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How to approach statistical data analysis in ophthalmology - a bucket of top tips with examples

Dr Gabriela Czanner, Lecturer in Ophthalmic Biostatistics

Department of Eye and Vision Science, Department of Biostatistics, University of Liverpool

Clinical Eye Research Centre, St. Paul's Eye Unit, Royal Liverpool Hospital

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Goal of this session

- To give an overview of
- … of statistical data analysis methods …
- … that occur in ophthalmology…
- … how the concepts connect with each other.
- … This should help in choosing the right statistical method.

This presentation shows 10 tips that I consider the most important

Why is statistics is needed?

- Statistics needed for ***objective evaluation*** of what is observed in clinical research
  - What the clinical data say
  - How certain we are of the message
  - How to assure data quality
  - Cost effectiveness of further studies
- Statistics as a ***communication tool***
  - Present findings convincingly
  - Understand and evaluate the findings of others

What statistical design and data analysis method we need?
Answer: “This depend on many factors. The most important factor is Research question. Tell me what is your ***research question***?”

Research question

Design of study

Collecting data

Data analysis

Report

General steps in a research project

Top tip 1. Understand that what we measure on eye or patient is a *variable*

What is a variable?

- It is something whose value can ***vary*** across subjects and within subjects e.g. when same subject measured repeatedly over time or on same visit
- E.g. BCVA is a variable
- Some variables can be named as ***primary or secondary outcomes***

How is a variable measured?

- E.g. How to measure visual acuity? Snellen Chart and Early Treatment Diabetic Retinopathy study Chart (ETDRS). No appropriate conversion exists.
- E.g. How to measure blood pressure? Can we measure it as dichotomised, e.g. as high and low?

Data are values you get when you measure a variable

- E.g. Age = 40.2, 40.5, 31.1, 51.2, 31.2 years
- Gender: Male and Female
- Quality of Fluorescein Angiography image: Excellent, Good, Fair, Poor

Thing to know: Often dichotomisation is not advised.

Cumberland PM, Czanner G, Bunce C, Dore C, Freemantle N, Garcia-Finana M. Ophthalmic statistics note: the perils of dichotomising continuous variables. *Br J Ophthalmol* 2014; 98:841-843.

Types of variables

- Categorical variables
  - Nominal
  - Ordinal
- Metric variables
  - Discrete
  - Continuous

Nominal — **Values in arbitrary categories**

E.g. Gender: Male/Female

Ordinal — **Values in ordered categories**

E.g. Quality of image (e.g. Excellent, Good, Fair, Poor)

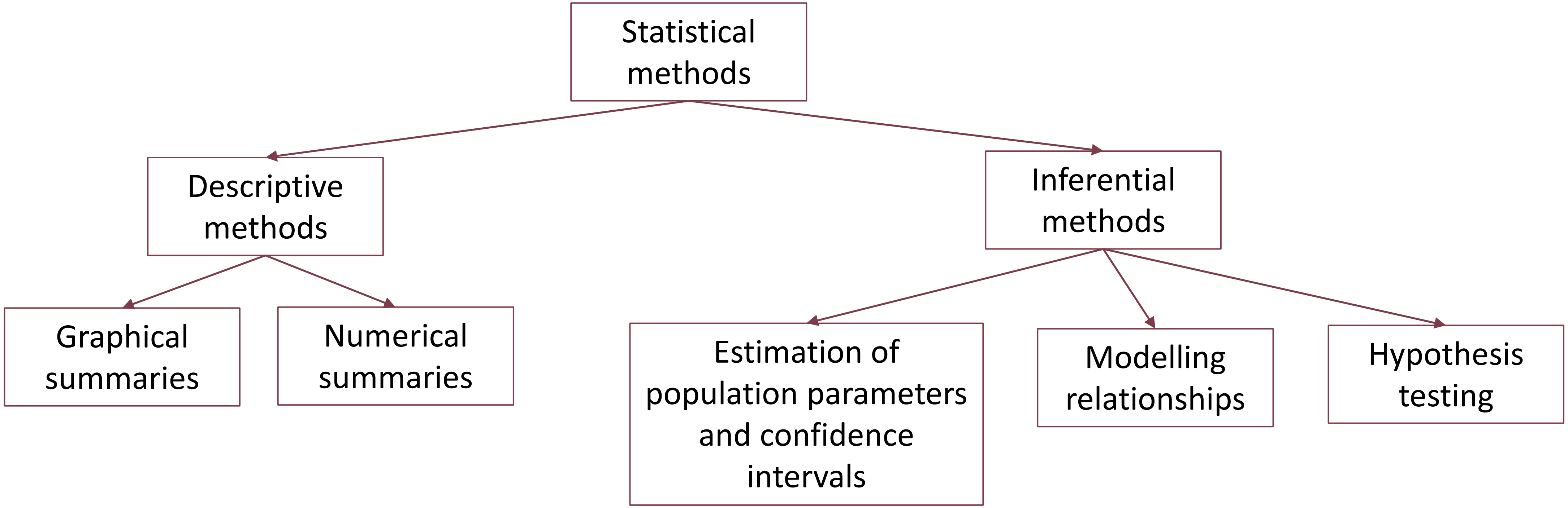
Discrete — **Integer values on proper numeric line or scale**

