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Statistical Evaluation of Correlated Measurement Data in Longitudinal Setting Based on Bilateral Corneal Cross-Linking

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ABSTRACT

Purpose: In ophthalmology, data from both eyes of a person are frequently included in the statistical evaluation. This violates the requirement of data independence for classical statistical tests (e.g. *t*-Test or analysis of variance (ANOVA)) because it is correlated data. Linear mixed models (LMM) were used as a possibility to include the data of both eyes in the statistical evaluation.

Methods: The LMM is available for a variety of statistical software such as *SPSS* or *R*. The application was applied to a retrospective longitudinal analysis of an accelerated corneal cross-linking (ACXL (9*10)) treatment in progressive keratoconus (KC) with a follow-up period of 36 months. Forty eyes of 20 patients were included, whereas sequential bilateral CXL treatment was performed within 12 months. LMM and ANOVA for repeated measurements were used for statistical evaluation of topographical and tomographical data measured by Pentacam (Oculus, Wetzlar, Germany).

Results: Both eyes were classified into a worse and better eye concerning corneal topography. Visual acuity, keratometric values and minimal corneal thickness were statistically significant between them at baseline ($p < 0.05$). A significant correlation between worse and better eye was shown ($p < 0.05$). Therefore, analyzing the data at each follow-up visit using ANOVA partially led to an overestimation of the statistical effect that could be avoided by using LMM. After 36 months, ACXL has significantly improved BCVA and flattened the cornea.

Conclusion: The evaluation of data of both eyes without considering their correlation using classical statistical tests leads to an overestimation of the statistical effect, which can be avoided by using the LMM.

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Analysis of variance; both eyes; corneal cross linking; keratoconus; correlated data; linear mixed model; R SPSS

Introduction

Due to research on the paired organ, clinical studies in the field of ophthalmology occupy a special position. For the statistical evaluation of measured values of different groups or points in time, the discussion about the inclusion of only one eye or both eyes has existed for a long time. Numerous publications have already identified this problem and pointed to incorrect evaluation methods.[1,2] However, only a few ophthalmology-specific publications showed a concrete way to take this source of error into account in statistical planning and evaluation.[3–6]

In ophthalmology, an unwritten law dictates that the measured values for clinical studies, such as corneal thickness, corneal topography, intraocular pressure (IOP), visual acuity, retinal thickness, or ERG signals of only one eye are to be included in the evaluation of two or more group comparisons. The convention is usually to include either the right, left or a randomly selected eye. This practice is justified by the correlation (dependence) that exists between the right and left eyes. Examples for the dependence of corneal metrics are given in reports by Xu et al. and Durr et al., who found a strong correlation between mean keratometry, maximal keratometry, corneal astigmatism or corneal thickness of the right and left eye in healthy subjects.[7,8] In addition, another study observed such inter-eye correlations in pairs of eyes with keratoconus regarding keratometry and corneal thickness, although keratoconus can also occur asymmetrically.[9,10]

For the classical statistical evaluation methods (*Student's t-Test*, *Mann-Whitney-Test, analysis of variance (ANOVA)*), the linear regression analysis and also for the calculation of the confidence interval or the standard error, the strict assumption applies that the measured values should be independent of each other. These statistical procedures do not consider correlated data. They, therefore, demand the exclusion of the second eye of a person. Although the data of both eyes are often collected, or at least could be collected, only half of the data is used for evaluation. Data from both eyes of a person are so-called cluster data because they are similar and interdependent. When analyzing cluster data, several measurement objects (e.g. right and left eye) are

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selected from a cluster (e.g. one person) and associated variables (e.g. IOP, corneal thickness) are collected. However, these cluster data cannot be analyzed with the statistical methods mentioned above.

Karakosta et al. investigated 161 publications in five ophthalmological journals (*Acta Ophthalmologica, American Journal Ophthalmology, Journal Cataract Refractive Surgery, Journal Glaucoma, Retina*) and found that inter-ocular or intra-personal correlation was correctly considered in only 7% of ophthalmological studies. 62% of the studies included only one eye of a person in the evaluation and 31% did not consider this correlation when including both eyes.[3] The sole use of classical statistical methods, therefore, means an immense renunciation of evaluable and informative data. One-third of the publications ignore the statistical assumption of the independence of both eyes of a person.[3] Often, more eyes than persons are included in statistical evaluations of ophthalmological studies (e.g. 400 eyes of 300 persons), although one of the classical evaluation methods is used.

