Fundus Topographical Distribution Patterns of Ocular Toxoplasmosis

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Short title: Fundus Distribution of Ocular Toxoplasmosis

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Word Count: 2921

Abstract

Background: To establish topographic maps and determine fundus distribution patterns of ocular toxoplasmosis lesions (OT).

Methods: In this retrospective study, patients who presented with OT to ophthalmology clinics from four countries (Argentina, Turkey, UK, USA) were included. Size, shape, and location of primary (1°)/recurrent (2°) and active /inactive lesions were converted into a two-dimensional retinal chart by a retinal drawing software. A final contour map of the merged image charts was then created using a custom Matlab program. Descriptive analyses were performed.

Results: 984 lesions in 514 eyes of 464 subjects (53% females) were included. Mean area of all 1° and 2° lesions was 5.96 ± 12.26 and 5.21 ± 12.77 mm^2, respectively. For the subset group lesions (eyes with both 1° and 2° lesions), 1° lesions were significantly larger than 2° lesions (5.52 ± 6.04 mm^2 vs. 4.09 ± 8.90 mm^2, $P = 0.038$). Mean distances from foveola to 1° and 2° lesion centres were 6336 ± 4267 and 5763 ± 3491 µm, respectively. The majority of lesions were found in temporal quadrant ($P <0.001$). Maximum overlap of all lesions was at 278 µm inferotemporal to foveola.

Conclusion: The 1° lesions were larger than 2° lesions. The 2° lesions were not significantly closer to fovea than 1° lesions. Temporal quadrant and macular region were found to be densely affected underlining the vision threatening nature of the disease.

What is already known on this topic: Toxoplasma retinochoroiditis is known to cause sight-threatening involvement posterior pole. However, there is no thorough analysis of distribution patterns of retinal toxoplasmosis and relations between primary and secondary lesions.

What this study adds: The current study reports topographic location of toxoplasma retinochoroiditis lesions and trends of change between primary and secondary lesions in terms of lesion size and location.

How this study might affect research, practice or policy: Determination of distribution patterns of ocular toxoplasma lesions may increase our understanding of disease pathophysiology and will guide future therapies.

Précis:

Topographic mapping of 984 lesions showed that all primary lesions had maximum overlap at a point 278 microns inferotemporal to foveola. Recurrent lesions were found to be significantly smaller in size.

Keywords:

INTRODUCTION

Ocular toxoplasmosis (OT) is the most common cause of posterior uveitis in immunocompetent subjects and the most frequent cause of infectious uveitis in many countries. The hallmark of ocular toxoplasmosis is focal necrotizing retinochoroiditis, ultimately resulting in characteristic atrophic and/or pigmented scars. [1] In typical cases active lesions are seen along with scars that resulted from prior affliction and characterized as whitish foci of retinochoroiditis, without well-limited borders. In postnatally acquired toxoplasmosis, as in congenital toxoplasma retinochoroiditis, recurrent lesions also can be located remote from scarred lesions, however new lesions are often located as satellites of pigmented toxoplasma scars. [2] It is generally considered that tissue cysts which had been already formed during prior active toxoplasma infection are responsible for causing these adjacent lesions. It has also been suggested that new retinitis lesions are not randomly distributed but rather might preferentially involve selected zones. [3]

Reactivation of the initial retinal condition presumably results from the rupture of quiescent parasitic cysts lying adjacent to pre-existing scars and may secondarily involve the choroid eventually leading to retinochoroiditis. [4] Unfortunately, there is still no practical way of knowing where the next satellite or recurrent retinochoroiditis lesion is going to be located. To our knowledge, there are no studies about the location of the recurrent lesions in comparison to previous toxoplasmosis activation scars. Indeed, there are few studies exploring the location of toxoplasma retinochoroiditis (primary or secondary) lesions in terms of retinal topography. Our primary objective in this study is to establish a topographic map and determine fundus distribution behaviours of primary ocular toxoplasmosis lesions as well as secondary or recurrent lesions.

