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Abstract:	Purpose To investigate the association between the vertical elevation of the external limiting membrane (ELM) and visual outcome in patients undergoing surgery for idiopathic full thickness macular hole (MH). Methods Retrospective observational study of a consecutive cohort of patients undergoing vitrectomy to treat MH. The greatest vertical height of the central external limiting membrane above the RPE (ELM height) was measured on spectral domain optical coherence tomography preoperatively. The relationship of ELM height to other pre and postoperative variables, including MH width and height, and visual acuity (VA) was analyzed. Results Data from 91 eyes of 91 patients who had undergone successful hole closure was included. The mean ELM height was 220 microns (range 100 -394). There were significant correlations between the ELM height and the diameter of the hole, hole height and worsening preoperative VA. For holes less than 400 microns in width, better postoperative VA was significantly predicted by a lower ELM height.

	Conclusion The ELM height varies widely in idiopathic MH. It is higher in eyes where the hole is wider and also when the hole itself is higher. For holes of less than 400 microns in width, a lower ELM height is a strong independent predictor of a good postoperative outcome.

Full title: The significance of preoperative external limiting membrane height on visual prognosis in patients undergoing macular hole surgery.

Abbreviated title: ELM height in idiopathic macular holes.

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Purpose

To investigate the association between the vertical elevation of the external limiting membrane (ELM) and visual outcome in patients undergoing surgery for idiopathic full thickness macular hole (MH).

Methods

Retrospective observational study of a consecutive cohort of patients undergoing vitrectomy to treat MH. The greatest vertical height of the central external limiting membrane above the RPE (ELM height) was measured on spectral domain optical coherence tomography preoperatively. The relationship of ELM height to other pre and postoperative variables, including MH width and height, and visual acuity (VA) was analyzed.

Results

Data from 91 eyes of 91 patients who had undergone successful hole closure was included. The mean ELM height was 220 microns (range 100 -394). There were significant correlations between the ELM height and the diameter of the hole, hole height and worsening preoperative VA. For holes less than 400 microns in width, better postoperative VA was significantly predicted by a lower ELM height.

Conclusion

The ELM height varies widely in idiopathic MH. It is higher in eyes where the hole is wider and also when the hole itself is higher. For holes of less than 400 microns in width, a lower ELM height is a strong independent predictor of a good postoperative outcome.

Precis

The vertical height of the ELM above the RPE was studied in a cohort of patients with idiopathic macular holes and found to vary widely. Its extent correlated with hole width and height. For small and medium sized holes, lower ELM height was a strong independent predictor of good postoperative outcome.

Introduction

Idiopathic macular hole (MH) formation is thought to occur secondary to the combined action of anteroposterior traction, a consequence of peri-foveal posterior vitreous detachment and tangential traction, and through contraction of myofibroblasts within epiretinal membranes around the rim of the macular hole.[1] The resultant inner retinal forces are transmitted to the outer retina via the central Muller cells and this results in an outer retinal dehiscence. This outer retinal traction results in the movement of the outer retina towards the inner retinal surface and on optical coherence tomography the external limiting membrane is seen to be elevated above the retinal pigment epithelium (RPE). Indeed photoreceptor components have been found in the inner retinal opercula[2] and on the peeled ILM of patients[3] undergoing MH surgery. We observed that the extent the ELM is elevated above the RPE varies between patients with MH, and has not been systematically studied or quantified. We hypothesized that the extent of the elevation may relate to the width and location of the initial central retinal dehiscence, and thus may be associated with visual prognosis. We conducted an observational study to describe this phenomenon and its association with other macular hole variables. We also aimed to assess its impact on visual outcomes following surgery.

Methods

This was a retrospective study of a cohort of patients who had undergone surgery by two surgeons for idiopathic full thickness macular hole. Consecutive patients undergoing primary surgery for MH of any width between January 2014 to June 2016 were included. Patients with traumatic macular holes, chronic holes for greater than 12 months, myopia greater than 6 dioptres, holes in association with other retinal pathology, previous retinal surgery or ocriplasmin treatment, second eyes, less than 3 months follow up, and those with inadequate imaging were excluded. Lamellar macular holes and pseudomacular holes were excluded.