Assuming that both eyes receive the same treatment and the correlation between both eyes is not taken into account, a treatment effect can be statistically overestimated due to a higher number of cases and the associated reduction of the confidence interval and *p*-value. With this study, we would like to draw attention to the fact that in ophthalmological research it is possible to include both eyes of a person in the analysis of study data. At the same time, we would like to recommend the use of the statistically correct evaluation of these data with the help of the software *SPSS* and *R,* taking into account, the inter-ocular correlation. Previously, we have demonstrated the statistical effect between ANOVA and the linear mixed model (LMM) in an observational study design.[11] In the current study, the ANOVA for repeated measurements and the LMM were applied in a longitudinal study design to evaluate the effect of accelerated corneal cross-linking (ACXL) in progressive keratoconus (KC).

As it is known, KC is an ectatic disease of the cornea that leads to a loss of vision due to corneal protrusion, corneal thinning and irregular astigmatism.[12,13] KC often occurs bilaterally and asymmetrically.[10,14–16] Corneal cross-linking (CXL) using riboflavin and ultraviolet light is the most common treatment for progressive KC to halt the progression.[13,17–19] The treatment aims at improving corneal biomechanical properties, where new bonds are created between proteins and molecules.[20] In 2003, the first clinical study was published by Wollensak et al., who had used a UV-light intensity of 3mW and an irradiation time of 30 min.[21] This protocol is known as Dresden or standard protocol (SCXL (3*30)). Subsequently, accelerated CXL protocols were developed to shorten the treatment time, thereby improving the patient's comfort and clinical workflow.[22,23]

Methods

Patients and measurements

Forty eyes of 20 KC patients were retrospectively enrolled in this monocentric study. All patients were treated sequentially with the ACXL protocol in both eyes within 12 months between 2013 and 2017. The study was approved by the Ethics Committee of the Technical University of Dresden according to the guidelines of the Declaration of Helsinki (EK104032018) and registered as a clinical trial (NCT04251143). Inclusion criteria were progressive KC, defined as an increase of maximal keratometry (K_{max}) of more than 1 D within 6, and up to 12 months. Exclusion criteria were previous corneal surgeries (e.g. cross-linking or intra-stromal rings or segments) or other types of ectasia (e.g. pellucid marginal degeneration or post-laser vision correction ectasia). The patients were not allowed to wear contact lenses for at least 10 days prior to each examination. Follow-up visits took place at 3, 12, 24 and 36 months after CXL. At each visit, corneal topography and tomography, as well as visual acuity, were measured, followed by a complete ophthalmologic examination. The corneal topography and tomography were determined using Scheimpflug tomography (Pentacam, Oculus, Wetzlar, Germany). The quality criteria were fulfilled if the quality score (QS) by Pentacam displayed "ok." In cases where an error was indicated via "model deviation" notification, three measurements were performed to confirm the reliability. Following parameters were assessed to evaluate the CXL outcome: K_{flat}, keratometry value of flat meridian in the central 3 mm zone, K_{steep}, keratometry value of steep meridian in the central 3 mm zone, K_{max}, maximal keratometry value in the 9 mm zone and MCT, minimal corneal thickness.

Surgical procedure

The ACXL was performed using an intensity of 9mW and an irradiation time of 10 min (ACXL (9*10), UV-X 2000, Avedro, Inc., Waltham, Massachusetts, USA).[24] The corneas were anesthetized by topical anesthetic eyedrops (proxymetacaine hydrochloride 0.5%). Epithelium removal was performed using a hockey knife. Afterwards, a solution of 0.1% riboflavin containing hydroxypropyl methylcellulose (pharmacy of the University Hospital Carl Gustav Carus) was applied on the corneas in a frequency of 3 min for 30 min. The post-treatment scheme included wearing a therapeutic soft contact lens until completed epithelialization, antibiotic drops (ofloxacin) for 7 days, dexamethasone sodium phosphate for 3 weeks and lubricants.