METHODS

Patient Selection

In this retrospective study, patients with ocular toxoplasmosis (OT) who presented with OT to ophthalmology clinics from four countries (Argentina, Turkey, UK, USA) were included. Ocular toxoplasmosis (OT) diagnosis was confirmed based on clinical findings where an active lesion was observed at least at one visit and positive serology and/or polymerase chain reaction for toxoplasmosis. Active OT was defined as the presence of an active whitish focal retino-choroidal

lesion in either eye. If there was a single OT lesion, whether active or inactive, the lesion was termed as primary. If there is a previous scar in either eye, OT was be considered recurrent or secondary.

Clinical evaluation

All patients who underwent a complete ophthalmologic evaluation including best-corrected visual acuity (BCVA), slit- lamp examination, fundus examination, colour fundus photography (Carl Zeiss Meditec, Inc., Jena, Germany), wide field OPTOS images, Heidelberg Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) at the initial visit were included. Patients presenting with a scarred lesion without evidence of an active lesion underwent a single-color photo image directed to the scar. In cases that presented with both a scarred lesion and an active lesion, the colour photos focused on the scar lesion as well as on the active lesion were obtained. Clinical data such as lesion status (active, scarred), presentation (primary, recurrent, asymptomatic, or treated old scar, distant or satellite lesion), presence of anterior uveitis, ocular hypertension, involvement of optic nerve, presence of vitreous haze, were also collected from medical charts of subjects, retrospectively. Clinical features such as anterior uveitis, ocular hypertension, periphlebitis, arteritic vasculitis, retinal haemorrhages, papillitis, vitreous haze score, exudative retinal detachment, hard exudates are collected from medical records of the patients and confirmed with fundus images where applicable. Vitreous haze scores are assessed from the optic nerve head centred colour fundus photographs.

Fundus mapping

Based on a thorough evaluation of all available fundus photographs, the best image of the lesion with less amount of blur, artefact and media opacity was chosen and included in the dataset. Quality of the photographs was assessed by an independent image grader. Images with less than satisfactory quality were excluded from the study. Based on fundus photographs, all visible toxoplasmosis lesions were drawn manually with azimuth equidistant projections on a standardized retinal drawing chart. Lesions were colour labelled as primary (1°) and recurrent/secondary (2°). 1° lesions were defined as the presence of an inactive only toxoplasma lesion or inactive lesion with an accompanying active lesion whether it is solitary or multifocal. As it is not possible to differentiate if the retino-choroidal scar is a result of a single activation or represents fusion or reactivations adjacent to it any pre-existing toxoplasma scar were assumed as 1° inactive lesion; if there is evidence of

previous toxoplasmosis scar in the fundus photo, then the newer (active) toxoplasmosis lesion was considered 2°. Subset group consisted of eyes that present both an inactive 1° and an active 2° lesion type at the same visit. After image labelling, size, shape, and location of all lesions were converted into a two-dimensional retinal chart. The drawing tools of the computer software PowerPoint™ (Microsoft Corp., Redmond, WA) were used for that purpose. Three trained retina and uveitis specialists (MH, MSO, GU) performed all drawings, and special care was taken to correct for circumferential distortion in the periphery. Lesion borders were drawn through the limit of retinal section where the retinal infiltrative part of the lesion ends. Each pixel of the color-coded images was divided in in three groups; 1° lesion, 2° lesion and non-infected by an automated MatLab™ algorithm. Descriptors like centroid (centre of mass of the binarized lesion), boundary (pixels lying on the margin of the lesion), area (number of pixels inside the margin, [margin inclusive] of the lesion X size of a pixel in μm^2), distances (fovea to the centroid of each lesion, fovea to the closest margin, disc centre to lesion centroid, disc centre to nearest lesion margin) were calculated. A final contour map of the merged images was then created aiming to display number of overlapping toxoplasmosis lesions in form of a heat map. Border-drawn images were converted to contour maps that have the same coordinates for optic nerve head and foveal centre. The map images have been finally superposed to form heat maps with colour coding of each pixel between no lesion (blue) and highest lesion overlap (yellow). The pixel with the highest lesion superpositioning is defined as "the maximum point of overlap. For the purposes of location and clinical correlation, the posterior pole was also divided into three zones as described by Holland et al. [5] and four quadrants with the optic nerve head at the centre. According to this retinal topographical classification, Zone 1 consists of the area of retina within 1500 μm from the edge of the optic nerve or within 3000 μm from the centre of the fovea. This area roughly corresponds to the posterior pole. Zone 2 extends from the edge of zone 1 anteriorly to a circle identified by the vortex vein ampullae. Zone 3 extends anteriorly from Zone 2 to the ora serrata. Quadrants were defined by dividing the posterior pole into four sectors: superior (between 45°-135°), temporal (between 135°-225°), inferior (between 225°-315°) and nasal (between 315°-45°) to the optic nerve head. The lesions then labelled according to the zone and quadrant location of their centre coordinates. Surface areas of Zone 1 (combined 3-mm diameter fovea-centred and 1.5-mm diameter

