analysis we back-transform the result (*e.g.*, from the 90% confidence interval of [–0.1832, +0.0432] we get [$e^{-0.1832}$, $e^{+0.0432}$] or [83.26%, 104.41%])

Why logarithmic transformation of the data?

Justification

- The basic equation of PK (after an extravascular dose) is
  $AUC = f \times D / CL$
- In BE we are interested in the fraction absorbed (f), which leads to
  $f = AUC \times CL / D$
  - which is a *multiplicative* model
  - We get an *additive* model (needed in ANOVA) by taking logs
    $\log(f) = \log(AUC) + \log(CL) - \log(D)$
- Actually we are interested in comparing f_{Test} with $f_{Reference}$
  - In the study we obtain AUC_{Test} and $AUC_{Reference}$
  - We assume (!) that $D_{Test} = D_{Reference}$ and $CL_{Test} = CL_{Reference}$
  - Given that, we get
    - $\log(f_{Test}) - \log(f_{Reference}) = \log(AUC_{Test}) - \log(AUC_{Reference})$ or
    - $f_{Test} / f_{Reference} = AUC_{Test} / AUC_{Reference}$

Why logarithmic transformation of the data?

Example

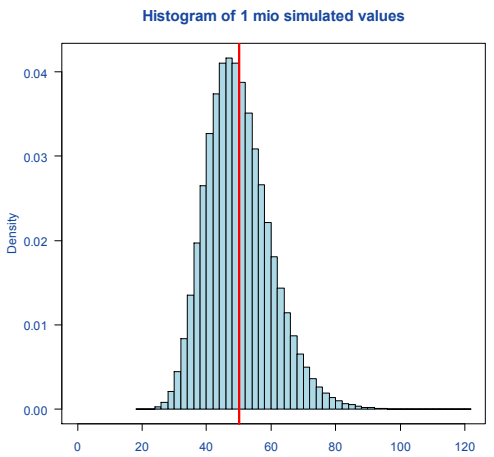
	Reference	log(*R*)	*Test*	log(*T*)	Δ log	Ratio T/R
AUC	200	5.2983	190	5.2470	-0.0513	95.00%
CL	0.2	-1.6094	0.2	-1.6094		
D	50	3.9120	50	3.9120		
f	80%	-0.2231	76%	-0.2744	-0.0513	95.00%

- The Test has a lower absorption (76%) than the Reference (80%)
  - We assume that the administered doses are equal, as are the clearances (property of the drug, not the formulation)
  - Then we can estimate $f_{Test}/f_{Reference}$ from the ratio of *AUC*s or the difference of log-transformed *AUC*s (Δ log)
- Practically the analysis is done on log-transformed data
  - We get $f_{Test}/f_{Reference}$ by the back-transformation of Δ log: $e^{-0.0513} = 95\%$

Why geometric means instead of arithmentic means?

In statistics we need an accurate ('unbiased') estimate of the location

- The best unbiased estimate of the location of the *normal distribution* is the *arithmetic* mean
- The best unbiased estimate of the location of the *log-normal distribution* is the *geometric* mean
  - Since we know that concentrations and most derived PK metrics (exception: t_{max}) follow a *log-normal* distribution we have to use their geometric means
  - The log-normal distribution is skewed to the right
    - The arithmetic mean is always larger than the geometric mean
    - If we would use the arithmetic mean, the estimate would be positively biased



Descriptive statistics (transformed and untransformed)

In order to describe the data accurately we have to use suitable descriptive statistics

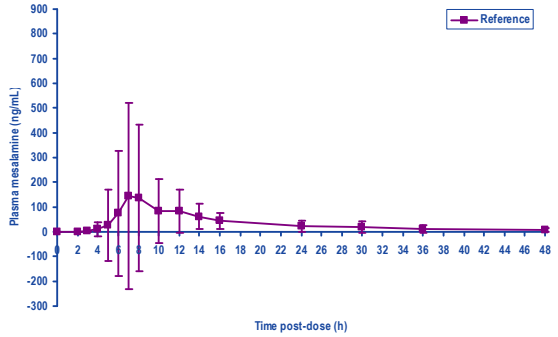
- If we report a certain location (mean, median, …) and a dispersion (standard deviation, CV, percentiles, …) we *implicitly* assume a specific distribution
- Arithmetic mean, standard deviation
  - normal distribution (*wrong* in PK…)
- Geometric mean, CV
  - log-normal distribution (concentrations, C_{max}, *AUC*, …)
  - back-transformed arithmetic mean of log-transformed data = geometric mean of raw data
- Median, percentiles, range
  - discrete distribution (t_{max}, t_{lag})

	raw	log
	1.0000	0.0000
	2.0000	0.6931
	3.0000	1.0986
arithm. mean	2.0000	0.5973
geom. mean	1.8171	
$e^{\text{arithm. mean(log)}}$		1.8171