Statistical analysis

The selection of only one eye of a person has the advantage that the conventional tests (e.g. student's *t*-test or ANOVA), which have been known for decades, can be used for statistical evaluation. However, this method also has significant disadvantages. Firstly, there is an ethical problem when data is collected that is not used for evaluation from the outset, which is also inefficient. If half of the data obtained is possibly omitted, the power (test strength) is reduced. If only one eye is selected, there is also the risk of a selection bias.[25] In principle, a random selection of one eye per person is possible – however, an analysis of the remaining unselected eyes should also be carried out to ensure that the results

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Table 1. Data structure (Long format) for each subject for statistical evaluation using linear mixed models in *SPSS* and *R*. "Group" represents the fixed effect (categorical independent variable), if two or more treatments were analyzed.

Subject	Group	Eye	Time point	K_{max} [D]
1	1	1	0	52.2
1	1	1	1	49.9
1	1	1	2	51.1
1	1	2	0	46.4
1	1	2	1	44.5
1	1	2	2	44.8
51	2	1	0	56.7
51	2	1	1	56.3
51	2	1	2	55.1
51	2	2	0	63.6
51	2	2	1	61.4
51	2	2	2	58.6

K_{max}, maximal keratometry value; Time point: 0: baseline measurement; 1: 12 months after treatment; 2: 36 months after treatment.

Table 2. Demographic data of keratoconus patients.

	Mean ± SD	95 % confidence interval (lower upper)	*p* value
Patients (*n*)	20		
Age [years]	25.7 ± 8.4	20.0 25.7	
Eyes right/left	20 (50 %)/20 (50 %)		
Gender (m./f.)	17 (85 %)/3 (15 %)		
BCVA [Logmar]			
Total	0.27 ± 0.22	0.20 0.34	
Worse eye	0.32 ± 0.21	0.21 0.42	**0.006**
Better eye	0.22 ± 0.23	0.11 0.33	
K_{flat} [D]			
Total	45.5 ± 3.8	44.3 46.8	
Worse eye	46.8 ± 4.2	44.8 48.8	**0.008**
Better eye	44.3 ± 2.9	42.9 45.6	
K_{steep} [D]			
Total	49.6 ± 4.4	48.2 ± 51.0	
Worse eye	51.1 ± 4.7	48.9 53.3	**0.002**
Better eye	48.1 ± 3.6	46.4 49.8	
K_{max} [µm]			
Total	57.4 ± 6.5	55.3 59.4	
Worse eye	60.2 ± 6.5	57.1 63.2	**<0.001**
Better eye	54.6 ± 5.3	52.1 57.0	
MCT [µm]			
Total	473.7 ± 41.8	460.4 487.1	
Worse eye	461.3 ± 37.9	443.5 479.0	**0.001**
Better eye	486.2 ± 42.7	466.2 506.2	
ARC [mm]			
Total	6.6 ± 0.5	6.4 6.7	
Worse eye	6.3 ± 0.4	6.07 6.48	**<0.001**
Better eye	6.8 ± 0.4	6.6 7.04	
PRC [mm]			
Total	4.9 ± 0.5	4.7 5.0	
Worse eye	4.6 ± 0.3	4.5 4.8	**<0.001**
Better eye	5.2 ± 0.5	4.9 5.4	

ARC, anterior radius of curvature based on ABCD grading system provided by Pentacam; BCVA, best corrected visual acuity; K_{flat}, keratometry value of flat meridian in the central 3 mm zone. K_{max}, maximal keratometry value in the 9 mm zone; K_{steep}, keratometry value of steep meridian in the central 3 mm zone; MCT, minimal corneal thickness; SD, standard deviation; m, male; f, female. Statistical significance was determined by paired *t* test and marked in bold ($p < 0.05$).

from both selection groups match. If only data e.g. from right eyes are used for the evaluation, then the evaluation should be repeated with the (left) partner eyes in order to test a possible influence of the body side. In any case, it is more accurate to ignore the data of one eye than to perform the evaluation without considering the dependency of both eyes. So far, however, the selection of only one eye has also been justified by the lack of available, suitable statistical programs. For some years now, corresponding statistical methods have been available that can consider correlated data, such as the Linear Mixed Model or Generalized Estimating Equations (GEE).