optic nerve head-centred areas) and Zones 2&3 together were calculated according to retinal surface area of 1363 mm^2 calculated by Nagra et al. [6], then lesion density within Zone 1 and Zones 2&3 were calculated by dividing number of lesions to the corresponding zone area.

Statistical analysis

Statistical analysis was performed with SPSS® 25 Software (IBM® Corporation, Armonk, NY, USA). Kolmogorov-Smirnov tests were performed to assess data distribution. Lesion distribution data for each zone and quadrant were given in frequency and percentage. Mean ± standard deviation (SD) of lesion area and distances to fovea and optic nerve were given in μm^2 and μm, respectively. Chi square test was performed to compare lesion distribution. Paired t-test was performed to compare parametric values of 1° and 2° lesions in subset group. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The study included 984 lesions in 514 eyes of 464 patients. Patient demographics and clinical features are shown in Table 1. Of 244 eyes who had active OT, 179 (43.7%) had anterior uveitis, 100 (24.4%) had periphlebitis, 54 (13.3%) had papillitis and 306 (75.0%) had vitritis with 1+ or more vitreous haze score.

Table 1. Patient demographics and clinical features of ocular toxoplasmosis cases.

Demographics		
Number of Patients	464	
Bilateral involvement	50	
No. of Affected Eyes	514	
Age (mean ± SD)	37.23 ± 15.62	
Females, n (%)	212 (45.7)	
Race, n (%)		
Caucasian	376 (81.0)	
African American	35 (7.5)	
Asian	12 (2.6)	
Hispanic or Latino	15 (3.2)	
Other	26 (5.6)	
Clinical features	n	(%)
Eye Involved		
OD	234	(50.4)
OS	180	(38.7)
OU	50	(10.8)
Disease Status (Eye)		
Active	244	(47.4)
Inactive	270	(52.6)

Presentation							
	Primary	774		(78.7)			
	Recurrent	210		(21.3)			
Ocular findings of active cases							
	Anterior uveitis	179		(43.7)			
	Ocular hypertension	50		(12.4)			
	Periphlebitis	100		(24.4)			
	Arteritic vasculitis	28		(7.0)			
	Retinal haemorrhage	56		(14.0)			
	Vitreous Haze Score $\geq$ 1+	306		(75.0)			
	Papillitis	54		(13.3)			
	Exudative RD	22		(5.5)			
	Hard exudates	27		(6.7)			

A total of 629 inactive (63.9%) and 355 active lesions (36.1%) were present. Centre point

lesion (centroid) distributions within posterior pole quadrants and zones are given in Table 2 and

Figure 1. The majority of both active (52.7%) and inactive (47.9%) lesions were found temporal to the

optic nerve head (all P <0.001). Near twenty-nine percent of lesion centroids were located in Holland

Zone 1, a distribution pattern which was reflected similarly for active (35.2%) and inactive (24.8%)

lesions, respectively. Lesion density in Holland Zone 1 was higher and calculated as 7.95 lesions/mm^2,

whereas lesion density in Holland zones 2 and 3 in total was found 0.53 lesions/mm^2.

Table 2. Centroid distribution of toxoplasma lesions within posterior pole quadrants and Holland

zones.