All patients had undergone trans-conjunctival 25 or 27-gauge vitrectomy in the same institution using the same technique and equipment (Alcon Constellation, Alcon, Fort Worth, USA) with wide field non-contact viewing and combined phacoemulsification and intraocular lens implantation if phakic. Brilliant Blue G [ILM Blue, Dorc international, The Netherlands] was used to stain the ILM and peeled using a pinch technique and 25g end gripping forceps (Grieshaber revolution DSP ILM forceps, Alcon Grieshaber AG, Schaffhausen, Switzerland). Either 25% SF6 or 20% C2F6 gas was used as a tamponade and the patients were instructed to remain in the face down position for 3 days postoperatively.

Preoperative and postoperative best corrected visual acuity (BCVA) at 3 months was measured using a standard Early treatment diabetic retinopathy study (ETDRS) letter chart and converted to the logarithm of the minimum angle of resolution (logMAR) for the purposes of statistical analysis.

Patients underwent Spectral domain optical coherence tomography (SD-OCT) on the Heidelberg Spectralis immediately preoperatively and at 3 months postoperatively. A high density central horizontal scanning protocol with 30-micron line spacing was used in the central 15 degrees. All scans used a 15 automatic real time setting enabling multisampling and noise reduction over 15 images.

Holes were considered closed, indicating anatomical success, if there was complete circumferential hole rim reattachment without a full thickness foveal neurosensory retinal defect demonstrated on SDOCT.

Image measurements

On the preoperative OCT, the minimum linear diameter (MLD) and maximum base diameter (BD) of the hole were measured as previously described, using the image measurements tools on the Heidleberg software.[4] The vertical height of the macular hole, , was measured by placing a tangent across the highest points on either side of the hole, and then dropping a perpendicular to the RPE at the midpoint of the hole. The maximum horizontal distance between the edges of the external limiting membrane (ELM) parallel to the RPE (termed ELM gap), and the mean of the maximum heights of the ELM edges perpendicularly from the RPE (termed ELM height), were measured. (Figure 1) The presence of vitreomacular adhesions (VMA) to the edge of the hole was noted. The derived variable 'ELM height ratio' (ELM height/hole height) was calculated. On postoperative OCT, the minimum retinal thickness at the foveal centre and the presence and maximum width of any ellipsoid zone and/or ELM defect was measured.

Statistical analysis

Descriptive and statistical analysis was performed using SPSS statistical package (SPSS, release 2.16). Preoperative and postoperative variables are presented in terms of mean, standard deviation and range when normally distributed, and percentage as appropriate. Association between continuous data were assessed using correlations and between categorical data using two sample t-tests. Stepwise multiple regression was used to analyse the effect of multiple variables. Statistical significance was considered with a p-value of 0.05 or less.

Results

Ninety-one eyes of ninety-one patients were analysed. Twenty-three holes were excluded as per the entry criteria, including 5 holes that did not successfully close with initial surgery.

There were 74 (81%) females, 40 (44%) right eyes and a mean patient age of 69 years. Thirty-one (34%) eyes had preoperative VMA. There were 24 small (<250 microns), 32 medium (250-400 microns) and 34 large (>400 microns) sized holes. The other baseline characteristics of the cohort are shown in table 1.

The ELM height ranged from 100 to 394 microns, with a mean of 220 microns. (Figure 2) There were significant correlations between the ELM height and the measures of hole width, namely BD, MLD and ELM gap (r=0.72, 0.58, 0.38 respectively, all p<0.01).

ELM height was also significantly correlated with hole height (r=0.66, p<0.01), ELM height ratio (r=0.89, p<0.01) and worsening preoperative visual acuity (r=0.29, p<0.01). There was no association between the presence of VMA and ELM height (p=0.67), nor any of the other preoperative variables.

There were highly positive correlations between the ELM gap measurement and MLD and BD (0.88 and 0.75, p<0.01).

Postoperative outcomes are shown in table 2. An ELM defect was present in 7 (8%) eyes postoperatively, but an ellipsoid layer defect was present in 79 (87%) eyes at the 3-month time-point chosen.
All outcome measures were significantly and highly correlated with each other. (Table 3)

Based on the high correlation between the various preoperative features and similarly the postoperative ones, exploratory regression analyses were performed using postoperative visual acuity as the outcome measure, and the variables of age, MLD, ELM height, hole height and preoperative visual acuity as predictors. As this analysis was exploratory the stepwise method of entry was used. For the whole sample a significant model was found in which post op visual acuity was predicted by a combination of MLD (explaining 32% of variance in post op VA) and preoperative visual acuity (explaining a further 7%). The total model was significant (F (2, 88) =28.86, p<.001).

