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Use of Hand-Held Optical Coherence Tomography during Retinopathy of Prematurity (ROP) Screening demonstrates an increased Outer Retina from early Postmenstrual Age in Preterm Infants with ROP.

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Abbreviated title: *Increased Outer Retina noted in ROP*

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Summary

Hand-held optical coherence tomography (HH-OCT) demonstrates increased foveal and parafoveal outer retina and reduced inner nuclear layer with retinopathy of prematurity (ROP) compared to no ROP in prematurity. There is future potential in developing a novel imaging biomarker for HH-OCT use during ROP screening.

Count 44 words

Abstract

Purpose: To identify structural markers of active retinopathy of prematurity (ROP) in foveal and parafoveal retinal layers using hand-held optical coherence tomography (HH-OCT).

Methods: We acquired HH-OCT images (n=278) from a prospective mixed cross-sectional longitudinal observational study of 87 participants (23-36 weeks gestational age (GA); n=30 with ROP, n=57 without ROP) between 31 to 44 weeks postmenstrual age (PMA) excluding treated ROP and features of cystoid macular edema (CME). Six retinal layer thicknesses from the fovea to the parafovea were analysed at five locations up to 1000 µm temporally and nasally.

Results: The mean outer retinal thickness (OUTRETL) during active ROP increased at the fovea and parafovea from PMA 33 to 39 weeks (p<0.001) while the parafoveal inner nuclear layer (INL) and retinal nerve fiber layer (RNFL) reduced (p<0.001). OUTRETL at the fovea from 33 to 39 weeks PMA was consistently thicker in infants with ROP across all levels of prematurity (GA).

Conclusions: Increased foveal and parafoveal outer retina measured using HH-OCT shows potential as a marker for ROP screening.

Count: 170 words

Introduction

Retinopathy of prematurity (ROP), associated with preterm birth, shows changes of foveal and macular structure during the neonatal period as demonstrated using hand held optical coherence tomography (HH-OCT) during ROP screening.[1-5] Preterms also have persistence of foveal inner retinal layers, increased central foveal thickness (CFT) and outer layer delays.[6-9] Additionally, inner nuclear layer (INL) intraretinal cystic spaces described as cystoid macular edema (CME) distort retinal structure and are present in up to 72% of preterm infants imaged before 42 weeks postmenstrual age (PMA).[1,2,4,5,10,11]

Emerging preterm OCT studies suggest ROP influence is distinct from that of prematurity on foveal and parafoveal structure,[10,12] and severity of prematurity is important in preterm retinal structure assessment.[12,13]

Due to the limitations for objective quantitative measurement of infant retinal structure, efforts to determine consistent structural markers have been explored using HH-OCT to distinguish characteristics in preterms infants with ROP during screening.[12,14]

Our aim was to assess ROP impact on individual foveal and parafoveal retinal layers using HH-OCT. We hypothesized that this would reveal pathologic structural changes related to ROP rather than prematurity. Demonstrating these differences opens future possibilities into identifying additional criteria that could be used during ROP screening and directing earlier treatment intervention in high-risk infants.

Methods

This was a prospective observational study completed between 2012 and 2015 in Leicester, United Kingdom, conducted under the tenets of the Declaration of Helsinki with approval by

a Local Ethics Committee (NRES committee, Nottingham East Midlands, United Kingdom). Patients were recruited from the Leicester Royal Infirmary neonatal and maternity unit, United Kingdom and all preterm babies from 31 to 44 weeks PMA who required ROP screening were eligible for inclusion in the study. An abnormal ocular examination other than diagnosis of ROP, treated ROP, and ROP stages 4 or 5 were exclusion criteria. For screening and imaging, ROP was defined as stages 1-3 using the UK guidelines[15] and eyes were dilated using Cyclomydril® eye drops.

Imaging was performed from 31 to 44 weeks PMA at 1 to 2 weekly intervals using HH-OCT (Envisu C-Class, Leica Microsystems, Wetzlar, Germany) scan sequence consisting of 500 A scans and 100 B scans covering a rectangular volume of 5.0 mm x 10.0 x 2.0 mm. Further details can be found in supplemental digital content 1 (**SDC1**).

Retinal layer analysis

Analysis of HH-OCT images was performed using customized layer segmentation macros written in ImageJ software (United States National Institutes of Health, Bethesda, MD, https://imagej.nih.gov/ij/, downloaded in December 2013).