Lesions	Total	Quadrants (n; %)				Holland zones (n; %)	
		Superior	Temporal	Inferior	Nasal	Zone 1	Zones 2&3
Cumulative	984	176 (17.9)	**488** **(49.6)**[*]	132 (13.4)	188 (19.1)	281 (28.6)	703 (71.4)
Active	355 (36.1)	74 (20.8)	**187** **(52.7)**[*]	45 (12.7)	49 (13.8)	125 (35.2)	230 (64.8)
Inactive	629 (63.9)	102 (16.2)	**301** **(47.9)**[*]	87 (13.8)	139 (22.1)	156 (24.8)	473 (75.2)
Primary	774 (78.7)	133 (17.2)	**385** **(49.7)**[*]	105 (13.6)	151 (19.5)	223 (28.8)	551 (71.2)
Secondary	210 (21.3)	43 (20.5)	**103** **(49.0)**[*]	27 (12.9)	37 (17.6)	58 (27.6)	152 (72.4)

Subset	296	60 (20.3)	**143 (47.3)**[*]	37 (12.5)	56 (18.9)	82 (27.7)	214 (72.3)
Primary	148	30 (20.3)	**70 (47.3)**[*]	18 (12.2)	30 (20.3)	42 (28.4)	106 (71.6)
Secondary	148	30 (20.3)	**73 (49.3)**[*]	19 (12.8)	26 (17.6)	40 (27.0)	108 (73.0)

*: Chi-square test within quadrants for lesion distribution. All $P < 0.001$.

Topographic measurements of OT lesions are given in Table 3. Heat maps depicting overlapping lesion areas in pixel units are given in Figures 2-4. The overall mean area of OT lesions was 5.80 ± 12.37 mm². The lesion range extended from the smallest lesion of 0.14 µm² to the largest lesion of 191.59 mm². OT lesion centroids were 6213 ± 4118 µm distant from fovea and 6541 ± 3750 µm distant from the optic nerve head, with a point of maximum overlap at 278 µm inferotemporal to the fovea (Figure 2). Overall, 2° OT lesions were smaller than 1° lesions (5.96 ± 12.26 vs. 5.21 ± 12.77 mm²) and active lesions were smaller than inactive lesions (5.29 ± 10.61 vs. 6.09 ± 13.26 mm²). 2° OT lesion centroids tend to be closer to foveal centre and optic nerve head when compared to 1° lesions.

Table 3. Topographic measurements of toxoplasma lesions.

Lesion type	n	Lesion centroid distance		PMO from fovea (µm)	Mean area (mm²)
		to fovea (µm)	to ONH (µm)		
All lesions					
Consolidated	984	6213 ± 4118	6541 ± 3750	278	5.80 ± 12.37
Inactive	629	6771 ± 4317	7025 ± 3883	327	6.09 ± 13.26
Active	355	5226 ± 3536	5684 ± 3340	415	5.29 ± 10.61
Primary	774	6336 ± 4267	6647 ± 3884	458	5.96 ± 12.26
Secondary	210	5763 ± 3491	6151 ± 3191	653	5.21 ± 12.77
Subset lesions					
Consolidated	296	5705 ± 3464	6018 ± 3048	466	4.80 ± 7.62

Primary	148	5849 ± 3590*	6061 ± 3074†	771	**5.52 ± 6.04‡**
Secondary	148	5561 ± 3338*	5975 ± 3032†	655	**4.09 ± 8.90‡**

*: Paired t-test; $P = 0.105$; †: Paried t-test; $P = 0.633$; ‡: **Paired t-test; $P = 0.038$**; ONH: Optic nerve head; PMO: Point of maximum overlap.

Comparative analysis of 1° (inactive) and 2° (active) lesions was performed on subset group which included eyes with both types of lesions (Table 2, Figure 3). The majority of both 1° and 2° lesions were found in temporal quadrant. The mean area of subset lesions was 4.80 ± 7.62 mm^2, for that 1° lesions (5.52 ± 6.04 mm^2) were found significantly larger than 2° lesions (4.09 ± 8.90 mm^2, $P = 0.038$). Lesion centroids were 5705 ± 3464 μm distant to fovea and 6018 ± 3048 μm distant to ONH, which showed no significant difference between two groups (1°: lesion to fovea: 5849 ± 3590 μm, lesion to ONH: 6061 ± 3074 μm; 2°: lesion to fovea: 5561 ± 3338 μm, lesion to ONH: 5975 ± 3032 μm; all $P >0.05$). Mean distance between lesion centroids of 1° and 2° lesions was found 2866 ± 2134 μm. Maximum overlap point for 1° OT lesions was found 771 μm inferotemporal to the fovea and 655 μm supertemporal to the fovea for 2° lesions, being 982 μm away from each other.

