Anova-Type Statistics,
a good alternative to parametric methods for analyzing repeated data from preclinical experiments

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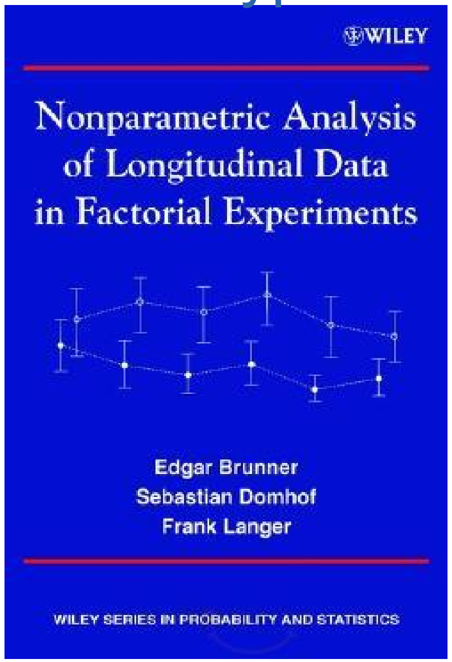
INTRODUCTION

- In preclinical field:
  - Frequent departure from hypotheses required by parametric analyses
    - Normal distribution
    - Variances homogeneity
  - Small sample size
  - Repeated design
  - Analyzing data = challenge

- A solution for some classical design:

  Anova-type statistics



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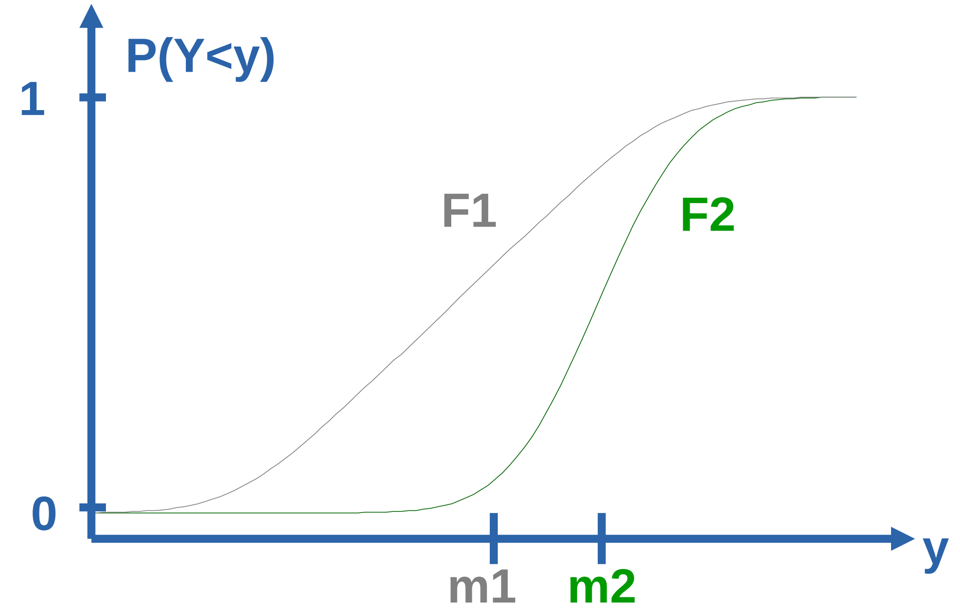
INTRODUCTION

- Anova-Type Method
- Simulation results
- Conclusion

ANOVA-TYPE METHOD (1)

Non parametric method



NON PARAMETRIC ANALYSIS:

Comparison of distributions: F1 and F2

PARAMETRIC ANALYSIS:

Comparison of positional parameters: m1 and m2

ANOVA-TYPE METHOD (2)

- **Distribution functions** of the response variable: $F_{11} \dots F_{it} \dots F_{aT}$
  - Group: i=1…a
  - Time: t=1…T

- Null hypothesis: **H0: CF=0**
  - C: Contrast matrix
  - $F=(F_{i\,t})$: Vector of distribution

- Weighted average of all the $F_{i\,t}$: $H=1/N \sum_i \sum_t n_i F_{i\,t}$

- **Relative marginal effect**: $p_{i\,t}=\int H \, dF_{i\,t}$
  - Ranks are used for estimating the p_{it} : $\hat{p}_{it} = \frac{1}{N}\left(\overline{R}_{it.} - \frac{1}{2}\right)$
  - Themselves used for testing the null hypotheses