Scans were optimized for obtaining a single high-quality scan at the central retina and the lateral distance settings were corrected to account for the smaller axial lengths in the infant population using a conversion table according to PMA and GA from the data presented by Maldonado and colleagues.[16]

Foveal B-scans were flattened using the Bruch's membrane as a reference line and translating individual A-scans vertically. Boundary detection of the internal limiting membrane (ILM) was performed automatically using the ABSnake plugin

(http://imagejdocu.tudor.lu/doku.php?id=plugin:segmentation:active_contour:start). Manual fine adjustment of the fitted line was used to generate the final segmentation.

Other retinal layer borders were manually segmented using semi-automatic with manual correction techniques and definitions from previously reported literature.[8,9,17-19] Individual layer thicknesses were calculated between border edges based on previous literature.[20,21] Details are shown in **Table 1**. Inner retinal layers were generally present across the central retina from 30 weeks' PMA using HH SD-OCT as previously reported.[7-9] The external limiting membrane (ELM) was often absent in early gestational age (GA) compared with later GA, limiting segmentation of the outer retina. Therefore, the combined outer retina was defined from the lower margin of the inner nuclear layer (INL) to the upper margin of the retinal pigment epithelium (RPE) and labelled as OUTRETL.

Duplicate images were discarded. Images with intraretinal hypo-reflected spaces distorting the macula within the INL were defined as CME and manually separated from those where CME was absent in accordance with peer reviewed literature.[10] Due to the disruption to retinal structure from CME, images showing CME present in one or both eyes were excluded from the study.

Locations 1000µm and 500µm nasal and temporal to the central fovea were chosen to be within the parafovea and foveal zone from histological studies of the developing eye.[22] Locations were measured from points taken on either side of the fovea up to 1000µm nasally and temporally.

Statistical Analysis

We fitted an additive mixed model to analyse the retinal thickness data on six retinal layers: retinal nerve fibre layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), INL,

OUTRETL and the RPE. The model predictor variables included GA, PMA, location and ROP status (presence and absence).

The model also considered the two-way interaction terms of GA (and PMA) with location, and GA with ROP. Since the two-way interaction terms were significant ($p<0.05$), we also considered three-way interaction terms with each retinal layer. To capture the non-linear shape of the retinal thickness in the entire nasal to temporal locations, the model incorporated the smoothing terms of the location by retinal layers, and location by ROP status. The smoothing term assumed the basis function as a Gaussian process model with 10 knots.

To account for the multiple measurements on the same infant, we incorporated the random intercept of infant assuming an equal correlation between the measurements on the same infant. We also incorporated the fixed effect of the eye to compare the left and right eyes. However, the final model excluded the eye as a predictor variable as it was not statistically significant. The model allowed for a participant to be ROP-positive and ROP-negative at different time points. An individual was considered ROP-positive when the ROP was detected in at least one eye.

The validity of the basis dimension was assessed using visual methods and the k-index, and model assumptions were checked using appropriate residual plots. Since the pattern of the residuals resembles that of a normal distribution with heavier tails, we considered the residual distribution using the scaled-t family for heavy-tailed data. We obtained and compared the predicted means for ROP for pre-specified values of GA, PMA and location, and the p-values for the comparisons were obtained after employing Bonferroni correction so that the overall type 1 error is less than 0.05. The additive mixed model was fitted using the R package mgcv (version 1.8.24) and all statistical analyses were conducted in R.

Results

There were 112 preterm infants aged between 23 to 36 weeks GA of which 25 demonstrated CME and excluded, leaving 87 participants and 278 images for analysis. No infant had undergone any treatment for ROP. The number of infants according to GA was as follows: 12 $\leq$ 24 weeks GA, 26 from 25 to 27 weeks GA, 29 from 28 to 29 weeks GA and 20 $\geq$ 30 weeks GA. **Table 2** shows participant and image demographics according to ROP. **Table 3** presents the summary statistics on preterm infants included in the study**.**

Changes in foveal and parafoveal retinal layers associated with retinopathy of prematurity as the retina develops (increasing postmenstrual age 33 to 39 weeks)

We explored the differences in foveal and parafoveal architecture associated with the presence of ROP and non-ROP for individual layers at five locations and for three time points (PMA 33, 36, 39 weeks).