To further explore the relationship between variables, the sample was divided into three subgroups of MLD<250 microns, MLD 250-400 microns and MLD >400 microns (i.e. small, medium and large). The analysis was repeated.[5]For small and medium sized holes, postoperative visual acuity was significantly predicted by ELM height alone (Small holes; 33% of variability in postoperative visual acuity explained by ELM height, F(1, 17)=8.39, p=0.01, Medium holes; 18% of variability, F(1, 31)=6.93, p=0.013). For large holes ELM height was not a significant predictor and only preoperative visual acuity significantly predicted outcome (29% of variability in postoperative visual acuity, F(1, 37)=15.17, p<0.001).

These results suggested that the relationship between ELM height and outcome was moderated by MLD. This was confirmed by performing a moderator analysis (PROCESS procedure of SPSS release 2.16, Andrew Hayes) with postoperative visual acuity as the predictor variable, MLD as the moderator variable, and ELM height as the output variable, to ascertain whether the significant relationship between ELM height and postoperative visual acuity systematically varied with MLD. There was a significant interaction between postoperative visual acuity and MLD (t=2.76, p=.007) resulting in a significant increase in the explanatory power of the model (R^2 increase due to interaction = .081, F(1,87)=7.63, p=.007). Analysis of simple slopes using the Johnson-Neyman technique indicated that the significant relationship between ELM height and postoperative visual acuity held at values of MLD less than 382 microns, with the effect being largest when MLD was smallest. (Figure 3)

Discussion

We found that the extent to which the outer retina is elevated above the RPE and towards the inner retina varies widely in MH. The absolute ELM height was strongly related to the proportion of the macular hole height that it extended, and was found to be higher in eyes where the hole was not only higher but also wider. Its extent was inversely related to preoperative visual acuity; the higher the ELM height, the poorer the preoperative vision. Importantly however in holes less than 400 microns in diameter, ELM height was inversely related to postoperative visual acuity.

There have been numerous publications relating the width of a macular hole to postoperative outcome.[6–8] In addition, the postoperative ELM defect is also closely related to postoperative visual acuity and, unsurprisingly, the extent of the preoperative ELM defect itself is very closely related to the size of the macular hole.[9–15] However, we are not aware of any publications specifically examining ELM height in idiopathic macular holes.

We found a strong correlation between all four of our postoperative outcome measurements and therefore concentrated on postoperative visual acuity. Better postoperative vision was associated with a thicker fovea postoperatively, smaller defects in the ellipsoid zone, and the absence of an ELM defect. The association between the postoperative visual acuity, the size of the foveal ellipsoid defect, and the presence of an ELM defect were particularly high, which is consistent with the findings from previous reports.[10–15]

Our findings on ELM height are consistent with larger holes having greater movement of the outer retina towards the inner retinal surface. We postulate that this relationship may be related to the width of the zone of dehiscence of the outer retina during hole formation and the Z-shaped configuration of the Muller cells. A wide zone of dehiscence with involvement of the peri-foveal Z-shaped fibres may lead to a wide hole and greater elevation of the outer retina. This explanation is also consistent with the bistable theory of MH formation proposed by Woon et al[16], where they proposed that an increasing hole size with tangential traction eventually leads to the peri-foveal retina everting anteriorly as the tangential ILM force overcomes the retinal-RPE adhesion. Rather than increasing hole size, it may be that the initial hole size is actually the main determining factor for the eversion.

It is postulated that the Muller cells located in the very centre of the fovea are of a different morphology to perifoveal ones. Centrally they are characterized by a thin cytoplasm, straight vertical course, and do not extend as far as the ELM, perhaps explaining some of the vulnerability of the fovea to dehiscence.[17–20] Gass[21] proposed the term 'Muller cell cone' to describe an inverted triangular shaped structure, based on the central ILM, which acts to bind the central fovea together. Dehiscence in this very central area with central VMA would only result in a small hole without direct traction on the outer retina, and without elevation of the outer retina to the inner retinal surface. In such cases it may be expected that surgical hole closure would result in good visual acuity; this is what we found. The most strongly predictive factor for visual acuity outcome in small and medium sized holes is a low ELM height. This relationship does not exist for larger holes greater than 400 microns in size. In these cases, preoperative visual acuity alone predicts outcome and not ELM height. We postulate that the reason for this is that hole size is partly related to chronicity, and in turn this is related to outer retinal atrophy which would reduce ELM height and confound the effect on visual outcome. Unfortunately, we did not systematically record hole duration so cannot comment specifically on this aspect, although we did only include holes of less than 12 months in duration by history. It is possible that chronicity will alter ELM height by atrophy but this would be less likely to affect the findings in narrower holes.