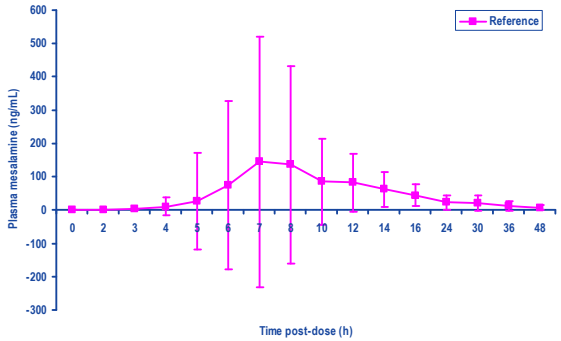
Descriptive statistics (transformed and untransformed)

Bad example from the FDA's files (mesalamine, n = 238)

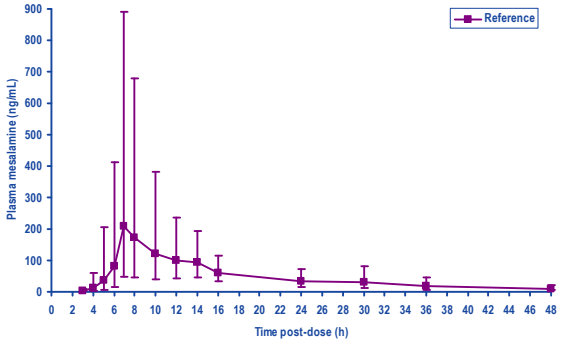
- Wrong: arithmetic means ± SD



line plot instead of XY-plot



- Correct: geometric means ± SD



What does the 90% confidence interval mean?

From the study (in statistical terms a 'sample') we

- estimate a mean treatment effect
  (in BE the point estimate of the Test/Reference ratio)
- The PE is the best unbiased estimate of the treatment effect
  in the population of patients

However, we don't know the 'true' value

- A confidence interval around the PE tells us
  where the 'true' value might be
- If we use a 90% confidence interval, a wrong decision (*i.e.*, falsely declaring BE of a product which is not) is possible with α
  - α is the probability of the Type I Error (the patient's risk) and commonly fixed at 5%
  - The 90% CI is based on 100(1 – 2α)

Excursion: Error(s)

All *formal* decisions are subjected to two 'Types' of Error.

- **α: Probability of Type I Error (aka Risk Type I)**
- **β: Probability of Type II Error (aka Risk Type II)**

Example from the justice system – which presumes that the defendant is *not guilty*:

Verdict	Defendant *innocent*	Defendant *guilty*
Presumption of innocence *rejected* (*guilty*)	wrong	correct
Presumption of innocence *accepted* (*not guilty*)	correct	wrong

Excursion: Hypotheses

In statistical terminology

- Null hypothesis (H_0): innocent
- Alternative hypothesis (H_a aka H_1): guilty

Decision	Null hypothesis *true*	Null hypothesis *false*
H_0 rejected	Type I Error	Correct (accept H_a)
Failed to reject H_0	Correct (accept H_0)	Type II Error

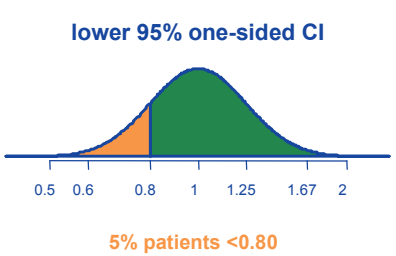
In BE the Null hypothesis is bioinequivalence ($\mu_T \neq \mu_R$)!