The Linear Mixed Model (LMM) is a statistical method for the evaluation of correlated interval scaled data.[6] Although the first formulation for the foundation of the LMM goes back to the British astronomer Airy in 1861, only in the past 30 years has a substantial advancement of statistical methods taken place, which consider correlated data.[26] This procedure is available both in commercial statistics programs, such as SPSS (IBM Statistics, Armonk, New York, USA) or SAS (Statistical Analysis System, SAS Institute, Cary, North Carolina), and in the freely available software R (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/) (packages Lme4 or nlme).[27] The LMM is a powerful and flexible method for statistical analysis, which assumes that the dependent variable (e.g. intraocular pressure, corneal thickness, or corneal curvature) is continuous and normally distributed. The normal distribution is assessed by the Q-Q plot or a corresponding statistical test (e.g. Kolmogorow-Smirnow test). If the data are not normally distributed, they can be transformed into a normal distribution (e.g. SQRT transformation). Furthermore, linearity must exist between the dependent variable and the fixed factors. Fixed factors are defined as factors that have a major influence on the outcome of the study purpose and can be specified within the statistic software. The linearity can be checked using a graphical representation between the residuals and the variables. A model is called an LMM if it contains mixed factors or effects (i.e. fixed and random factors simultaneously). The term *effect,* which is often used in the following, represents the totality of factors, covariates, and their combinations. The categorical independent variables (e.g. treatment group) are called fixed effects and the continuous independent variables (e.g. age) are called covariates.

In our examples, the random effect is modeled only by the random factor "person" (from which the two correlated data of both eyes were collected). This guarantees that the variation and the p-value are calculated under consideration of this inter-ocular correlation. In repeated measurements (longitudinal data), the individual data labeled as "person" has to be additionally defined as a "subject" to ensure the independence from other "subjects (persons), however, taking into account the correlation of the longitudinal data within the "subject." The dependent variable is the measured value, e.g. K_{max}. The correlated data (e.g. K_{max} values) of all eyes are included in the analysis. In the case of a balanced dataset (data from right and left eyes of all participants are available), the mean values of the variable do not change due to the consideration of the correlation between both eyes, but the standard error, confidence interval, and *p*-value do. If unbalanced data are available (isolated data of one eye of a person are missing), differences can also occur in the mean values of the variable.

The correlated data can be evaluated with the help of the LMM, both as a cross-sectional study design[11] and for longitudinal studies. The basic arrangement of the data for evaluation with the LMM is shown in Table 1, whereby the

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Table 3. Pearson correlation (r) and intraclass correlation coefficient (ICC) between the worse and better eye.

	r (*p* value)	ICC (*p* value)
BCVA [Logmar]	0.828 (**<0.001**)	0.814 (**<0.001**)
K_{flat} [D]	0.356 (0.081)	0.328 (0.051)
K_{steep} [D]	0.570 (**0.003**)	0.543 (**0.002**)
K_{max} [µm]	0.562 (**0.003**)	0.543 (**0.002**)
MCT [µm]	0.692 (**<0.001**)	0.690 (**<0.001**)

BCVA, best corrected visual acuity; ICC, intraclass correlation coefficient; K_{flat}, keratometry value of flat meridian in the central 3 mm zone. K_{max}, maximal keratometry value in the 9 mm zone; K_{steep}, keratometry value of steep meridian in the central 3 mm zone; MCT, minimal corneal thickness; r, Pearson correlation coefficient. Line represents the bisection line.
Statistical significance is marked in bold ($p < 0.05$).

values of the repeated measurement variable are displayed in a single column below one another (in long format) in the table. Examples of different study designs are presented in the Supplementary material (example 1: group comparison at one level of time; example 2: longitudinal study; example 3: a longitudinal study with group comparison), with the corresponding *SPSS* and *R* codes, where the LMM can be applied.

The statistical analysis was carried out with the *SPSS 25* and *R* software. Normal distribution was present for all parameters investigated using Kolmogorow-Smirnow test and Q-Q plots. Statistical significance was achieved at a *p*-value < 0.05. In this study, the algorithm of example 2 (Supplementary material) was used to analyze the data between baseline and each follow-up examination. Due to multiple comparisons, the *p*-values were adjusted by least significance difference (LSD) correction in both LMM and repeated measures ANOVA. The results were presented as mean values and 95% confidence intervals (95-CI). To assess the Pearson correlation and intraclass correlation coefficient (ICC, model = random, type = consistency, confidence interval = 0.95) between both eyes, they were classified once as "worse" and "better" eye of each patient based on topographical findings. Worse eye means a more advanced level of KC based on the topographic map and K_{max} value. Better eye means the eye with less developed KC based on the topography as well. Paired *t*-test was performed to analyze differences between the worse and better eye.