Our findings also support the theory recently presented by Chung and Byeon[22] who proposed that there are two types of macular hole based on the area of dehiscence during hole formation. They divided holes in two types: type A where the zone of dehiscence affected only the Muller cell cones, and type 2 where the zone was wider involving the Z-shaped eccentric Muller cells. They predicted that type A holes would have better preoperative outcomes; our finding that smaller holes with low ELM height have a better prognosis concurs with this prediction.

It is uncertain exactly which factors dictate the width of the zone of dehiscence at onset. Shin et al[23] have recently shown that foveal pit size in the fellow eye is highly related to macular hole size. With the well-established symmetry in foveal shape between eyes, they postulated that foveal shape was a significant determinant of macular hole size. In turn, this may explain differences in hole size between races and sexes.[23-25] We did not evaluate foveal floor size in the fellow eye so cannot assess whether this had an impact in our cases. It is accepted that most macular holes result from anteroposterior vitreous traction resulting from perifoveal vitreous separation.[1] The width of the fovea that dehisces to form the hole may relate to the width of the zone of vitreofoveal traction when the hole first forms. However, we did not find an association between MH size and the presence of VMA when the patient underwent surgery and similarly, the ELM height did not increase with the presence of VMA. Interestingly Tsai et al. found that impending macular holes with a more vertical angle of vitreous insertion had wider zones of vitreomacular adhesion and a higher rate of foveal detachment, and stage 2 macular holes with the same vertical angle of vitreous insertion were higher, had wider base diameters and had less improvement in postoperative visual acuity improvement than those with lower more horizontally inserted vitreous attachment.[26] We did not assess the angles of vitreous insertion but it would be interesting to see if ELM height was related to vitreous insertion angle and VMA width prior to hole formation.

There are several limitations to this study. The study analyzed one preoperative OCT and did not evaluate changes in ELM height with time, which may alter as the macular hole forms. As mentioned we did not systematically record hole duration which may have affected our findings. Two surgeons conducted the operations, however they used the same surgical technique and no difference in outcome between them were found.

In conclusion, we found that the extent to which the ELM was elevated towards the inner retina varied widely in MH. In holes that are less than 400 microns in MLD, a low ELM height was a strong independent predictor of a good postoperative outcome, likely relating to differences during initial foveal dehiscence and hole formation. Further study to understand this observation is needed.

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Figure legends

Figure 1: Spectral domain optical coherence tomography images of a macular hole. Upper panel shows measurement of minimum linear diameter and base diameter. Lower panel shows measurement of external limiting membrane (ELM) height and gap (ELM highlighted by a white line). The mean of the two measured ELM heights was used for analysis.

Figure 2: Spectral domain optical coherence tomography images of four macular holes with high (on left hand side of image) and low (on right hand side) ELM heights. The two upper and two lower holes have similar minimum linear diameters. In all images, the ELM is highlighted by a white line.

Figure 3: The shape of the relationship between postoperative visual acuity (VA) and external limiting membrane (ELM) height at different levels of minimum linear diameter (MLD). When MLD is small there is a steep relationship, while with increasing MLD, the relationship between ELM height and postoperative visual acuity gradually flattens and becomes non-significant. For both MLD and ELM, 1-5 refer to quintiles with 1 being the lowest 20%, to 5 being the highest 20%.