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ANOVA-TYPE METHOD (3)

- **Illustration for a response measured on 3 timepoints following known distribution:**

PARAMETRIC:

LEGEND:

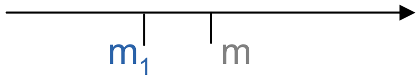
m_1: mean at time 1
m: mean over time

m_2: mean at time 2
m: mean over time

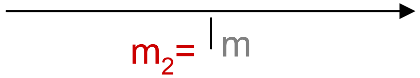
m_3: mean at time 3
m: mean over time

Difference of means

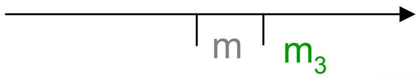
Time 1: F1~N(-2,1) — $m_1 - m = -2$



Time 2: F2~N(0,1) — $m_2 - m = 0$



Time 3: F3~N(2,1) — $m_3 - m = 2$



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ANOVA-TYPE METHOD (3)

- Illustration for a response measured on 3 timepoints following known distribution:

ANOVA-TYPE:

LEGEND:

_: distribution function at T1
_: average distribution

_: distribution function at T2
_: average distribution

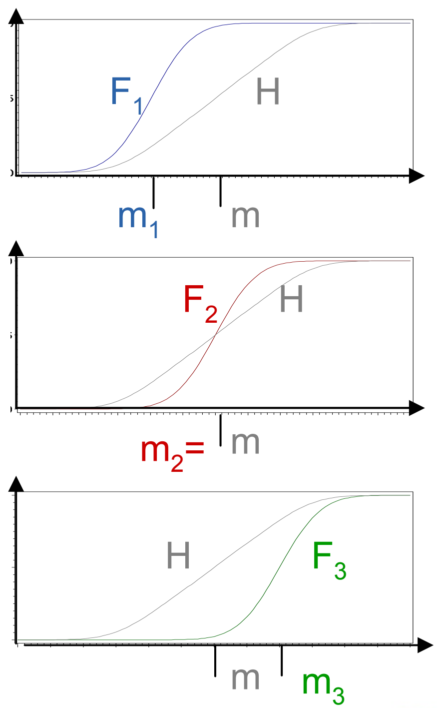
_: distribution function at T3
_: average distribution

Relative marginal effect

Time 1: F1~N(-2,1) — p_1=0.194

Time 2: F2~N(0,1) — p_2=0.5

Time 3: F3~N(2,1) — p_3=0.806



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ANOVA-TYPE METHOD (4)

- Test of H0: CF=0
  - Anova Type Statistic: $F_n(C) = \frac{n}{tr\left(C'[CC']^- C\hat{V}_n\right)} \hat{p}'C'[CC']^- C\hat{p}$
    - C : contrast matrix
    - $\hat{p}$: vector of estimated relative marginal effects
    - $\hat{V}_n$: estimated covariance matrix
  - $F_n(C)$ ~>Fisher(DFnum,DFden)
    - » DFden= ∞
    - » When C does not depend on the repeated factor, Box approximation for DFden
  - The covariance matrix is allowed to be singular since only its trace is used.

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ANOVA-TYPE METHOD (5)

- SAS implementation:
  - Proc rank
  - Followed by proc mixed:

Estimation of the covariance matrix

Anova-Type Statistics and pvalues

```
proc mixed  data=dataset ANOVAF METHOD=MIVQUE0 ;
        class Time Treatment id ;
        model RANK = Time Treatment Time* Treatment / ddfm=kr;
        repeated Time / subject=id(Treatment );
        Contrast "Comparison" / …..;
        run;
```

For obtaining non-parametric tests for factors levels comparisons

ANOVA-TYPE METHOD (6)

