One eye or two

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Introduction

Research in Ophthalmology is unusual in comparison to other many medical specialities by having two targets where frequent and accessible data can be collected and analysed. The major factor is that two eyes are not independent as they belong to the same individual. As many statistical methods assume independence of observations, historically many researchers have arbitrarily chosen one eye for analysis. Another approach to maintain independence of observations is to generate subject level index variables using information from both eyes. This was the approach taken by Anagnostou *et al.*(1) in this issue of Journal of Neuro-ophthalmology. Specifically, for measurements taken in both eyes of subjects with three possible grades (i.e. 0,1,2 in the right eye and 0,1,2 in the left eye), each combination was considered to yield nine potential subject level outcomes for two analyses.

Statistical methods exist which allow for the analysis of both eyes, however these require acknowledging and accounting for the intra-participant correlation between eyes. While the potential for leveraging data is promising, ensuring appropriate application of statistical methods is critical to ensure sound analysis and appropriate conclusions. The purpose of this article is to provide a statistical overview for researchers and readers of clinical studies to allow for critical appraisal of the statistical methods chosen, in terms of using both or either eye in analysis with an emphasis on interventional clinical trials.

Benefits of using one eye

In a review considering the one- vs two- eyed problem, the ophthalmic literature between 2009 and 2012 was collated, and the authors found that 64% of studies only obtained

data from one eye, with the most common methods of eye selection being the right (35%) followed by the worst (23%). (2) Therefore, by only analysing one eye and keeping the methodology employed consistent, there is an opportunity for direct comparison with historical studies.

The requisite analysis of one eye may be more straightforward because independence of observations (one observation per subject) is maintained. Thus, more traditional statistical methods being employed (e.g. *t*-tests, analysis of variance (ANOVA) and linear regression).

There are certain study settings where it is appropriate to analyze only one eye. For example, in the instance where only one eye is affected by disease at the time of the study (such as occurs in acute optic neuritis or non-arteritis anterior ischaemic optic neuropathy) or if the intervention only affects one eye (e.g. certain topical applications); in these cases, analyses using both eyes would not be appropriate. Here it would not be appropriate to assume that any intervention will affect each eye analogously and analyses should instead focus on only the affected eye or treated eye.

In studies of diseases where both eyes are affected and analyses only focuses on one eye, there are multiple choices regarding the selection of the eye. These include: arbitrarily selecting the left or right eye; randomly selecting the left or right eye; using the worst (most disease affected) eye; or using the best (least disease affected eye). Each of these selections have their own advantages, for example arbitrarily choosing an eye *a priori* or having an eye selected via a randomised process mitigates against selection bias, while focusing analyses around the worst eye allows for the evaluation of the intervention in the most serious

scenario. However, all the selections have a disadvantage as there is potential for selection bias as to which eye should be chosen and which method of selecting the eye should be used.

Drawbacks of using one eye

Analysis of one eye may make implicit assumptions regarding generalisability. These may be that both eyes will act similarly (in the case where one eye is randomly chosen) or that if an intervention works well in the most serious scenario then conclusions will be generalisable to the less severe scenario (in the case where the worst eye is used). As data are not collected or analysed in such a way to address this, such assumptions remain unverifiable.

The key limitation to only using one eye in analysis of clinical trials, is that such methods are not information efficient. Data are usually available from both eyes (even if not explicitly collected as part of the trial), yet half of it is somewhat arbitrarily discarded prior to analysis. This may mean that a larger sample size is needed to ascertain the same precision as in the case where both eyes are analysed.

Benefit of using both eyes

Research data that is generously provided by patients and carefully collected should have the benefit of not being cut in half at analysis where possible. By analysing both eyes, more data are contributed by the same number of participants, making better scientific use of a precious resource. The increase in data by utilising both eyes reduces uncertainty by providing more precise effect estimates and reducing uncertainty (such as narrowing of the confidence interval).

Utilising all the data contributed per participant in any study may be more ethical for several factors. Firstly, as each participant is contributing more data, it is possible to ascertain the same (or greater) statistical power with fewer participants. This could mean that fewer participants would be needed over all to deliver the results of any given trial and therefore allow for faster recruitment and overall shorter trial length. This ultimately would result in quicker ascertainment regarding the efficacy of an intervention by analysing both eyes. This reduction in sample size without sacrificing statistical power would facilitate trials in rare diseases, where single eye sample sizes may be infeasible due to the low prevalence in the general population. Furthermore, the arbitrary choice of which eye to analyse is negated, reducing any potential selection bias.