E.g. Number of scars in a retina (e.g. 0, 1, 2, 3)

Continuous — **Continuous values on proper numeric line or scale**

E.g. Age of the patient measured as continuous

Top tip 2. There are two broad categories of statistical methods



Descriptive statistics is the discipline of quantitatively describing the data.

Inference is the process of deducing properties of an underlying distribution by analysis of data.

Thing to know: If our study is to test a hypothesis, we still need to employ both descriptive and inferential statistical methods.

Top tip 3. The descriptive statistical methods

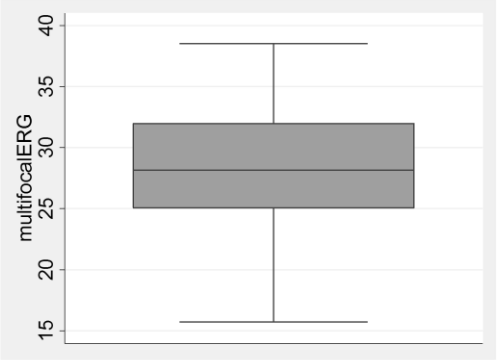
How to do a good *numerical* description of the data measured on continuous scale?

- If the distribution skewed - report median and quartiles
- If the distribution is symmetric - report mean and standard deviations

How to do a useful *graphical* description of data?

- Histogram and boxplot for continuous variables
- Barcharts for nominal and ordinal variables
- Scatter plots to explore associations between continuous variables

Fig. Boxplot of mfERG central density in early DR (n=20)



Why we need descriptive methods?

- Demographics tables for reports
- Quality assurance, e.g. outliers due typo errors
- May find new unexpected patterns or associations
- Explore the distribution of outcome variables, to support selection of inferential statistical methods (e.g. many methods require normal distribution)

Thing to know: Descriptive statistics show or summarize data in a meaningful way such that patterns might emerge from the data e.g. new unknown associations. Descriptive statistics do not, however, allow us to make conclusions beyond the data.

More info: Initiative STRATOS for observational studies, Topic Group 3 “Initial data analysis” http://www.stratos-initiative.org/

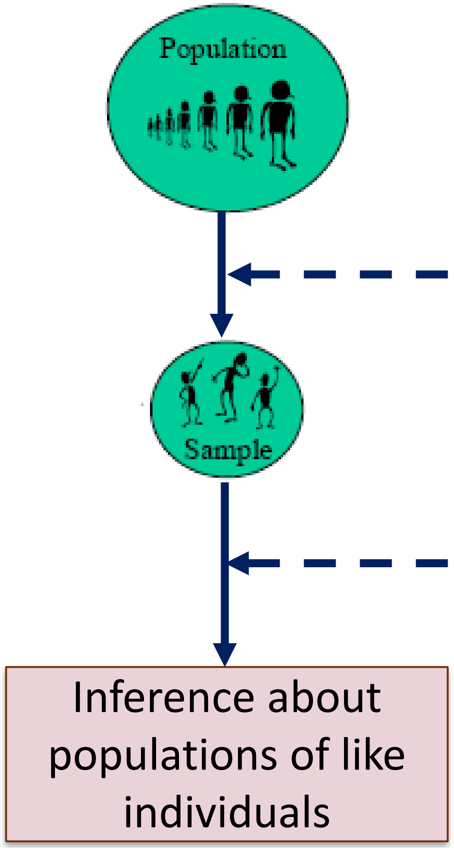
Top tip 4. The inferential statistical methods

The main principle

We use data collected on a sample to make ***statistical inference*** about the population of interest.