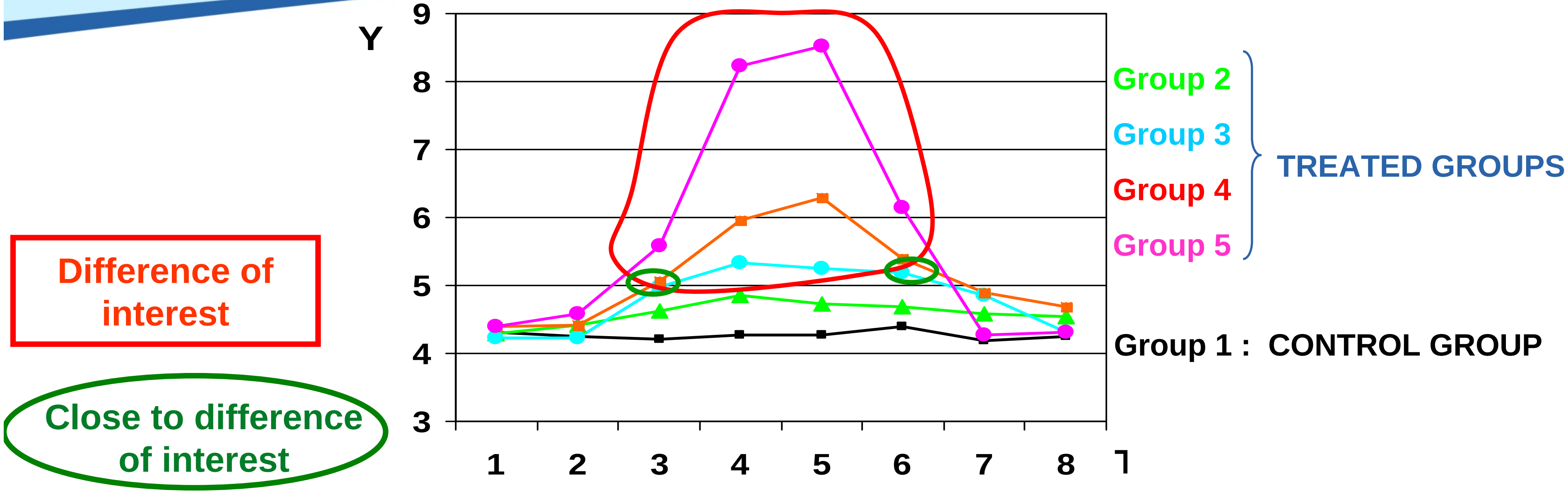
- Anova-Type main advantages:
  - Easy implementation under SAS software
  - No underlying hypothesis on the response distribution function (shape, variability…)
  - It is allowed to have no variability in some groups
    - The covariance matrix being allowed to be singular

SIMULATION STUDY (1)

- Design:
  - 5 groups;
    - **group1**=control group;
    - **group2**, **group3**, **group4**, **group5**= treated groups
  - 10 animals by group;
  - 8 times of measurements (repeated measures)

- Homogeneous variances case:
  - Standard deviation chosen so that a difference of 20% of the control group should be significant with 80% power and 5% alpha.

SIMULATION STUDY (2)



GROUP	T1	T2	T3	T4	T5	T6	T7	T8
2 vs 1	-0.5%	3.9%	10.0%	13.9%	10.5%	6.7%	9.4%	6.7%
3 vs 1	-1.7%	-0.1%	18.2%	24.8%	23.0%	17.9%	15.7%	1.3%
4 vs 1	1.7%	4.1%	20.4%	39.6%	47.1%	22.5%	16.9%	10.2%
5 vs 1	1.7%	7.9%	32.8%	93.0%	99.3%	39.5%	2.0%	1.2%

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SIMULATION STUDY (3)

- Simulation of several datasets from the previous design with departures from the homogeneous variances case
  - Over- or under- responding subjects
    - The means are unchanged but the medians and variances are modified
    - **Reference analysis**: Anova on data with over-responding data replaced
  - Variances heterogeneity on group factor
    - The means and medians are unchanged but the variances are modified
    - **Reference analysis**: MIXED model with group= option
  - Variance generated as a function of exp(mean)
    - The means and medians are unchanged but the variances are modified
    - **Reference analysis**: MIXED model with Repeated / local=exp(MEAN)
- Challenged analyses:
  - Anova on raw data, on normal scores, on ranks
  - Anova-Type
  - **Reference analysis**