Results

The demographic data of the study cohort are presented in Table 2. Forty eyes of 20 KC patients who have shown bilateral progression were enrolled in this study. The mean age was 25.7 ± 8.4 years. There were more males (85%) than females (15%). Concerning visual acuity (BCVA) and topographic (*K*-values)/tomographic data (MCT), worse eyes had statistically significant higher *K*-values ($p < 0.05$) as well as significantly lower BCVA ($p = 0.006$), MCT ($p = 0.001$), an anterior radius of curvature ($p < 0.001$) and posterior radius of curvature ($p < 0.001$). In these eyes, 60% had secondary signs of KC in biomicroscopy (Fleischer Ring and Vogt's striae) whereas 40% did not show biomicroscopic findings. In better eyes, 40% showed secondary signs whereas 60% had no biomicroscopic findings.

Correlations between both eyes

Table 3 shows the correlations of BCVA, K-values and MCT between the two eyes (worse and better eye). There is no significant correlation between both eyes concerning K_{flat} ($r = 0.356$, $p = 0.081$). For K_{steep}, K_{max}, MCT and BCVA, it is found a significant positive correlation between the worse and better eye of a subject with an observed correlation coefficient (r) greater than 0.5 in all analyzed parameters ($p < 0.05$). The strongest correlation was found for BCVA ($r = 0.828$, $p < 0.001$) and MCT ($r = 0.692$, $p < 0.001$). The ICC showed similar results with high accordance between both eyes for BCVA, K_{steep}, K_{max} and MCT ($p < 0.05$). Figure 1 represents the scatterplots between worse and better eyes for all parameters.

Application of the LMM in comparison to repeated measures ANOVA

In order to show the effect of using the LMM in a dataset including both eyes of each patient, a mean value comparison was carried out between the baseline examination and a follow-up time point of 3, 12, 24 and 36 months. Simultaneously, both eyes of each patient were analyzed with repeated measures ANOVA as well. Table 4 shows the results of both the repeated measures ANOVA and the LMM concerning the BCVA, *K*-values and MCT.

A lower mean BCVA (logmar scale) after 3 (0.23), 12 (0.21), 24 (0.19) and 36 (0.17) months was found compared to baseline (0.27). Statistical significance was found after 12, 24 and 36 months using the repeated measures ANOVA ($p < 0.05$). However, the LMM showed statistical significance after 36 months in comparison to baseline ($p = 0.040$). The mean values and mean differences at each follow-up time were equal between repeated measures ANOVA and LMM; however, 95-CI were different between both methods.

For K_{flat}, no statistically significant changes were observed after CXL at any follow-up ($p > 0.05$). For K_{steep}, a slight decrease from 49.6 D at baseline to 49.3 D, 49.1 D and 48.8 D was observed after 12, 24 and 36 months, respectively. The repeated measures ANOVA showed statistical significance between baseline and 36 months (95-CI = 0.20–1.38, $p = 0.003$), which contrarily was not shown using the LMM (95-CI= −0.001–1.6, $p = 0.051$). K_{max} showed a slight increase 3 months after CXL that was not significant ($p > 0.05$). At 12, 24 and 36 months, the mean differences (baseline minus follow up) was 1.13 D (ANOVA: 95-CI = 0.05–2.21, $p = 0.035$; LMM: 95-CI = 0.04–2.22, $p = 0.037$), 1.33 D (ANOVA: 95-CI = 0.33–2.33, $p = 0.003$; LMM: 95-CI = 0.01–2.65, $p = 0.047$) and 1.74 D (ANOVA: 95-CI = 0.69–2.79, $p < 0.001$; LMM: 95-CI = 0.23–3.24, $p = 0.012$) indicating an apical flatting of the cone (K_{max}), respectively. MCT decreased significantly 3 months after CXL, by 15 µm ($p < 0.001$) and recovered to baseline at 12, 24 and 36 months (all $p > 0.05$) with a slight remaining decrease of approximately 8 µm. Both methods showed equal results concerning MCT. In 16 of 40 eyes, a slight persistent haze was observed after ACXL. None of the eyes had progressed after ACXL within 36 months; however, 2 eyes of 2 patients