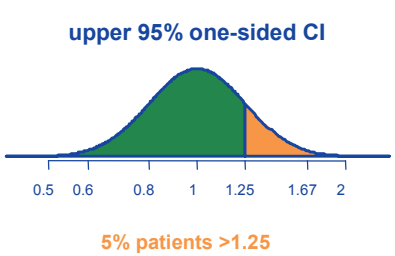
Decision	Null hypothesis *true*	Null hypothesis *false*
H_0 rejected	Patient's risk (α)	Correct (BE)
Failed to reject H_0	Correct (not BE)	Producer's risk (β)

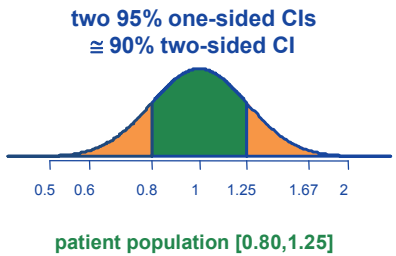
Excursion: Type I Error

α: Patient's risk to be treated with an inequivalent formulation (H_0 *falsely* rejected)

- BA of the test compared to reference in a *particular* patient is considered to be risky *either* below 0.80 *or* above 1.25.
  - If we keep the risk of *particular* patients at α 0.05 (5%), the risk of the entire *population* of patients (where BA <0.80 *and* >1.25) is 2α (10%) – expressed as a confidence interval: 100(1 – 2α) = 90%.
  - However, since in a particular patient BA cannot be <0.80 *and* >1.25 *at the same time*, the patient's risk from a 90% CI is still 5%!



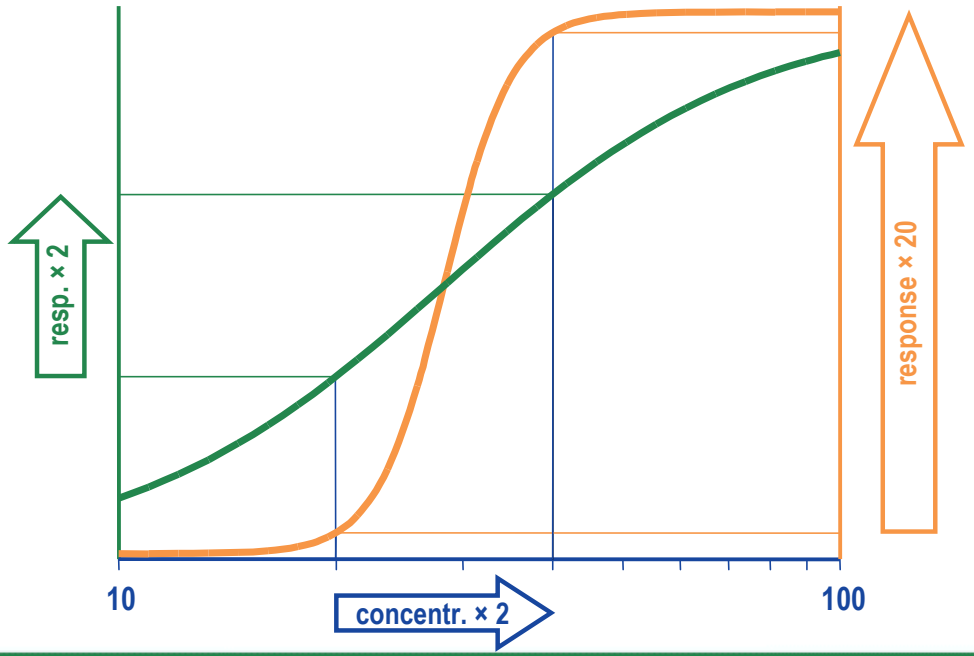




What does ±20% mean and where does it come from?

Clinically not relevant difference

- Based on PK/PD but extrapolated to similarity of safety and efficacy in the patient population
  - Depends on the dose-response curve! NTID (steep curve), HVD (flat curve):



What does ±20% mean and where does it come from?

Clinically not relevant difference

- Predefined by the authority
  - A difference Δ of $\leq$20% is considered to be clinically not relevant for 'uncomplicated drugs'
    - The limits [*L*, *U*] of the acceptance range for BE are fixed to $\log(1-\Delta) = \log((1-\Delta)^{-1})$ or $L \sim -0.2231$ and $U \sim +0.2231$, which are back-transformed 80 – 125%
  - Smaller Δ for Narrow Therapeutic Index Drugs (NTIDs)
    - EMA Δ 10% leads to BE-limits of 90.00 – 111.11%
    - FDA Scaled (narrowed) based on the variability of the reference
  - Larger Δ for Highly Variable Drugs / Drug Products (HVD(P)s)
    - EMA Δ >20% scaled based on the variability of the reference (CV_{wR}), which leads to BE-limits expanded to up to 69.84 – 143.19%
    - HC like EMA, but BE-limits of up to 66.7 – 150.0%
    - FDA Scaled based on the variability of the reference (no upper limit)

What does ±20% mean and where does it come from?