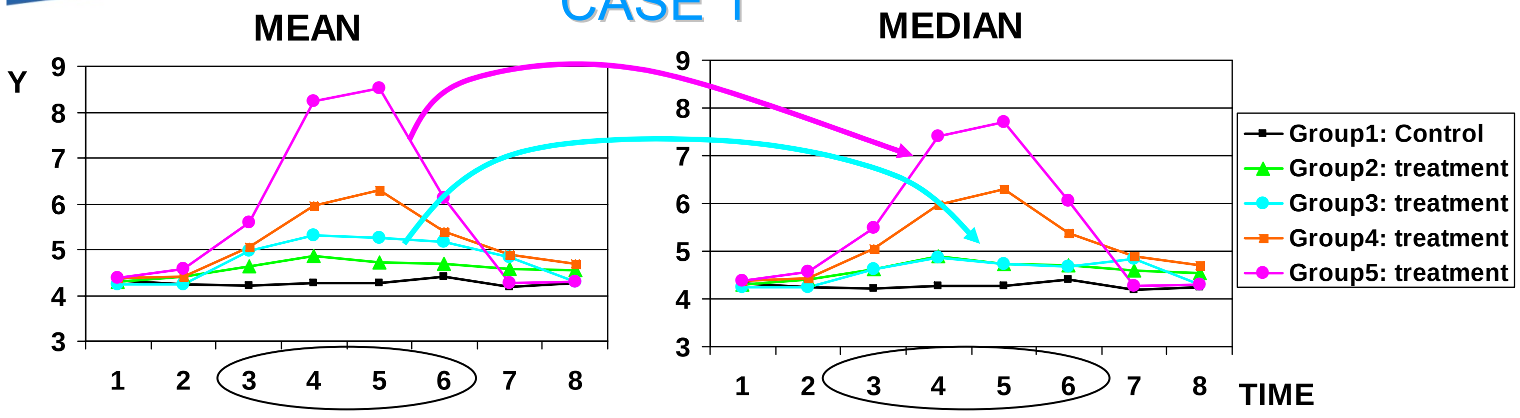
SIMULATION STUDY (4)

- Power: probability to reject H0 when H1 is true
  - Simulation of datasets with:
    - Difference of interest between the groups
    - Variances heterogeneity
  - For each comparison, the power is estimated as the proportion of significant p-values among all 1000 simulations.

- Alpha risk: probability to reject H0 when H0 is true
  - Simulation of datasets with:
    - **NO** difference between the groups
    - Variances heterogeneity
  - The alpha risk is globally estimated for all comparisons as the proportion of the simulations with at least one significant difference among all 1000 simulations.

Simulation of samples with over/under responding subjects (1)

CASE 1



2 over-responding subjects

2 over-responding subject

Mean>Median

POWER of the comparison GROUP3 vs GROUP1

METHOD	T3	T4	T5	T6
RAW DATA	22%	67%	56%	25%
NORMAL SCORES	35%	63%	48%	21%
RANKS	33%	59%	40%	17%
ANOVA-TYPE	44%	71%	50%	26%
REFERENCE	7%	20%	8%	2%

All analyses are too powerful in comparison with the reference analysis

Anova on data with over-responding data replaced

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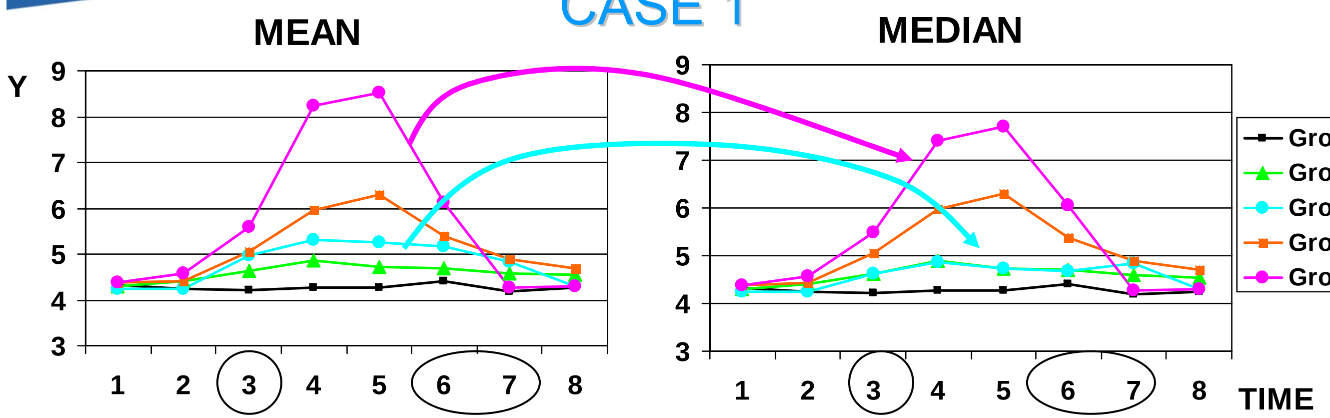
Simulation of samples with over/under responding subjects (2)

CASE 1



2 over-responding subjects

2 over-responding subject

Mean>Median

POWER of the comparison GROUP4 vs GROUP1

METHOD	T3	T6	T7
RAW DATA	36%	58%	18%
NORMAL SCORES	78%	83%	62%
RANKS	84%	91%	69%
ANOVA-TYPE	86%	92%	70%
REFERENCE	84%	94%	66%