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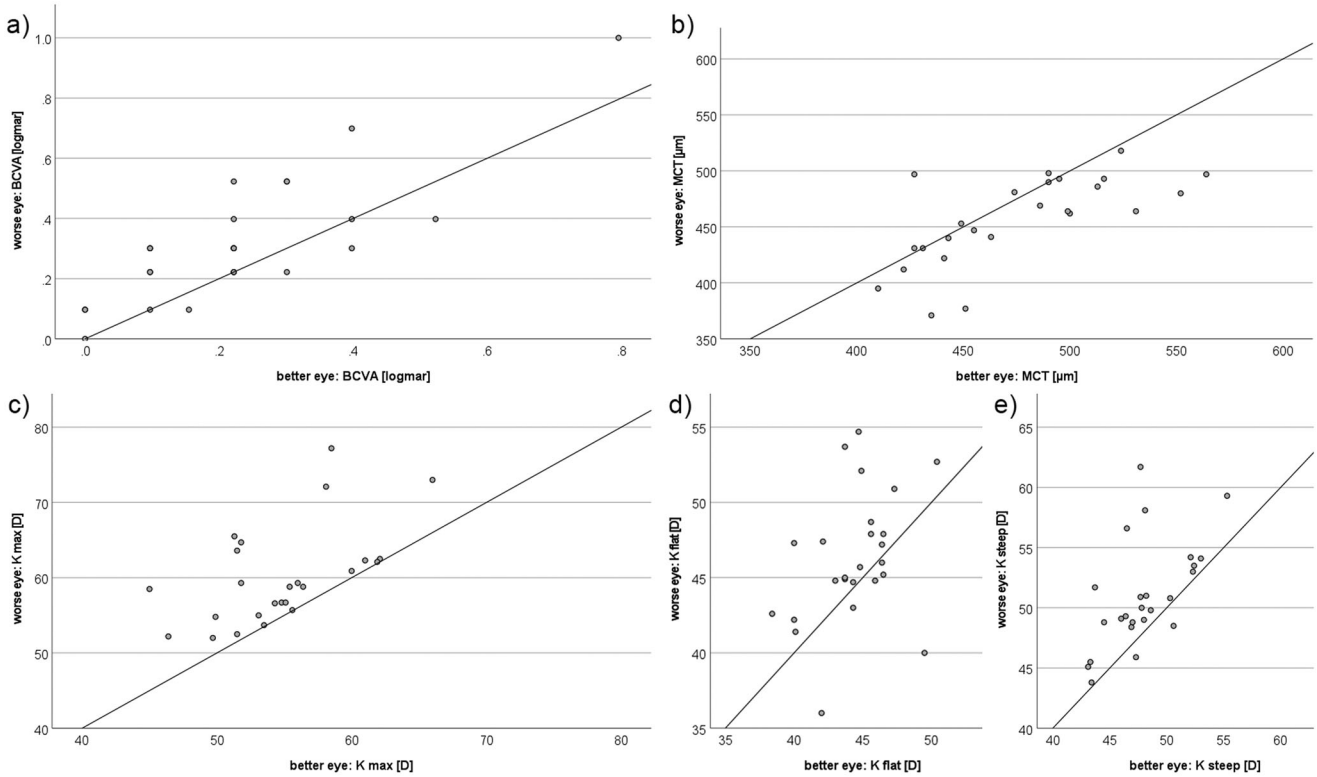

Figure 1. Scatterplots of (a) BCVA, (b) MCT, (c) K_{max}, (d) K_{flat}, (e) K_{steep} between the worse and better eye.

showed progression and were cross-linked again after 36 months.

Discussion

The main concern of our study is to point out the problem of the correlation of data of both eyes of a person. A recent analysis of ophthalmological studies showed no improvement in the use of statistical methods to adjust for the correlation between both eyes in the last 20 years.[28] In order to achieve statistically properly sized confidence intervals and p-values, knowledge of the possible applications of statistical tests is indispensable. So far, the LMM has not been able to assert itself among researching ophthalmologists. However, only the correct selection of suitable statistical methods can prevent larger data losses, possible false consequences from statistically incorrect study results or the exclusion of studies for evaluation purposes, for example for new treatment methods. In some current ophthalmological publications, this correction of the correlated data has already been exemplarily observed.[29–32] In principle, however, any statistical evaluation of data on paired organs should take into account, the intra-personal (in ophthalmology inter-ocular) correlation by applying appropriate modern statistical methods.