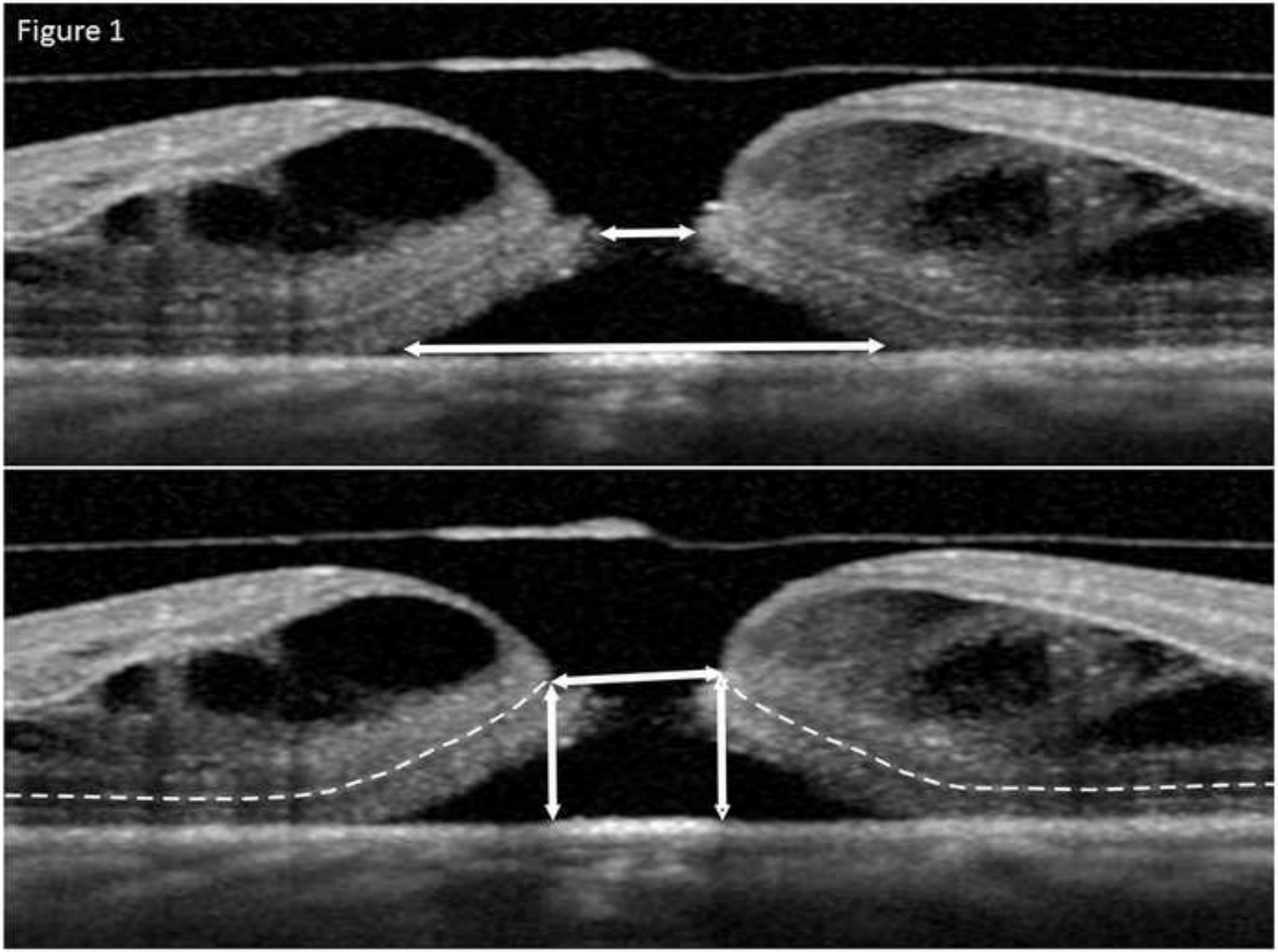
Figure 1

Figure 2