Figure 1 summarizes the changes associated with ROP in each of the foveal / parafoveal retinal segmented layers, at (A) 33 weeks, (B) 36 weeks, and (C) 39 weeks PMA at the fovea and 1000 µm and 500 µm temporally and nasally, using mean GA (27.5 weeks). **Table 4** reports the mean difference in retinal thickness between the presence and absence of ROP for each layer along with 95% confidence intervals and the adjusted *p*-values (Bonferroni adjustment).

Our analysis found the OUTRETL was significantly thicker during active ROP compared with non-ROP across timepoints PMA 33, 36 and 39 weeks at mean GA 27.5 weeks (see **Figure 1**; **Table 4** sections A, B and C). This was noted across the fovea and parafovea from 1000µm nasally to 1000µm temporally.

There was also a reduction in mean INL thickness for infants with active ROP compared with non-ROP measured in the temporal and nasal parafovea at PMA 33 weeks. By late PMA 39 weeks this reduction included the fovea.

At 33 weeks PMA, RNFL and RPE thickness reduced at the parafovea, but the mean thickness difference was not statistically significant ($p>0.05$) between ROP and non-ROP at PMA 36 and 39 weeks.

Supplementary Video 1 **(SDC2)** demonstrates the dynamic mean retinal thickness changes at mean GA (27.5 weeks) with PMA from 33 to 39 weeks between ROP and non-ROP infants for the RNFL, GCL, IPL, INL, OUTRETL and RPE. ROP is marked in red, non-ROP is marked in blue. The locations are marked as nasal (parafovea -1000µm and -500µm) and temporal (parafovea 1000µm and 500µm) and the fovea (0).

Changes in foveal and parafoveal layers associated with retinopathy of prematurity investigating degree of prematurity (gestational ages 24, 27.5, 30 weeks) as the retina develops (postmenstrual ages 33, 36 and 39 weeks).

Since we found significant differences between retinal layers for ROP at 33 to 39 weeks PMA using mean GA, we wanted to see if degree of prematurity also had an effect on the retinal layers. **Figure 2** summarizes the changes associated with ROP for various GA (24, 27.5 and 30 weeks) at 33-, 36- and 39-weeks PMA in each of the segmented retinal layers.

Tables 5, 6 and 7 (**SDC3**, **SDC4** and **SDC5** respectively) report the mean difference in retinal thickness between the presence and absence of ROP along with 95% confidence intervals and adjusted *p*-values (Bonferroni adjustment) at (A) 24 weeks, (B) 27 weeks and (C) 30 weeks GA at the five locations for PMA of 33, 36 and 39 weeks.

At 33 weeks PMA the OUTRETL consistently remained thicker at the fovea and at 500µm nasal and temporal to the fovea for all GA. We also found thicker GCL and IPL in extreme preterm infants (GA 24 weeks) with ROP compared with the non-ROP group across all locations. Conversely in preterm infants of GA 30 weeks with ROP, this pattern reversed with thinner GCL and IPL (see **Figure 2** GA 24 and GA 30 weeks).

When investigating the retinal layers at later time points (PMA 36 and 39 weeks) for 24-, 27- and 30-weeks GA, the OUTRETL was still greater in active ROP compared with non-ROP. The increased OUTRETL was greater for 30 weeks GA compared with 24 weeks GA for both ROP and non-ROP (see **Figure 2;** Tables 6 and 7 **SDC4** and **SDC5** respectively).

Examining the effect of location on retinal layers with GA, at the fovea the INL increased with severity of prematurity (see **Figure 2** GA 24 weeks compared with GA 30 weeks fovea, INL). However, GCL and IPL were reduced at the fovea in 24 weeks GA compared with 30 weeks GA in non-ROP, whereas both increased at the fovea in GA 24 weeks compared with 30 weeks GA in ROP.

Discussion

As the retina develops from early to late PMA (33 to 39 weeks), the preterm outer retina is significantly thicker at the fovea and parafovea for GA 30 weeks, and least for GA 24 weeks. This is would concur with the delays reported in outer retinal development for extreme prematurity.[6] However, when ROP was present, the outer retina was increased further for all gestational ages.

We found increased foveal and parafoveal INL at 24 weeks GA compared with 30 weeks GA. This is in keeping with other reports suggesting an effect on the foveal depth and

thickness from severity of prematurity.[12,13] Examining the additional effect of ROP, at GA 27 and 30 weeks, INL thickness measured less when compared with non-ROP.