As a further advantage, the LMM also allows the evaluation of incomplete data records of repeated measurements. This means that measured values do not have to be available for all measurement times (unbalanced design). Therefore, the LMM is even better suited for the evaluation of longitudinal studies than the analysis of variance (ANOVA) with repeated measurement, in which entire patient or eye datasets must be completely excluded in the case of even a single missing measured value, which can lead to a considerable reduction in the number of cases. In addition, covariates such as age or gender can be included in the LMM statistics, which is not possible with the known *t*-test. As an example, only three basic examples were shown here (Supplementary material). An extension of the Linear Mixed Model to special evaluation targets is possible at any time. It is always advisable to have the evaluations carried out or at least validated by an experienced statistician.

The main issue regarding both eyes of the same person as independent for the evaluation in classical statistical procedures is that this leads to a doubling of the number of cases. Subsequently, the power increases and confidence intervals, as well as the p-values, seem to decrease. This is, however, an error and can result in an overestimation of the statistical effect.[33,34] This effect was shown previously in another dataset including both eyes of each participant that ANOVA has led to an overestimation of the statistical effect compared to using the LMM by our group.[11] The data were related to corneal biomechanical properties in healthy eyes obtained by the Corvis ST (Oculus, Wetzlar, Germany). It could be shown that when including data from both eyes and thus existing inter-ocular correlation, the use of the LMM instead of the ANOVA resulted in an increase of the p-values, which in turn is accompanied by increased standard error values. For some analyzed parameters, even the existence of statistical significance changed while the mean values hardly changed applying the LMM. In the current study, we have evaluated the effect of inter-ocular correlated data using (repeated) ANOVA and LMM on a longitudinal

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Table 4. Comparison of topographic and tomographic parameters between base ine and 3, 12, 24 and 36 months after CXL based on Tab e 2, examp e 2.

	Base ine	3 months			12 months			24 months			36 months		
	Mean (95-C)	Mean (95-C)	Mean difference (95-C)	*p*	Mean (95-C)	Mean difference (95-C)	*p*	Mean (95-C)	Mean difference (95-C)	*p*	Mean (95-C)	Mean difference (95-C)	*p*
BCVA [ogmar]													
ANOVA	0.27 (0.20–0.34)	0.23 (0.16–0.30)	0.04 (−0.02–0.10)	0.603	0.21 (0.14–0.26)	0.07 (0.01–0.13)	**0.020**	0.19 (0.12–0.26)	0.08 (0.01–0.15)	**0.020**	0.17 (0.10–0.24)	0.10 (0.04–0.17)	**<0.001**
LMM	0.27 (0.20–0.34)	0.23 (0.14–0.33)	0.04 (−0.02–0.09)	0.515	0.21 (0.12–0.30)	0.07 (−0.01–0.13)	0.134	0.19 (0.11–0.30)	0.08 (−0.01–0.14)	0.121	0.17 (0.09–0.28)	0.10 (0.002–0.16)	**0.040**
K_{flat} [D]													
ANOVA	45.5 (44.3–46.7)	45.6 (44.3–46.9)	−0.09 (−0.49–0.29)	1.0	45.2 (44.1–46.3)	0.33 (−0.20–0.86)	0.738	45.3 (44.1–46.5)	0.26 (−0.32–0.84)	1.0	45.1 (43.9–46.3)	0.43 (−0.27–1.13)	0.761
LMM	45.5 (44.1–47.0)	45.6 (44.2–47.1)	−0.1 (−0.47–0.27)	1.0	45.2 (43.7–46.7)	0.33 (−0.19–0.85)	0.744	45.3 (43.8–46.7)	0.26 (−0.37–0.89)	1.0	45.1 (43.6–46.6)	0.43 -(0.29–1.15)	0.931
K_{steep} [D]													
ANOVA	49.6 (48.2–51.0)	49.8 (48.3–51.3)	−0.22 (−0.71–0.27)	1.0	49.3 (47.9–50.6)	0.32 (−0.25–0.89)	1.0	49.1 (47.8–50.4)	0.48 (−0.07–1.04)	0.137	48.8 (47.5–50.1)	0.79 (0.20–1.38)	**0.003**
LMM	49.6 (47.9–51.3)	49.8 (48.1–51.5)	−0.22 (−0.63–0.19)	1.0	49.3 (47.6–51.0)	0.32 (−0.25–0.89)	1.0	49.1 (47.4–50.8)	0.48 (−0.21–1.18)	0.492	48.8 (47.1–50.5)	0.79 (−0.001–1.6)	0.051
K_{max} [D]													
ANOVA	57.4 (55.3–59.4)	57.5 (55.3–59.7)	−0.16 (−1.15–0.84)	1.0	56.2 (54.3–58.2)	1.13 (0.05–2.21)	**0.035**	56.0 (54.0–58.1)	1.33 (0.33–2.33)	**0.003**	55.6 (53.7–57.5)	1.74 (0.69–2.79)	**<0.001**
LMM	57.4 (55.0–59.7)	57.5 (55.2–59.8)	−0.16 (−0.94–0.63)	1.0	56.2 (53.9–58.5)	1.13 (0.04–2.22)	**0.037**	56.0 (53.7–58.3)	1.33 (0.01–2.65)	**0.047**	55.6 (53.3–57.9)	1.74 (0.23–3.24)	**0.012**
MCT [µm]													
ANOVA	474 (460–487)	459 (444–475)	15 (6–23)	**<0.001**	467 (451–482)	7 (−4–18)	0.630	466 (451–482)	8 (−2–17)	0.274	465 (449–482)	8 (−3–20)	0.370
LMM	474 (454–493)	459 (440–479)	15 (8–21)	**<0.001**	467 (447–486)	7 (−2–16)	0.267	466 (447–486)	8 (−3–19)	0.492	465 (446–485)	8 (−4–21)	0.510