The result of the inference is summarized in

- Confidence interval
- P-value



e.g. All eyes in UK with early or late DR

Sampling chance

e.g. a random sample of 30 eyes with early DR and 30 eyes with late DR

Inferential statistical data analysis method applied e.g. two-sample t-test

e.g. a statement on difference in <u>central choroidal thickness</u> between early DR and late DR.

EXAMPLE: We are comparing mean central choroidal thickness between early and late DR. We recruited 20 early DR and 20 late DR eyes. In two-sample t-test the p-value=0.12. Does it mean that the thickness is same?

Thing to know: Absence of evidence is not evidence of absence.

BunceC, Patel KV, Xing W, Freemantle N, Doré CJ. Ophthalmic statistics note 2: absence of evidence is not evidence of absence, *Br J Ophthalmol* 2014;98:703-705; Nuzzo R. Statistical errors. *Nature*, 506, 2014

Example - Colour blindness

- We are interested in whether there is an association between colour blindness and gender.

- We asked 240 men and 260 women about color-blindness. The results of a survey are as follows:

	Normal colour vision	Colour blind	Total
Male	221	19	240
Female	254	6	260
Total	475	25	500

Example - Colour blindness

In sample 8% males and 2% females are colour blind

Does this mean that women are at less risk of becoming colour blind than men?

We rewrite research question into null hypothesis

- H_0: there is no association between colour blindness and gender

How to test the null hypothesis?

- We can use a method of comparison of proportions (or Chi-squared test of association or Fisher exact test – see slides with Top Tip 6)
- Results reported as Confidence Interval or P-value

Confidence interval for the difference of two population proportions

When studying the difference between two population proportions, the difference between the two sample proportions, $\hat{p}_1 - \hat{p}_2$ can be used as an unbiased point estimator for the difference between the two population proportions, $p_1 - p_2$. This is used with the general formula:

estimator ± (reliability coefficient) x (standard error)

Distribution

When the central limit theorem applies, the normal distribution is used to obtain confidence intervals. The standard error is estimated by the formula:

$$\sigma_{\hat{p}_1-\hat{p}_2} = \sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}$$

Confidence interval

The 100(1-alpha) percent confidence interval for $p_1 - p_2$ is given by:

$$(\hat{p}_1-\hat{p}_2) \pm z_{(1-\alpha/2)} \sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}$$

- **Interpretation of the interval**

The degree of confidence depends on the value of . When = .05, then we have a 95% confidence interval. We can then state the probabilistic and practical interpretations of the interval.

Probabilistic interpretation. We say that we are 95% confident that the difference between the two population proportions, $p_1 - p_2$, lies between the calculated limits since, in repeated sampling, about 95% of the intervals constructed this way would contain $p_1 - p_2$.

Practical interpretation. In a specific example, we would expect, with 95% confidence, to find the difference between the two population proportions between the two limits.

Example - Colour blindness

- Confidence interval: range of plausible values for the "true" difference (usually use 95% certainty)
- Method of comparison of proportions
  - General formula (in large samples)

$$\text{estimate} \pm 1.96 \cdot \text{standard error}$$

95% CI for $p_1 - p_2$ is (2%, 9%)

- Is there is significant difference in proportion of colour blindness between the 2 groups?

$\Rightarrow$ there is a significant difference in the proportion with colour blindness between the 2 groups at a 95% confidence level *(because value 0 is not in CI)*

Top tip 5. Clinical and statistical significance are different concepts

Statistical significance

- It rules out chance as an explanation for the observed difference of means
- Summarized by p-value