Analysis on raw data is not powerful enough

Anova on data with over-responding data replaced

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Simulation of samples with over/under responding subjects (3)

CASE 1

2 over-responding subjects in group 5

2 over-responding subject in group 3

Mean>Median

Alpha risk: probability that at least one comparison is significant

METHOD	T1	T2	T3	T4	T5	T6	T7	T8
RAW DATA	1%	1%	6%	47%	47%	12%	0%	0%
NORMAL SCORES	3%	3%	9%	14%	13%	6%	3%	4%
RANKS	4%	4%	9%	10%	9%	6%	4%	4%
ANOVA-TYPE	4%	4%	8%	10%	9%	6%	3%	4%
REFERENCE	6%	4%	4%	5%	5%	5%	4%	5%

Too conservative

Too high

Good conservation

Anova on data with over-responding data replaced

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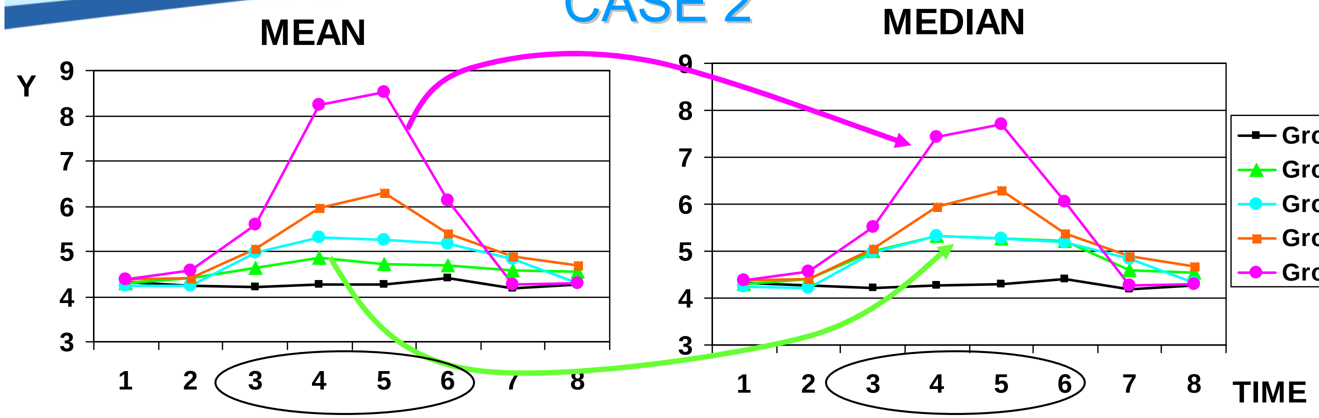
Simulation of samples with over/under responding subjects (4)

CASE 2



2 over-responding subjects

Mean>Median

2 under-responding subject

Mean<Median

POWER of the comparison GROUP2 vs GROUP1

METHOD	T3	T4	T5	T6
RAW DATA	3%	9%	2%	0%
NORMAL SCORES	19%	43%	26%	9%
RANKS	52%	80%	71%	42%
ANOVA-TYPE	69%	91%	86%	63%
REFERENCE	92%	100%	98%	93%

Anova-Type fits better than the other analyses to the reference analysis

Anova on data with over/under-responding data replaced

Simulation of samples with over/under responding subjects (5)

CASE 2

2 over-responding subjects in group 5 — Mean>Median

2 under-responding subject in group 2 — Mean<Median

Alpha risk: probability that at least one comparison is significant

METHOD	T1	T2	T3	T4	T5	T6	T7	T8
RAW DATA	0%	0%	1%	55%	86%	2%	0%	0%
NORMAL SCORES	3%	3%	7%	15%	14%	9%	3%	3%
RANKS	4%	4%	8%	10%	8%	8%	5%	4%
ANOVA-TYPE	4%	4%	7%	9%	7%	7%	4%	4%
REFERENCE	5%	5%	4%	6%	6%	6%	5%	5%