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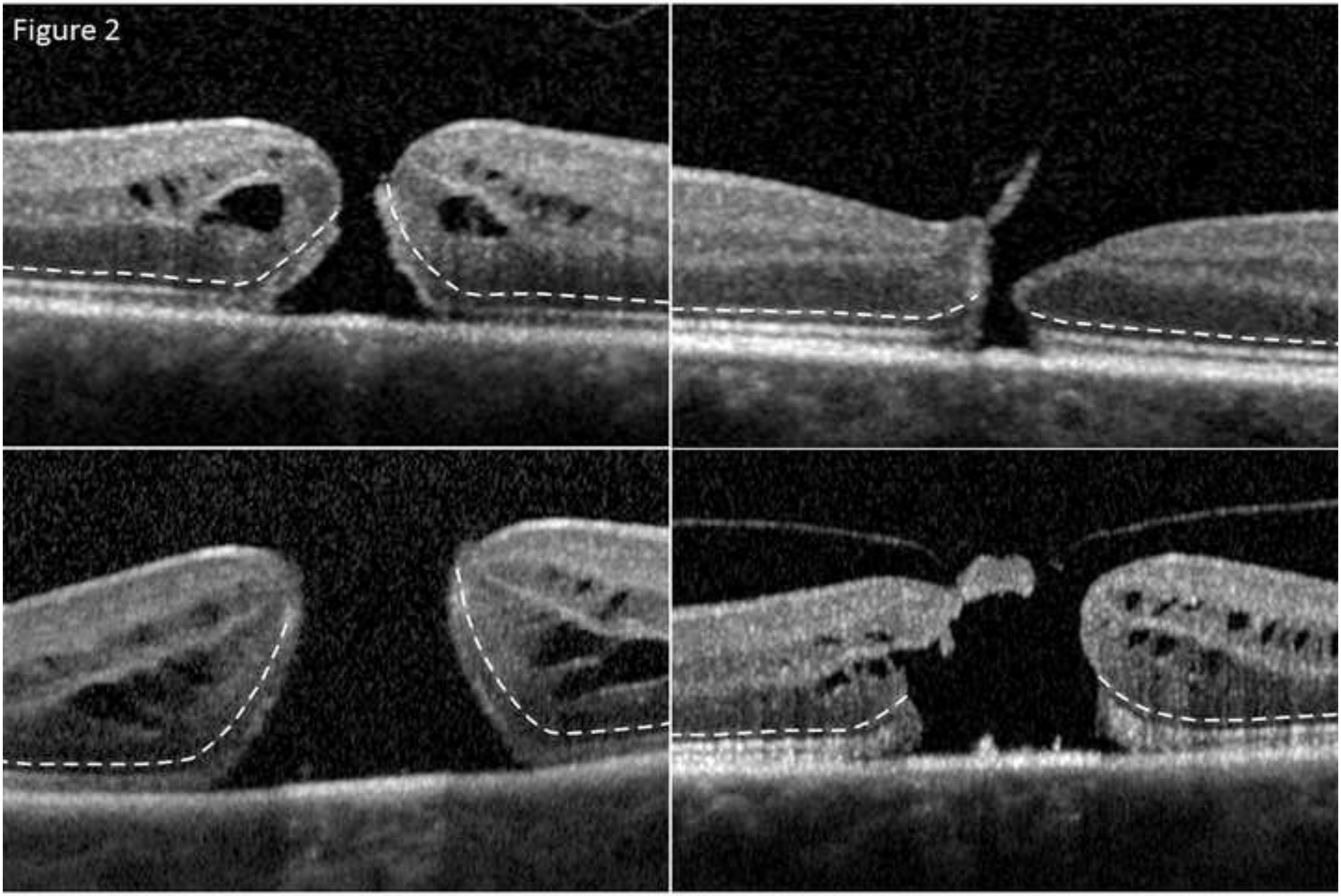