Difference of lesion centroid locations between 1° and 2° lesions is given as a vectorial diagram in Figure 4. Secondary lesions were found on the same quadrant in 112 (75.7%) of primary cases with similar percentages for superior (70%), temporal (81.4%), inferior (61.1%) and nasal (76.7%) quadrants.

DISCUSSION

The study presents topographic characteristics of ocular toxoplasmosis lesions in the retina. Among very few studies which aim to assess distribution patterns of OT lesions, to our knowledge, this is the first and largest descriptive study which depicts OT lesion characteristics with comprehensive, quantitative data including lesion area, lesion centre location, distance of nearest affected location and retinal topographic assessment of disease-affected areas.

As indicated by highly varied lesion centroid distances to fovea and ONH, OT lesions appear to show a scattered configuration around the posterior pole. However, lesion distribution data showed a predominant preference of temporal quadrant for each type of lesion, eventually including macular region of the posterior pole, which is confirmed by the fact that 25-35% of lesions were centred within

Holland zone 1 that include peripapillary and central foveal areas. Although less than one-third of lesion centroids have been located in Holland zone 1, it corresponds to an involvement with much higher lesion density (7.95 lesions/mm^2) when compared with the lesion density in Holland zones 2 and 3 taken together (0.53 lesions/mm^2). This finding is further supported by lesion heat maps that were depicted in Figures 2-3, showing that the central foveolar area within 1000 μm diameter was densely affected by both active and inactive lesions.

The comparative analysis of primary and secondary lesions of subset data might give an insight over recurrence characteristics of ocular toxoplasmosis. Regarding high variance of lesion distances to fovea and ONH and no significant difference in fovea and ONH-to-lesion distances between primary and secondary lesions, it could be argued that secondary lesions tend to occur in a more random distribution with no apparently specific type of pattern, including centripetal/centrifugal or quadrantal shifts. Nevertheless, it is also remarkable that secondary lesions tend to appear at the same quadrant as the primary lesions; as shown in Figure 4. Another finding shows that secondary lesions affect smaller areas than primary lesions. This finding could be due to several potential reasons: 1) It is more likely for patients to present with recurrences earlier, thus the size at presentation for secondary lesions would be smaller; 2) two lesion types could be observed at different phases in the disease course; for that secondary lesions could have been defined earlier than primary lesions which might have had time to progress or heal thus enlarge; 3) lesions that were considered to be primary might in fact represent multiple lesions within the same scar, which would exaggerate their size. As shown in Figure 3, while a narrow area near foveal center is affected in high density, it could be interpreted that the secondary lesions are dispersed in a relatively larger but less densely affected region within the macular vascular arcades.

Few studies that have previously investigated topographical characteristics of OT lesions are present in current literature. Also, studies have noted that macula is predominantly affected relative to its surface area when compared to whole retina. [7-10] This pattern was attributed to the fact that CD68+ macrophages, which participate to immune defence against *T. gondii* infection, are found less densely in macular area when compared to periphery. [3,11] There is no strong evidence for whether macular preference of ocular toxoplasmosis is related to congenital or acquired onset of disease. [3] A recent

study held by Duraffour et al. analysed the areas of scarred and active lesions of toxoplasmic retinochoroidis. [12] Similar to our study, they showed an apparent increase of mean areas in scarred lesions when compared with active lesions (1.79 ± 2.36 against 1.32 ± 1.59 optic disc area), however the difference was not found significant. Apart from assessment of lesion areas and qualitative topographical data given in previous studies, the current study is unique in quantitative depiction with heat map analysis for localization of retino-choroidal lesions that occur in OT.

There are several limitations of our study. Due to its retrospective design, we were not able to document all ocular toxoplasmosis cases that were included in the study with a colour fundus photograph on their active lesion phase, however all cases were confirmed to have an active toxoplasmosis lesion on at least one visit, including a laboratory evidence of toxoplasma infection with either serology or PCR testing. Classification of lesions in the subset group as primary and secondary was based on a single time-point assessment of appearance rather than longitudinal analysis. There was no direct evidence to define primary lesions in the subset group, for that the inactive lesions were indirectly defined as primary lesions with presence of an active lesion at the same eye, which was considered secondary. We were also unable to confirm if inactive lesions were due to a singular primary lesion or they have also included recurrences. Similarly, we were not able to assess the effect of possible confounding factors including congenital or acquired status of disease, time interval between primary and recurrent involvement, or type of treatment over size and location of OT lesions. Future studies with larger sample size and clinical input could provide strong spatial correlation capacity to predict the course of the disease in terms of localizing recurrent lesions. Furthermore, such an algorithm, if it were possible, would become a powerful tool as a guide to disease management for that detecting a high likelihood for second affectation close to fovea, disc or macular region may necessitate timely and aggressive intervention to prevent vision loss while the lack of a strong spatial correlation may provide more flexible treatment options which includes initiation of prophylaxis for those at higher risk of visual loss.