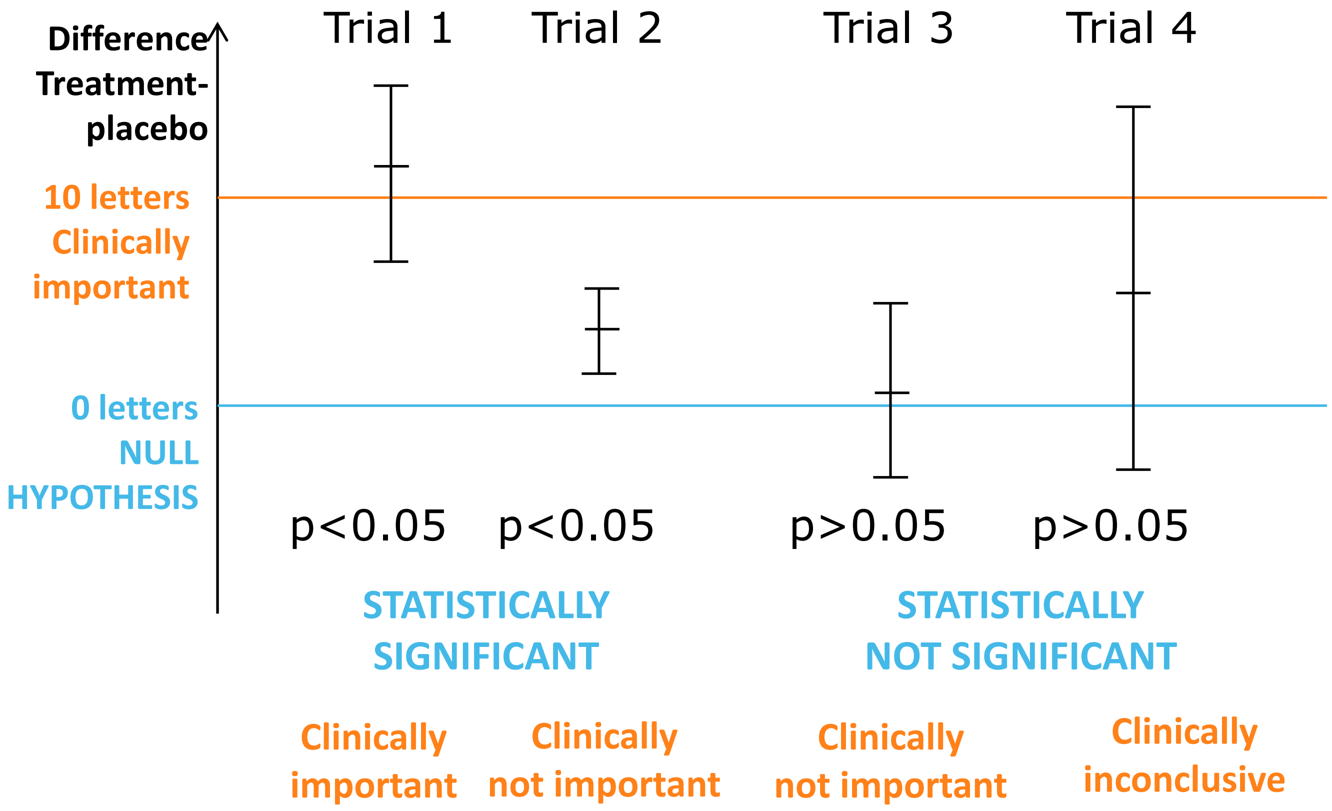
Clinical relevance

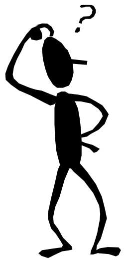
- It tells if the observed difference is of clinical value
- Summarized by confidence interval

Kay R, 2014. *Statistical Thinking for Non-Statisticians in Drug Regulation*, Wiley Blackwell, 2nd edition.

Thing to know: Presentation of p-values together with confidence intervals is good practice.

Example: In a collection of 4 placebo-controlled trials in BCVA a difference of 10 letters in terms of mean increase of visual acuity is to be considered of clinical importance; anything less is unimportant. The results are given in the figure below.





Top tip 6. Statistical methods *to compare groups* with one variable

Comparing two groups (e.g. two treatments)

Type of data

Categorical	Continuous Normal	Continuous Skewed

Tests for differences, independent data:

Chi-square χ^2 test Fisher's exact test	t-test	Mann-Whitney U test

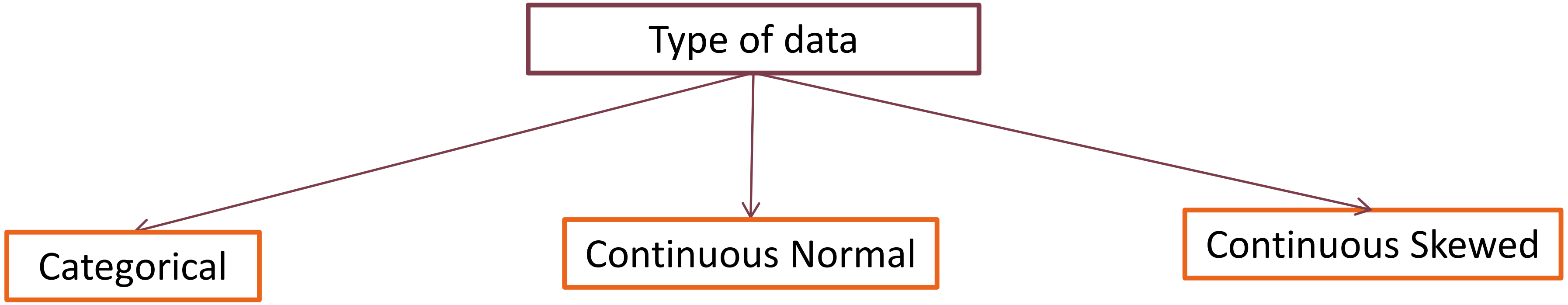
Tests for differences, non-independent (paired) data:

McNemar's test	Paired (single sample) t-test	Wilcoxon test

Thing to know: If continuous data are skewed then applying a transformation (*log* or *sqrt*) can make them Normal, in such case a t-test can be used which has stronger power (than a parametric test).

Source: adapted from teaching materials of Department of Biostatistics, University of Liverpool

Comparing three of more groups (e.g. multiple treatments)



Tests for differences, independent data:

Compare two groups at a time. This leads to multiple comparisons.

One-way ANOVA

Assumptions: Normal distribution of data in each group; Independence of units of analysis; Equal variability in each group

Kruskal-Wallis test

Thing to know: Be aware of multiple comparison problem and seek proper adjustments.

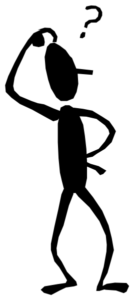
Cipriani V, Quartilho A, Bunce C, Freemantle N, Dore C. Ophthalmic statistics note 7: multiple hypothesis testing-to adjust or not to adjust. Br J Ophthalmol 2015;99:1155-1157.

Source: adapted from teaching materials of Department of Biostatistics, University of Liverpool

Top tip 7. Choosing the right statistical inferential method

Several factors to consider

- ***Research question or objectives*** of the analysis
  - e.g. Estimating prevalence, comparison of groups, instruments comparisons, estimate associations/risk factors, prediction of treatment failure?
- ***Type of data***
  - E.g. how the data are measured (nominal, ordinal, scale, count)
- ***Design paired or independent***
  - Data are ***independent*** if each patient was given only one treatment
  - Data are ***paired*** if each patient was given both treatments (cross-over design)
  - If BCVA of an eye measured at two time points
- ***Distribution of the data***
  - i.e. is it ***symmetric*** or ***skewed?*** *Is it* ***Normal?***



Example:

We want to compare IOP for female and male. We collected IOP on one eye per patient, 20 eyes in each gender group. What statistical method to choose?

Answer:

Objective: compare means in two groups

Type of data: continuous

Design: independent

Distribution: needs to be checked with measured IOP data in each group.

What data analysis method to use?