Too conservative

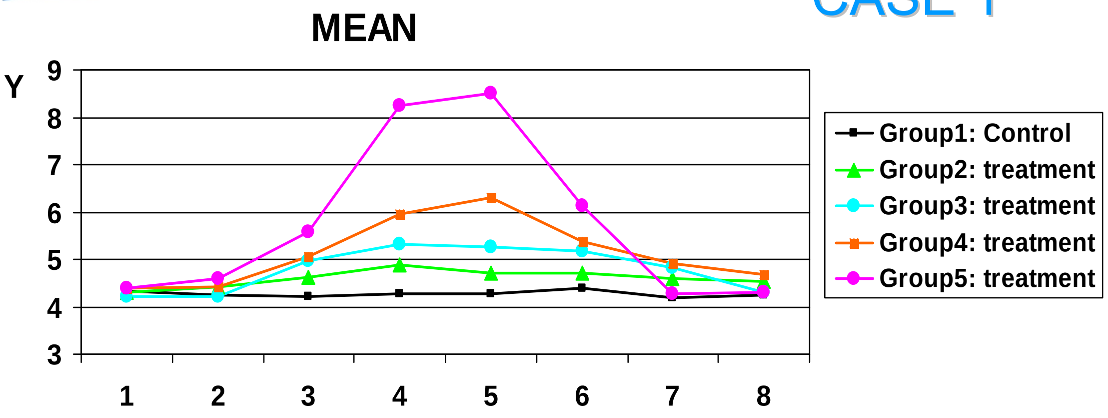
Too high

Good conservation

Anova on data with over/under-responding data replaced

Simulation of samples with variances heterogeneity on group factor (1)

CASE 1



Standard Deviation

sd= 2 in Group 1

sd= 0.5 in Group 2

sd= 0.6 in Group 3

sd= 0.7 in Group 4

sd= 0.8 in Group 5

POWER of the comparison GROUP4 vs GROUP1

METHOD	T1	T2	T3	T4	T5	T6	T7
RAW DATA	8%	10%	31%	79%	91%	39%	25%
NORMAL SCORES	5%	8%	35%	86%	95%	47%	25%
RANKS	6%	8%	30%	86%	95%	48%	20%
ANOVA-TYPE	8%	9%	33%	88%	95%	52%	20%
REFERENCE	1%	3%	11%	52%	71%	16%	9%

All analyses are too powerful in comparison with the reference analysis

MIXED model with group= option

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Simulation of samples with variances heterogeneity on group factor (2)

CASE 1

Standard Deviation **sd= 2 in Group 1**

sd= 0.5 in Group 2; 0.6 in Group 3 ; 0.7 in Group 4; 0.8 in Group 5

ALPHA RISK: probability that at least one comparison is significant

METHOD	Minimum	Maximum
RAW DATA	15%	18%
NORMAL SCORES	8%	14%
RANKS	9%	13%
ANOVA-TYPE	8%	12%
REFERENCE	3%	5%

very high for analysis on raw data

high for analyses on normal scores, ranks and Anova-Type

close to 5% for the reference analysis

MIXED model with group= option

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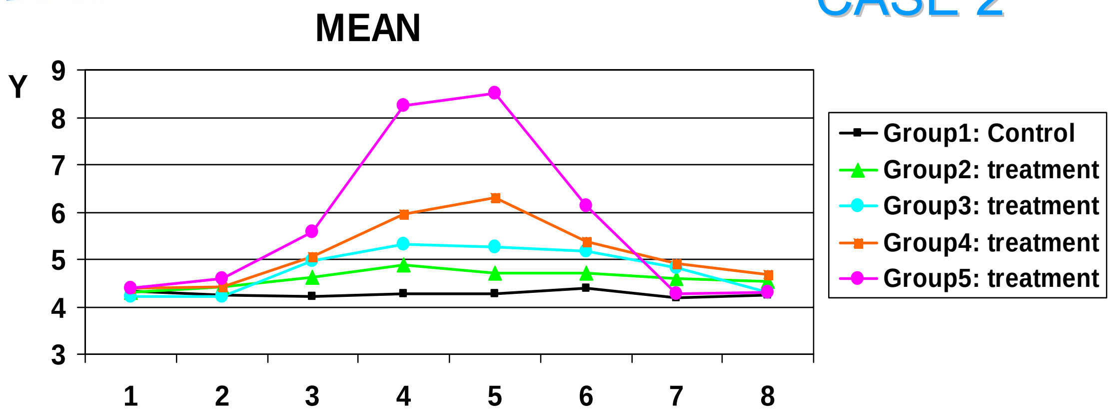
Simulation of samples with variances heterogeneity on group factor (3)