This was not found for 24 weeks GA, where the INL was even greater suggesting that ROP was an additional significant factor perhaps depending on stage of ROP, since the risk of ROP is greatest in extreme prematurity. However, we did not have enough infants in stages 1-3 or plus disease for the various GA to investigate this further (see **Table 2**) and could not exclude sub-clinical CME.

In the absence of ROP, the GCL and IPL reduced at the fovea more for 24 weeks GA compared with 30 weeks GA reflecting degree of prematurity. When exploring the presence of ROP, this pattern reversed, with earlier born infants measuring a thicker mean GCL and IPL at the fovea compared with later born infants. Our findings are in agreement with Mangalesh et al[10] that ROP plays a role in addition to severity of prematurity in retinal development with PMA when measured till 42 weeks PMA. However, we have also shown that dynamic changes occur from early to late PMA with ROP and GA.

ROP and increased outer retinal layers

Reduced outer retina at the fovea relates to severity of prematurity where the photoreceptors are delayed during development[6] but few studies have separated active untreated ROP from prematurity in the analysis of individual retinal layer structure and measurements using HH-OCT during the developing postnatal period.[10] In a large study by O Sullivan and colleagues[13] the authors concluded that more premature infants had thicker foveal outer retinal layers. Out of 204 eyes, 26 were treated and the impact of ROP was unclear on outer retinal analysis. In contrast, we specifically wished to understand the impact of ROP and GA in multivariate analyses and excluded treated infants. We found that ROP influences the outer

retina, with increased thickness in preterm infants with ROP since ROP risk is greatest in very preterm born babies. We suggest that our findings are in accordance with O Sullivan et al but in addition we have delineated the processes that can affect foveal outer layers with respect to ROP as well as GA.

In ROP pathogenesis, the photoreceptor outer layers are metabolically active driving oxygen requirement. Image acquisition prior to 32 weeks PMA was not possible so it is unclear if infants developed greater outer retina thickness and were more at risk of developing ROP, or if the increased outer retina developed in the presence of active ROP. However, the outer retina in preterm infants without ROP measured less compared with ROP suggesting that outer retinal layer increase is associated with ROP presence. Possibly, this increase results from factors released due to either parafoveal or peripheral retinal ischemia or other unknown mechanism.

Reduction of inner nuclear layer during ROP

We hypothesized that HH-OCT of retinal structure during ROP may reveal surrogate markers of changes distinct from prematurity and that the INL reduction in our study could represent ischaemia reflecting ROP presence.

In adults, paracentral maculopathy due to small retinal vessel intermediate and deep vascular plexus ischemia has been described in association with several vascular retinopathies and identified by characteristic lesions found using spectral domain OCT (SD-OCT).[23] The location of the lesions is described to be in either the INL or below the OPL.[24,25] However, in developing preterm infants, the outer retina is immature and the OPL is either poorly visualized or absent on imaging.

The deep vascular networks are located above and below the INL, while the intermediate capillary plexus component is located at the INL and IPL interface.[26,27] The INL contains the bodies of Müller cells which express vascular endothelial growth factor (VEGF) and are known to respond to photoreceptor injury or stress.[28] Retinal ischemia, as seen in oxygen-induced retinopathy (OIR) in rats, increases Müller cell expression of VEGF type A[29] and in a vascular/avascular retinal OIR mouse model, decreased inner retinal thickness was associated with reduced capillary density and the development of large vessel tortuosity.[30]

The reduction in INL in our study may have been found in comparison to an abnormal increase in INL due to sub-clinical CME in our images. Preterm infant CME has been observed primarily in the INL.[11] We did not calculate a predictor measure for INL for the exclusion of CME as we did not have sufficient data to do this using a ROC curve[13] however our analysis was powered for ROP and individual layers, and applied equally to both groups (with and without ROP). Images with CME were identified in our study accordance with peer reviewed literature[2,7,10] and specifically excluded.

We also found increased INL related to increased severity of prematurity as reported by O'Sullivan and colleagues[13] but in our study, additionally, the INL was reduced in ROP compared with non-ROP, suggesting ROP is a factor in this change rather than prematurity alone for the infants with ROP.