CONCLUSION

In conclusion, the current descriptive study attempted to describe certain topographical features of various OT lesions. Most lesions were located temporal to the optic nerve head, with a relative predominance of central macular area. Secondary lesions tend to be smaller than primary lesions. All OT lesions are mostly overlapped inside the vascular arcades at the posterior pole with primary OT lesion maximum overlap point being slightly closer to foveal centre (458 μm) when compared to secondary lesions (653 μm). In eyes which active and inactive lesion were seen together, the mean of the lesion centres were 465 microns away from each other. Further studies with novel imaging technologies such as adaptive optics focusing specifically on these topographic locations can potentially provide insights regarding toxoplasma cyst formations. As shown in our study, although ocular toxoplasmosis is a self-limiting disease in some cases and the majority of lesions are treated, macular region is densely affected, which emphasizes its importance as a vision threatening condition.

Data accessibility: Data can be provided upon request.

Ethics statement: The study adhered to the tenets of the Declaration of Helsinki and approved by the Institutional Review Board of Gazi University, Ankara, Turkey (ID: 25901600/5447).

Contributorship statement:

Conceived and designed the study: MH, MSH, AKD, BAS, CP and QDN.

Analysed the data: MH, MSH and CK.

Acquisition of data: MH, MSH, PCO, MSO, HBO, GU, MC, XL, AKD, MNR, DNC, BAS, PAK, CP and QDN.

Administrative, technical or material support: MSH, AKD, BAS, PAK, CP and QDN.

Wrote the paper: MH, MSH and CK.

Critically revised the manuscript: MH, AKD, BAS, PAK, CP and QDN.

MH and QDN had full access to all study data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

QDN is guarantor.

Statistical analyses were performed by MSH and CK.

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Figure legends

Figure 1: Centroid distributions of toxoplasma lesions. *Left*: Distribution of centroids in both Holland zones and posterior pole quadrants. Red and green dots are centroids located in Holland zones 1 and 2&3 respectively. Dashed diagonal lines define quadrant borders with the optic nerve head on their intersection. *Right*: Distribution of centroids in posterior pole quadrants. Left side of the graph represents temporal quadrant of the posterior pole. Black cross defines centre of the fovea.

Figure 2: Heat maps of all toxoplasma lesions where each lesion is presented as a "shadow" that covers an area at the posterior pole. The superposition of lesions is given with a colour coding of pixels between no lesion (blue) and highest lesion overlap (yellow) with a scale bar of point overlap added next to each heat map. The pixel with highest number of overlaps is marked as the maximum point of overlap. *On top*: Consolidated heat map of all lesions. *Middle left*: Primary lesions. *Middle right*: Secondary lesions. *Bottom left*: Inactive lesions. *Bottom right*: Active lesions.

Red cross: foveal centre. *Red circle:* optic nerve head centre. *Asterisk:* Maximum point of overlap.

Figure 3: Heat maps of subset lesions which include both active and inactive toxoplasma lesions. *Left*: Consolidated heat map of subset lesions. *Middle*: Heat maps of primary (inactive) lesions. *Right*: Heat maps of secondary (active) lesions.

Red cross: foveal centre. *Red circle:* optic nerve head centre. *Asterisk:* Maximum point of overlap.

Figure 4: Vectorial diagram of lesion centre differences between primary and secondary OT lesions in the subset group. Vectors are originated from primary lesion centre and directed towards secondary lesion centre points for each eye. Quadrants were colour-coded with blue (superior), orange (temporal), grey (inferior) and yellow (nasal), while mean centroid displacement in each quadrant was depicted with black arrows.