We may use two-sample t-test if the histograms of IOP confirm normality of data

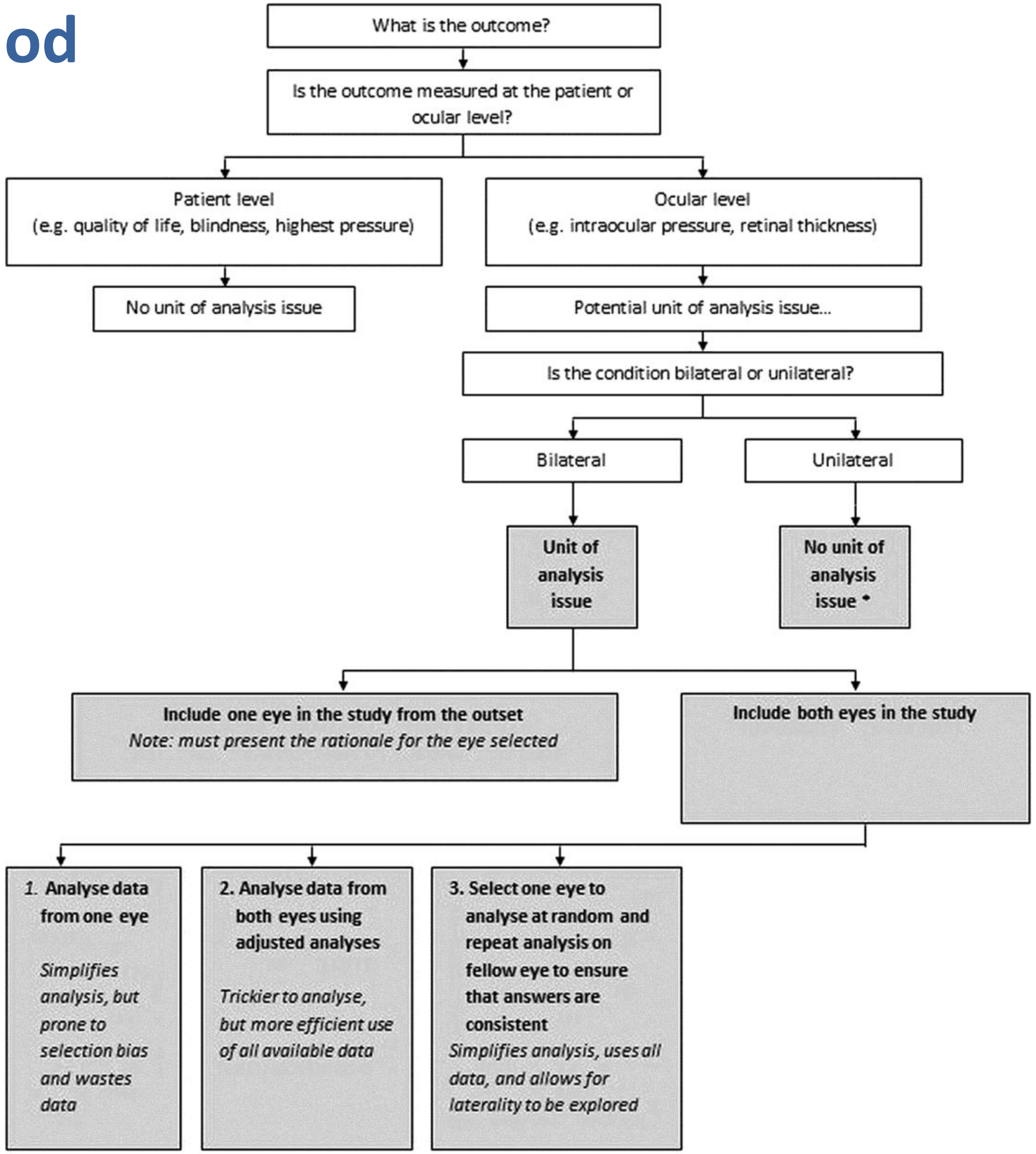
Thing to know: Always check if the *assumptions* of the statistical method are *satisfied.* If the assumptions are violated, then the estimates, confidence intervals and p-values can not be trusted.

Source: adapted from teaching materials of Department of Biostatistics, University of Liverpool

Top tip 8. Choice of inferential statistical method depends on *unit of analysis*

- **Eyes** exist in **patients**,
  - and typically patients have **two** eyes.
- Challenge for the study design
  - Do we use one eye?
  - Or both?
- Challenge for analysis
  - Measurements from two eyes of same patient may be ***correlated*** and hence carry less information than two eyes from two different patients; and standard data analysis methods are not appropriate.
- If eye is the unit of analysis
  - Then correct statistical method to analyse your data depends on whether you did measurements from both eyes or just one eye.

Thing to know: Always report if the unit of your analysis is eye or patient.



**unless repeat measures on or within an eye*

Bunce C, Patel KV, Xing W, Freemantle N, Dore CJ, Ophthalmic statistics note 1: unit of analysis, *Br J Ophthalmol* 2014;98: 408-412.

Top tip 9. Statistical methods for *association between two variables*

Association

- It is a synonym for ***dependence*** or ***relation*** (e.g. relation between age and choroidal thickness on OCT)
- It is a very general relationship: one variable ***provides information*** about another.
- One particular type of association is a linear association, also called ***correlation***.