Limitations

There is paucity of information regarding the developing preterm retinal vasculature and structural disparities found using HH-OCT could represent unrelated vascular characteristics between the two groups. Perifoveal vascular development, especially the deep vascular complex, may not have been fully developed at the time of imaging between 33- and 39-

weeks PMA. While it is possible that additional delays to this network developing due to ROP may reflect HH-OCT findings, the suggestion of retinal ischemia and reduced INL remains speculative.

O'Sullivan et al[13] calculated a threshold INL thickness as a surrogate measure in order to distinguish between CME and non-CME therefore reducing the possibility of contamination in retinal layer measurements. Our segmentation and analysis algorithm differs and we did not have enough CME data to define our criteria in the same way. Furthermore, as the resolution of portable OCT improves there will be less subjectivity in subclinical CME detection, offering further insight. Including more infants with earlier GA and ROP stages 1 to 3 in order to investigate a dose response between ROP severity and the OUTREL would strengthen the study conclusions.

Conclusions

Our study demonstrates the dynamic differences between PMA, prematurity and ROP on foveal and parafoveal development. Extreme prematurity demonstrates a differential between ROP and non ROP foveal INL. When the effects of ROP were examined with extreme prematurity, we found reduced INL and increased outer retina, acknowledging that subclinical CME could still be present within the INL. Although ROP severity could not be characterised, we believe our study presents possibilities that merit further investigation.

SDC1 Methodology -http://links.lww.com/IAE/C82

SDC2 video 1 -http://links.lww.com/IAE/C83

SDC3 Table 5 -http://links.lww.com/IAE/C84

SDC4 Table 6 -http://links.lww.com/IAE/C85

SDC5 Table 7 -http://links.lww.com/IAE/C86

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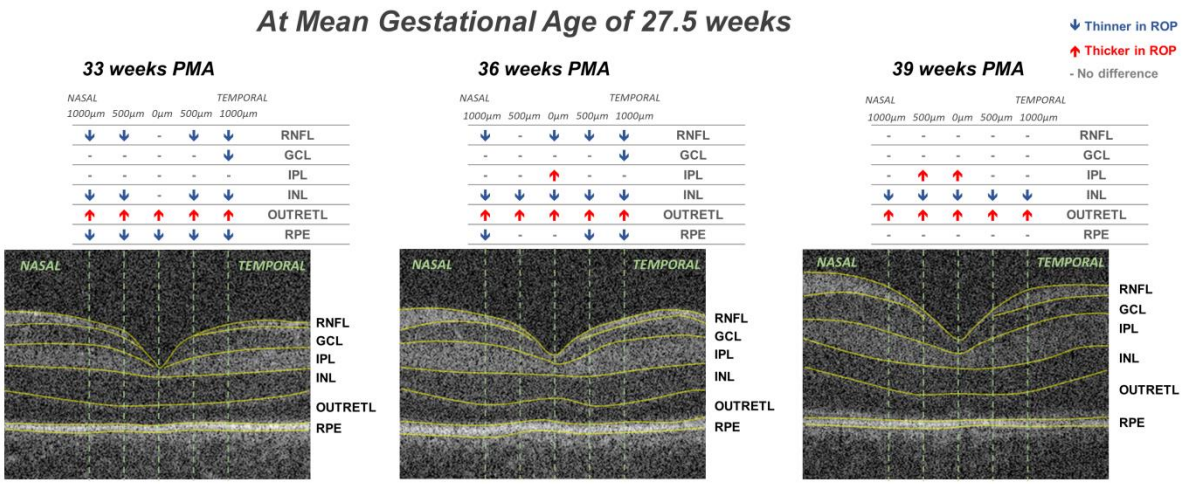
Figure Legends

Figure 1. A summary of changes in foveal / parafoveal retinal layers associated with retinopathy of prematurity as the retina develops.