Clinically not relevant difference

- Bioequivalence *is not* a scientific concept
  - state a hypothesis
  - perform experiments in order to challenge the hypothesis
  - accept the hypothesis as long as it is not falsified
- Assuming ±20% to be not clinically relevant was an *ad hoc* concept
- However, empiric evidence of more almost 40 years showed that it 'works' (*"No dead people lie in the streets…"*)
- It is a common misconception that BE-limits of 80–125% can lead to approval of products which differ by 45%
  - A survey of 1,636 BE studies submitted to the FDA within 1996–2005 showed Δ of 3.19% (±2.72) for AUC_t and 4.50% (±3.57) for C_{max}
  - In a strict sense switching *between* generics is *not* supported by (A)BE; nevertheless, it seems to work in practice

Calculation of point estimate and its 90% CI

Example (2×2 crossover, 8 subjects, 1 dropout, CV_{intra} ~10%)

		period								
subject	sequence	1	2	1 (log)	2 (log)			LSM (1)	LSM (2)	
1	TR	92.4	97.1	4.526	4.576		T	4.575	4.448	
2	TR	86.4	98.0	4.459	4.585		LSM (T)	4.511		mean (T) 4.520
4	TR	114.0	97.9	4.736	4.584		GLSM (T)	91.0		g. mean (T) 91.9
7	TR	97.4	94.6	4.579	4.550					
3	RT	100.9	94.9	4.614	4.553		R	4.589	4.574	
5	RT	101.1	71.3	4.616	4.267		LSM (R)	4.581		mean (R) 4.580
6	RT	93.4	92.1	4.537	4.523		GLSM (R)	97.6		g. mean (R) 97.5
8	RT	105.2	–	–	–					

n_1 (sequence TR)	4	degr. of freedom (n_1+n_2–2)	
n_2 (sequence RT)	3	5	
Mean Squared Error (MSE)	0.0108184	(from ANOVA)	
Standard Error (SE) of Δ	0.056173	$= \sqrt{[0.5 \times MSE \times (1/n_1 + 1/n_2)]}$	
$t_{\alpha = 0.05,\ df}$	2.0150		
90% CI = Δ ± $t_{\alpha = 0.05,\ df}$ × SE			
Δ = LSM (T) – LSM (R)	-0.0700	93.24%	PE (GMR = e^{Δ})
lower 90% CL	-0.1832	83.26%	90% CI
upper 90% CL	0.0432	104.41%	

Calculation of point estimate and its 90% CI

Example (2×2 crossover, 8 subjects, 1 dropout, CV_{intra} ~10%)

- Important
  - Always use the Geometric Least Square Means – *not* the geometric means of treatments
    - Only if a design is *balanced*, *i.e.*, there are an equal number of subjects in each sequence, GLSM equals the geometric mean
    - In the example (unbalanced; $n_1 = 4$, $n_2 = 3$):
      LSM (T) 4.511 (GLSM 91.0) → PE 93.24%
      LSM (R) 4.581 (GLSM 97.6)
      mean (T) 4.520 (geom. mean 91.9) → PE 93.19%
      mean (R) 4.580 (geom. mean 97.5)
  - Always use the formula which takes subjects / sequence into account
    - There is a 'simple' formula which is *only* correct if a study is balanced, namely SE = $\sqrt{(MSE/n_{ps})}$, where $n_{ps} = (n_1 + n_2)/2$
    - In the example ($n_{ps} = 3.5$!):
      The 90% CI will be wrong (83.36–104.29% instead of 83.26–104.41%)

Calculation of point estimate and its 90% CI

Where to find the MSE in software's output

- **SAS**

The GLM Procedure

Dependent Variable: AUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	10.8915670	0.5732404	1.86	0.1891
Error	16	4.9439802	0.3089988		
Corrected Total	35	15.8355472			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	1	1.0469949	1.0469949	3.39	0.0843
Period	1	0.1958572	0.1958572	0.63	0.4376
Seqence	1	1.3052864	1.3052864	2.50	0.1332
Subject(Sequence)	16	8.3434285	0.5214643	1.69	0.1528