Two examples of specific study designs that use data from both eyes in subjects are discussed in detail below.

Studies where both eyes are affected by disease of interest and a subject level intervention is administered

In this instance, one of the following situations will hold: both eyes will be affected equally (such as often occurs in toxic optic neuropathy); the disease will affect each eye differentially, and this difference will be deterministic (such as occurs in glaucoma or Leber's hereditary optic neuropathy); or the disease will affect each eye differentially, and this difference will be random (such as occurs in multiple sclerosis and acute optic neuritis). Provided the mechanism of action of the intervention can be assumed to act equally over both eyes, analysis using both eyes will lead to an increase in the effective sample size (equivalently, an increase in statistical power). The extent to which effective sample size is increased depends on the intra-ocular correlation. If outcomes are perfectly correlated, the

effective sample size does not increase at all i.e. the two-eye analysis will be no more efficient than a single eye analysis. (3) The theoretical maximal increase in effective sample size is 100% (i.e. effective sample size is doubled), occurring when outcomes in the eyes are completely independent. However, in reality, outcomes from different eyes within a patient will always be correlated to some extent, and thus the expected increase in effective sample size is somewhere between these two extremes. (4)

To perform two eye analyses, the data for each eye needs to be paired and come from a common source (e.g. each participant contributing both a left and right eye). This creates a nested data structure within participants thus requiring more complex methods of analysis. To properly account for this nesting structure, hierarchical extensions to well-known regression modelling methods (also referred to as multi-level, random effects, or mixed effect models) which account for the nesting of data through random effects should be used. Many common statistical tests (e.g. linear regression, t-test, etc. etc.) can be written as linear models, and hierarchical extensions to linear models can be employed. (5) In instances where linear models are not appropriate, hierarchical extensions exist to other types of modelling such as repeated measures analysis, logistic regression models, and time-to-event models, all of which can be performed using either frequentist or Bayesian methodology. (6-8)

Due to the increased computational intensity of the planned analysis when using hierarchical models for two eyes, ascertainment of characteristics of trials designs (including sample size calculations and derivation of type I and II errors) can be more complex. Existing sample size formulae rarely have extensions to allow for hierarchical models, and instead simulations are often warranted. These simulations can be multifaceted and time consuming.

Studies where both eyes are affected by disease of interest and an eye level intervention is administered

Dependent on the mechanism of action of the intervention, it may here be appropriate to use intra-participant controls. This is feasible where it can be assumed that the intervention will only affect one eye (such for some topical treatments) and not for systemic drug interventions. By using each participant's untreated eye as their own control, the intra-participant variation will be reduced and so it may be possible to again ascertain more precise effect estimates. In this situation, analogous to the analyses of both eyes, more involved analysis methods, such as hierarchical models would need to be used.

The assumption that a local treatment will only affect one eye can however, be compromised, as was demonstrated in "REVERSE". REVERSE was a randomized, double-masked, sham-controlled, multicenter, phase 3 clinical trial that evaluated the efficacy of a single unilateral intravitreal injection of rAAV2/2-*ND4* in subjects with visual loss from Leber's hereditary optic neuropathy that reported an unexpected bilateral improvement in visual function after unilateral injection suggesting bilateral effect. (9) This effect has been noted in other ocular disease such as uniocular intravitreal injections in bilateral diabetic macular oedema (10) and topical treatments to lower intraocular pressure (11).

Other study designs

Two eye data can be analysed for other study designs as well. For example, generalized estimating equation or mixed effect models and bootstrapping approaches can generate point estimates and confidence intervals of sensitivity, specificity and predicative value studies of diagnostic accuracy using two eye data that may be congruent between eyes in some subjects

and incongruent between eyes in different subjects. (12) These methods have broader application to other observational studies.

Conclusion

Statistical analysis plans in ophthalmic disease need to carefully consider the rational for analysing one eye or two. Authors should clearly describe the methods used and the rationale for their statistical choices. Where the disease affects both eyes, generally both eyes should be analysed as this will allow a more precise estimate of effects, consequently increased power and reduced bias from selecting a single eye; however statistical tools must be employed to avoid bias. Using a single eye analysis because it is seemingly the default option may not be in patients' best interest who dedicate their time and energy to participate in clinical studies.

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