Mean differences and *p* va ues represents the ana ysis between each fo ow up time point to base ine.
ANOVA, repeated measures ana ysis of variance; BCVA, best corrected visua acuity; C , confidence interva ; K_{flat}, keratometry va ue of f at meridian in the centra 3 mm zone. K_{max}, maxima keratometry va ue in the 9 mm zone; K_{steep}, keratometry va ue of steep meridian in the centra 3 mm zone; LMM, inear mixed mode ; MCT, minima cornea thickness. Statistica significance is marked in bo d ($p < 0.05$).

study design. The dataset was balanced as no data was missing. The analysis was applied to topographical and tomographical data before and after ACXL (9*10).

First, both eyes of each patient were simply categorized into the worse and better eye by evaluating the topographic maps and K_{max}. It was shown that worse eyes had statistically significant higher BCVA (logmar scale) and *K*-values as well as lower MCT. However, a significant positive correlation was shown between the worse and better eye concerning the analyzed parameters, except K_{flat}. Additionally, ICC showed good accordance ($0.4 < ICC < 0.75$) between worse and better eye for K_{steep}, K_{max} and MCT. BCVA had excellent accordance ($ICC > 0.75$) between both eyes, whereas K_{flat} showed poor accordance ($ICC < 0.4$). Therefore, the existing inter-ocular correlation of both eyes should be considered when performing statistical analysis if both eyes were included. The usage of LMM ensured that BCVA were not statistically significant at 12 and 24 months after CXL, whereas repeated measures ANOVA showed significance at these follow-up time points. The change was not based on different mean values; instead, 95-CI was changed. In addition, K_{steep} showed a significant change after 36 months that in turn was not significant if LMM was applied. In the case of K_{max}, both methods showed a significant decrease of K_{max} after 12, 24 and 36 months indicating a flattening of the keratoconic cornea and a stabilization effect. Our results are in accordance with other studies concerning the ACXL (9*10) protocol.[35–37] The ACXL (9*10) is an advancement of the SCXL protocol[21] with the benefit of a shorter treatment time to improve the patient's comfort and clinical workflow. However, experimental investigations comparing the efficacy of different protocols have shown that ACXL (9*10) significantly improves the corneal stiffness in comparison to controls; however, the stiffening effect was more pronounced in SCXL.[22,23,38–41] In a meta-analysis, it was shown that SCXL has greater efficacy in corneal flattening after treatment in comparison to ACXL. Both protocols though are suitable for the treatment of progressive KC.[42–44]

Conclusion

In the future, LMM should be the statistical method of choice for correctly evaluating correlated data in research ophthalmology. This longitudinal dataset has shown the influence of correlated data on the statistical effect. Under the presented considerations, an overestimation of treatment effects could be avoided in the future and possibly lead to a better acceptance of statistical analysis during approval processes of new treatments or treatment modalities by the government or official authorities.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, RH, upon reasonable request.

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