Figure 3

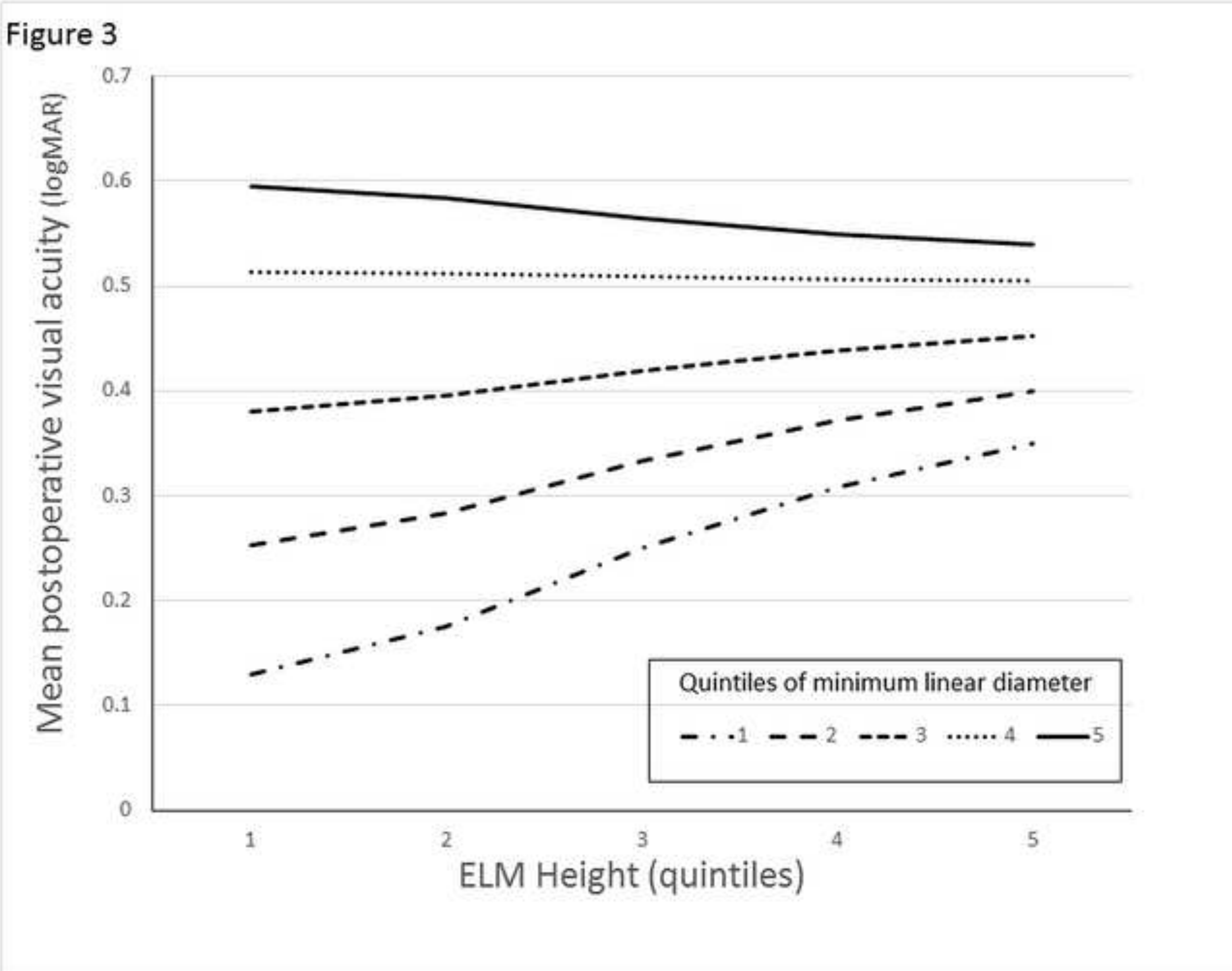
Figure 3
Mean postoperative visual acuity (logMAR)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
ELM Height (quintiles)
Quintiles of minimum linear diameter
1
2
3
4
5

Table 1: Baseline characteristics

	Mean	Standard deviation	Range
Age (years)	69.9	7.3	48-84
Preoperative Va (logMAR, Snellen)	0.90 (20/159)	0.22 (20/33)	0.44-1.5 (20/55-20/632)
MLD (microns)	369.3	166.9	32-774
BD (microns)	740.7	269	100-1331
Hole height (microns)	383.7	53.3	274-503
ELM gap (microns)	430.6	171.3	78-840
ELM height (microns)	220.1	63.7	100-394.5
ELM height ratio	0.57	0.12	0.31-0.92

Va; visual acuity, ELM; External limiting membrane, BD; Base diameter, MLD; minimum linear diameter.

Table 2: Postoperative outcomes.

	Mean	Standard deviation	Range
Postoperative Va (logMAR, Snellen)	0.39 (20/49)	0.27 (20/37)	0-1.3 (20/25- 20/399)
Minimum foveal thickness (microns)	156.3	53.4	27-302
Ellipsoid defect (n=79) (microns)	189.1	358.9	0-2208
ELM defect (n=7) (microns)	38.9	177.3	0-988

Va; visual acuity, ELM; External limiting membrane

Table 3: Correlations between postoperative outcomes.

	Postoperative Va	Minimum foveal thickness	Ellipsoid defect
Minimum foveal thickness	-0.37		
Ellipsoid defect	0.64	-0.46	
ELM defect	0.53	-0.52	0.77

Va; visual acuity, ELM; External limiting membrane

All values are significant at p<0.01.