- **Phoenix/WinNonlin**

WINNONLIN LINEAR MIXED EFFECTS MODELING / BIOEQUIVALENCE
8.0.0.3176
Core Version 30Jan2014

Model Specification and User Settings
Dependent variable: AUC

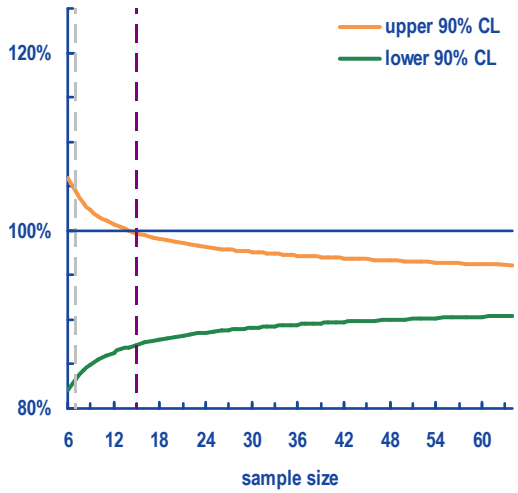
Partial Sum of Squares

Hypothesis	DF	SS	MS	F_stat	P_value
Sequence	1	1.30529	1.30529	2.50312	0.1332
Sequence*Subject	16	8.34343	0.521464	1.68759	0.1528
Treatment	1	1.04699	1.04699	3.38835	0.0843
Period	1	0.195857	0.195857	0.633844	0.4376
Error	16	4.94398	0.308999		

Excursion: Treatment effect

Statistical *significant* ≠ clinically *relevant*

- For any given T/R-ratio and variability one will get a significant treatment effect (in the ANOVA $p < 0.05$) if the sample size is only large enough
  - The confidence interval narrows with $\sqrt{N}$, *i.e.*, if one uses a four times larger sample size, the CI will be ~half as wide
  - If the CI does not include 100% any more, treatments will *significantly* differ
  - However, if the 90% CI is within the acceptance range, this difference is *clinically not relevant*



Excursion: Period effect

In crossover-studies the period effect is not relevant

- Due to the randomization all treatments will be affected by a true period effect to the same degree
- Period effects mean out, *i.e.*, are handled in the ANOVA
- Previous example, all data in the 2nd period multiplied by ten
- Exactly the same PE and 90% CI

		period						
subject	sequence	1	2	1 (log)	2 (log)		LSM (1)	LSM (2)
1	TR	92.4	971	4.526	6.878	T	4.575	6.750
2	TR	86.4	980	4.459	6.888	LSM (T)	5.663	
4	TR	114.0	979	4.736	6.887	GLSM (T)	287.9	
7	TR	97.4	946	4.579	6.852			
3	RT	100.9	949	4.614	6.855	R	4.589	6.876
5	RT	101.1	713	4.616	6.569	LSM (R)	5.733	
6	RT	93.4	921	4.537	6.825	GLSM (R)	308.8	
8	RT	105.2	–	–	–			
		Δ = LSM (T) – LSM (R)		-0.0700	93.24%	PE (GMR = e^{Δ})		
		lower 90% CL		-0.1832	83.26%	90% CI		
		upper 90% CL		0.0432	104.41%			

Excursion: Sequence effect

In crossover-studies an *equal* sequence effect is not relevant

- However, a *true* sequence effect (better: unequal carry-over) *will* bias the treatment effect
- There is no statistical method to correct for unequal carry-over
- Can only be avoided *by design*, *i.e.*, a sufficiently long enough wash-out between periods
- Previous example, unequal carry-over (TR –5, RT +5)
- Biased PE and CI

		period						
subject	sequence	1	2	1 (log)	2 (log)		LSM (1)	LSM (2)
1	TR	92.4	92.1	4.526	4.523	T	4.575	4.505
2	TR	86.4	93.0	4.459	4.533	LSM (T)	4.540	
4	TR	114.0	92.9	4.736	4.532	GLSM (T)	93.7	
7	TR	97.4	89.6	4.579	4.495			
3	RT	100.9	99.9	4.614	4.604	R	4.589	4.521
5	RT	101.1	76.3	4.616	4.335	LSM (R)	4.555	
6	RT	93.4	97.1	4.537	4.576	GLSM (R)	95.1	
8	RT	105.2	–	–	–			
		Δ = LSM (T) – LSM (R)		-0.0149	98.52%	PE (GMR = e^{Δ})		
		lower 90% CL		-0.1281	87.98%	90% CI		
		upper 90% CL		0.0983	110.33%			

Basic Statistics for BE

Thank You!
Open Questions?

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