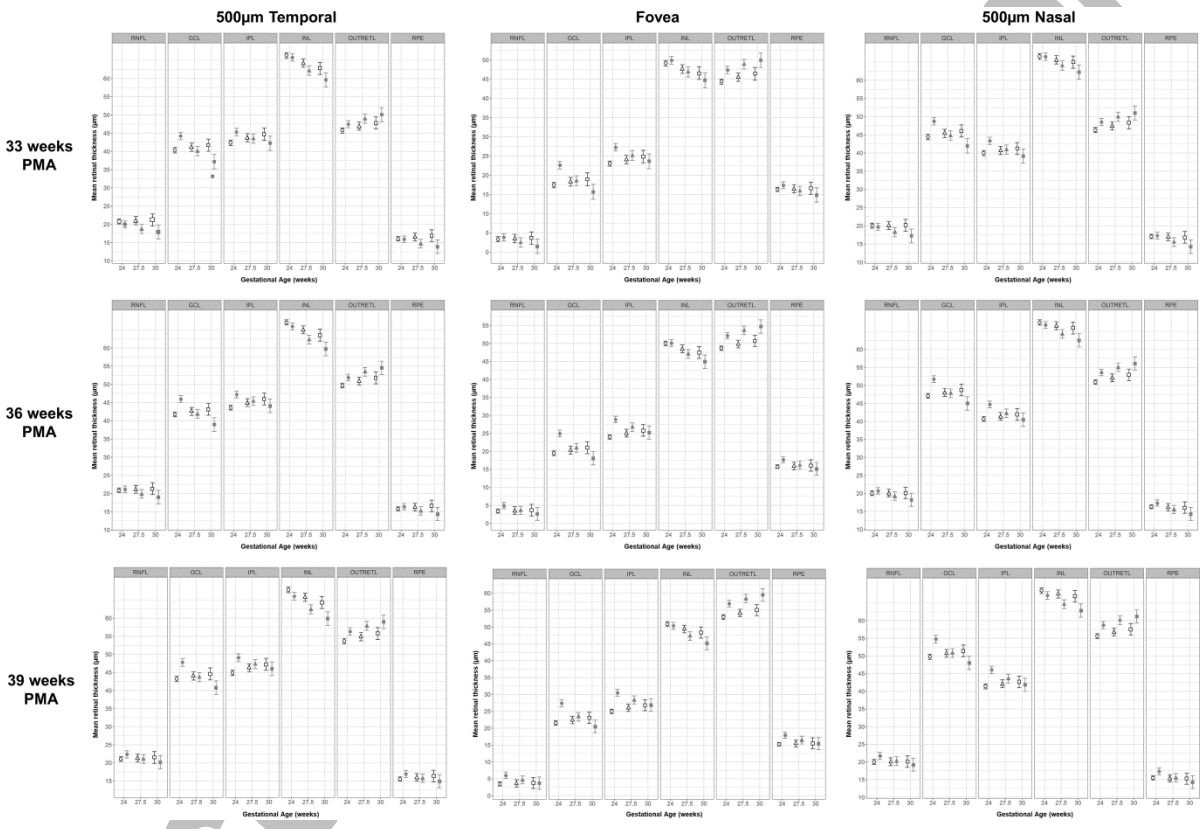


Differences are recorded at postmenstrual ages (PMA) of 33, 36 and 39 weeks where blue arrows indicate thinner layers in the ROP groups and red arrows that layers are thicker. An example of a segmented B-scan at each age is shown below.

RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OUTRETL – outer retinal layers, RPE – retinal pigment epithelium.

HH-OCT – hand-held optical coherence tomography

Figure 2. Foveal and parafoveal layer changes between 500 µm nasal and temporal retina associated with retinopathy of prematurity (ROP, shaded grey) and non-ROP (unshaded) at 24-weeks (circles), 27.5-weeks (triangles) and 30-weeks (squares) gestational age (GA) for 33-, 36- and 39-weeks postmenstrual age (PMA). Results are shown with 95% confidence intervals.



SDC1 Methodology *using hand-held spectral domain optical coherence tomography (HH SD-OCT).*

SDC2 Video 1 *mp4 D*ynamic mean retinal thickness changes at mean GA (27.5 weeks) with PMA from 33 to 39 weeks between ROP and non-ROP infants for macular retinal layers

SDC 3. Table 5. The predicted mean difference in retinal thickness (µm) between presence and absence of retinopathy of prematurity for foveal and parafoveal retinal layers at gestational age: (A) 24, (B) 27.5, and (C) 30 weeks. Results are shown with 95% confidence intervals **using 33 weeks postmenstrual age** (PMA) at 5 locations (fovea 0, 1000 µm and 500 µm nasal and temporal to the fovea). RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OUTRETL – outer retinal layers, RPE – retinal pigment epithelium, padj – adjusted p-value.