CASE 2



Standard Deviation

sd= 0.4 in Group 1

sd= 0.4 in Group 2

sd= 1 in Group 3

sd= 1.5 in Group 4

sd= 2 in Group 5

POWER of the comparison GROUP3 vs GROUP1

METHOD	T3	T4	T5	T6
RAW DATA	7%	21%	15%	7%
NORMAL SCORES	23%	43%	36%	13%
RANKS	40%	62%	56%	30%
ANOVA-TYPE	44%	72%	65%	35%
REFERENCE	42%	73%	66%	45%

MIXED model with group= option

Anova-Type fits better than the other analyses to the reference analysis

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Simulation of samples with variances heterogeneity on group factor (4)

CASE 2

Standard Deviation **sd= 0.4 in Group 1**

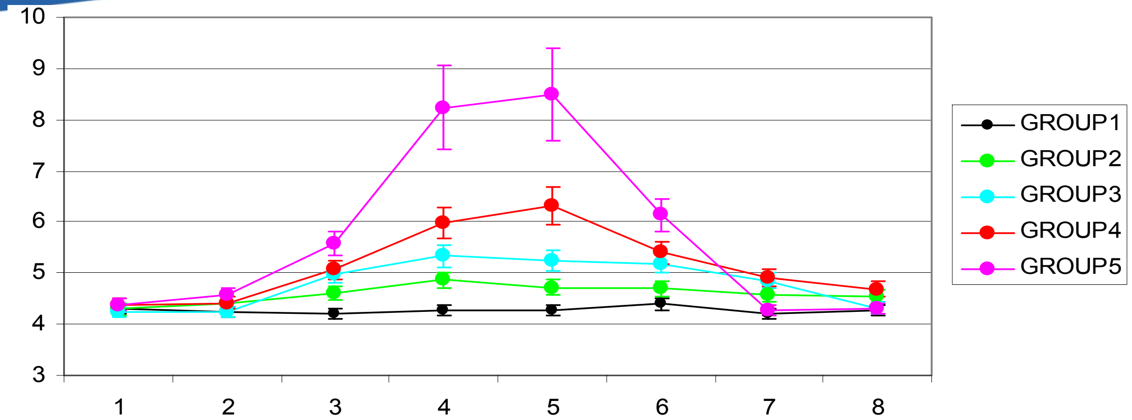
sd= 0.4 in Group 2; 1 in Group 3 ; 1.5 in Group 4; 2 in Group 5

ALPHA RISK: probability that at least one comparison is significant

METHOD	Minimum	Maximum
RAW DATA	4%	5%
NORMAL SCORES	2%	3%
RANKS	3%	4%
ANOVA-TYPE	3%	4%
REFERENCE	6%	7%

MIXED model with group= option

Simulation of samples with variances being exponential of the mean (1)



$$Var(Y) = \sigma^2 \exp[\gamma.Mean(y)]$$

POWER of the comparison GROUP2 vs GROUP1

METHOD	T3	T4	T5	T6
RAW DATA	1%	10%	2%	0%
NORMAL SCORES	44%	64%	42%	16%
RANKS	56%	75%	54%	23%
ANOVA-TYPE	73%	88%	72%	41%
REFERENCE	45%	71%	44%	16%

AnovaType is the most powerful method

MIXED model with Repeated / local=exp(MEAN)
WARNING: Unaccurate variances estimations ?