Type of data

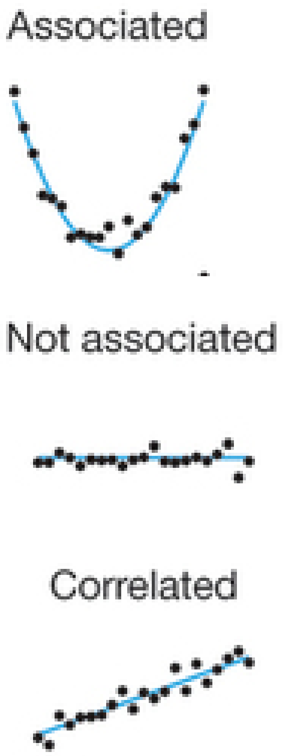
Both variables ordinal

Spearman rank correlation coefficient to assess an increasing or decreasing association

Both variables continuous

Pearson correlation coefficient and Linear Regression to assess a linear association

Statistical modelling for more complex associations



Things to know: 1. High correlation or association does ***not*** mean causation. **2.** Be aware of ***confounders*** when assessing the correlation. If confounders not taken into account, then paradoxical correlations values may arise. **3.** When assessing agreement between two measurement methods, the correlation should ***not*** be used.

Altman N, Krzywinski M. Points of Significance: Association, correlation and causation. Nature Methods 2015 12, 899-900.

Source: adapted from teaching materials of Department of Biostatistics, University of Liverpool

Top tip 10. Statistical methods for *relation between <u>several</u> variables*

These are more advanced statistical methods used in ophthalmic clinical research:

- Called ***"statistical modelling"***, or ***"multivariate modelling"***
- **Most commonly used methods**
  - One –way, Two-way ANOVA
  - ANCOVA
  - Simple and Multiple linear regression
  - Logistic regression
  - Survival models
  - Linear and nonlinear mixed models, also called "hierarchical models"
- **Why to use them? Can allow to:**
  - adjust for confounders
  - more complex (than linear) relationships
  - non Normal outcome
  - better utilisation of missing data
  - imbalanced designs…

Main principle

Characterise the relation between outcome and predictors via a model. The model is fitted to the data. E.g.

BCVA = 50 - 8 Diabetes - 0.4 Age + Error

Outcome **Predictors (or Covariates)**

↓ Assumptions of the model are checked

Use the model:

- **To answer research questions**
  E.g. Effect of diabetes on BCVA?
- **To make predictions for individual patients (or eyes)**
  E.g. BCVA at age 41 yrs in diabetes

Thing to know: More sophisticated methods can answer same research questions as simple methods (such as t-test), while adjusting for the effect of confounders.

Summary

- The best practice is to do analysis in stages:
  - **Stage 1. Do descriptive summaries of data**
    - Numerical and graphical summaries
    - E.g. two histograms of BCVA: one for each Diabetes group
  - **Stage 2. Main data analyses**
    - **In association studies** we apply methods of statistical inference
      - Adjust for confounders e.g. linear regression of BCVA with Diabetes as main risk factor while adjusting for age
      - Some research communities require that you also report unadjusted inference e.g. BCVA vs diabetes without adjusting for age
    - **In prediction or discrimination studies** we apply relevant data methods
    - **In methods of measurement comparison** studies we use relevant methods.
      - E.g. Bland-Altman plot

Discussion

- **Good design of the study is important**
  - Statistical data analysis (inference) methods however sophisticated can not “rescue” a poor study design
- **Choice of statistical inferential methods**
  - It is a complex task
  - Decision need to be done early at designing stage, and described in the statistical analysis plan
- **There are other statistical methods not mentioned here**
  - Bayesian statistical methods, Methods for genetics data
  - Multivariate statistical methods (Principal factor analysis, Cluster analysis, Factor analysis)
  - Statistical methods for imaging data … and many more!

Recommended reading

Books

- *Practical statistics for medical research* by Douglas G. Altman [A general and intermediate level book.]
- *Medical statistics from scratch* by David Bowers [A perhaps more readable intermediate level book.]
- *Statistical Thinking for Non-Statisticians in Drug Regulation*, Kay R, 2014. Wiley Blackwell, 2nd edition. [Very thorough intermediate level book.]

Journals' with series on how to do statistics in clinical research

- BJO has ***Ophthalmic Statistics Notes*** (http://www.brcophthalmology.org/ophthalmic-statistics-group-osg)
- American Journal of Ophthalmology has ***Series on Statistics***
- British Medical Journal has series ***Statistics Notes***

Guide on how to do clinical research in ophthalmology

- *Clinical Research. A primer for Ophthalmologists.* International Council of Ophthalmology. February 2009, http://www.icoph.org

Nature notes

- http://www.nature.com/collections/qghhqm/

BMJ resources

http://www.bmj.com/about-bmj/resources-readers/publications/statistics-square-one/13-study-design-and-choosing-statisti

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Contact details/for more information
Gabriela Czanner
czanner@liverpool.ac.uk