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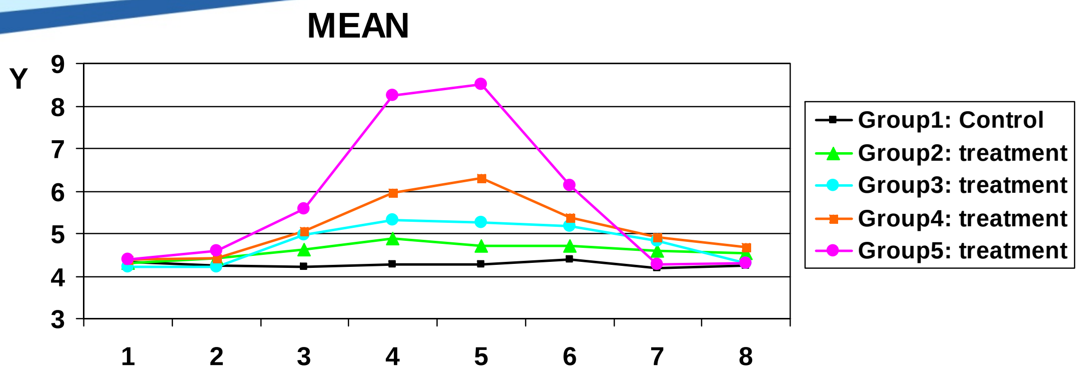
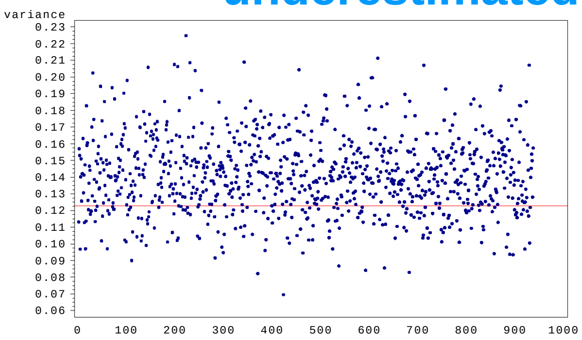
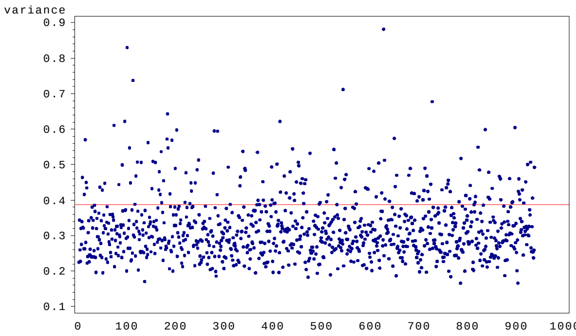
Simulation of samples with variances being exponential of the mean (1bis)



$$Var(Y) = \sigma^2 \exp[\gamma.Mean(y)]$$

- Mixed model with variance modeled as a function of exp(mean): should be a reference analysis
  - Does is make good estimations of the variances for small samples?
  - **NO: some of the variances are overestimated, the other are underestimated**





— :Theoretical Variance
•••: Estimated Variance

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Simulation of samples with variances being exponential of the mean (2)

No difference between the groups, but same variances heterogeneity as for the power study

ALPHA RISK: probability that at least one comparison is significant

METHOD	T1	T2	T3	T4	T5	T6	T7	T8
RAW DATA	0%	0%	0%	32%	41%	0%	0%	0%
NORMAL SCORES	0%	1%	2%	13%	15%	4%	1%	1%
RANKS	1%	1%	4%	10%	9%	5%	2%	2%
ANOVA-TYPE	1%	1%	3%	10%	9%	5%	2%	2%

Very high variability: huge alpha risk for analysis on raw data

analysis on ranks and Anova-Type have the best alpha risk.

When the variability is small, analysis on raw data is too conservative

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CONCLUSION (1)

- When heterogeneity of variances corresponds to a group structure, it is better to use the SAS group= statement.

- When over- or under-responding subjects are present in the dataset, Anova-Type is the most appropriate method:
  - Good power
  - Good alpha risk conservation

- When heterogeneity of variances could be modeled using complex model, Anova-Type is a good answer for analyzing such data easily.

CONCLUSION (2)

- Case studies:
  - Analysis of repeated discrete data
  - Usual method:
    - Friedman test
    - Kruskal-Wallis test at each time
    - Proportional odds models (genmod procedure)
  - Anova-Type Statistics is an excellent solution:
    - No hypothesis on the shape of the data distribution
    - Enables the variances to be null for some factors levels
    - Very powerful for relevant differences

REFERENCES

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- Singer, J. M., Poleto, F. Z., and Rosa, P. (2004). Parametric and nonparametric analyses of repeated ordinal categorical data. Biometrical Journal 46, 460–473.

- **PAPERS where ANOVA-TYPE is used:**
  - Bunn, Terry L., Slavova, Svetla, Spiller, Henry A., Colvin, Jonathan, Bathke, Arne and Nicholson, Valerie J. (2008)'The Effect of Poison Control Center Consultation on Accidental Poisoning Inpatient Hospitalizations with Preexisting Medical Conditions, Journal of Toxicology and Environmental Health, Part A,71:4,283 - 288.
  - Ciraulo, D.A., Hitzemann, R.J., Somoza, E., Knapp, C.M., Rotrosen, J., Sarid-Segal, O., Ciraulo, A.M, Greenblatt, D.J., Chiang, C.N. (2006). Pharmacokinetics and Pharmacodynamics of Multiple Sublingual Buprenorphine Tablets in Dose-Escalation Trials. J. Clin. Pharmacol., 46, 179

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