SDC 4 Table 6. The predicted mean difference in retinal thickness (µm) between presence and absence of retinopathy of prematurity for foveal and parafoveal retinal layers at gestational age: (A) 24, (B) 27.5, and (C) 30 weeks. Results are shown with 95% confidence intervals **using postmenstrual age of 36 weeks** at 5 locations (fovea 0, 1000 µm and 500 µm nasal and temporal to the fovea). RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OUTRETL – outer retinal layers, RPE – retinal pigment epithelium, padj – adjusted p-value.

SDC 5 Table 7. The predicted mean difference in retinal thickness (µm) between presence and absence of retinopathy of prematurity for foveal and parafoveal retinal layers at gestational age: (A) 24, (B) 27.5, and (C) 30 weeks. Results are shown with 95% confidence intervals **using postmenstrual age of 39 weeks** at 5 locations (fovea 0, 1000 µm and 500 µm nasal and temporal to the fovea). RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OUTRETL – outer retinal layers, RPE – retinal pigment epithelium, padj – adjusted p-value.

Table 1. *Retinal layer borders and definitions used in the study*

Retinal nerve fibre layer	RNFL	ILM border to RNFL/GCL junction
Ganglion cell layer	GCL	RNFL/GCL junction to GCL/IPL junction
Inner plexiform layer	IPL	GCL/IPL junction to IPL/INL junction
Inner nuclear layer	INL	IPL/INL junction to anterior OUTRETL border
Outer retinal layers	OUTRETL	Anterior OUTRETL border to anterior border of RPE
Retinal pigment epithelium	RPE	Anterior to posterior borders of the RPE

Table 2. Participant and image characteristics according to ROP

	Always had ROP	**Never had ROP**	**Mixed (ROP or no ROP)**
Number of children			
Total 87 (100%)	19 (22%)	57 (65%)	11 (13%)
Male	7	33	7
Female	12	24	4
Caucasian	9	29	4

Non-Caucasian	10	28	7
Single birth	14	52	9
Multiple birth	5	5	2
Mean (± SD) *GA, BW, PMA			
GA (weeks)	26.1±1.98	28.6±2.43	26.6±1.79
BW (grams)	807±170	1154±423	864±179
PMA (weeks)	36.5±2.63	36.1±2.45	36.0±3.18
Number of images			
Total 278 (100%)	73 (26%)	156 (55%)	49(18%)
stage 1	12	-	11
stage 2	57	-	17
stage 3	4	-	2
Right eye	40	73	12 ROP 10 no ROP
Left eye	33	83	18 ROP 9 no ROP

Gestational age (GA), Birthweight (BW), postmenstrual age (PMA), retinopathy of prematurity (ROP)

Table 3. *Summary statistics on preterm infants according to ethnicity, multiplicity of birth and sex. PMA = postmenstrual age, GA = gestational age, SD = standard deviation.*

Ethnicity								
	PMA at Exam (weeks)			**GA at Birth (weeks)**			**Birth Weight (grams)**	
	Non-caucasian	Caucasian		Non-caucasian	Caucasian		Non-caucasian	Caucasian
Total	46	41		46	41		46	41
minimum	31.29	31.57		23.43	24.00		635	500
maximum	41.85	41.85		35.71	34.00		3540	1750
mean	35.73	36.25		27.84	28.59		1077	1091
SD	2.76	2.47		2.70	2.48		498	341
median	35.71	36.85		27.93	28.71		970	1100
p value	p=0.36			p=0.18			p=0.88	

Multiplicity of birth								
	PMA at Exam (weeks)			**GA at Birth (weeks)**			**Birth Weight (grams)**	
	single birth	multiple birth		single birth	multiple birth		single birth	multiple birth
Total	75	12		75	12		75	12
minimum	31.29	31.57		23.43	24.43		500	555
maximum	41.85	40.00		35.71	29.57		3540	1370
mean	35.99	35.86		28.32	27.42		1093	1020
SD	2.54	3.26		2.72	1.67		450	272
median	36.43	35.71		28.57	27.21		1042	1025

p value	p=0.89			p=0.13			p=0.45	
Sex								
	PMA at Exam			**GA at Birth**			**Birth Weight**	
		(weeks)			(weeks)			(grams)
	All infants	Female	Male	All infants	Female	Male	All infants	Female	Male
total	87	40	47	87	40	47	87	40	47
minimum	31.00	31.57	31.29	23.43	23.43	24.29	500	555	500
maximum	43.85	39.57	41.85	35.71	34.71	35.71	3540	3540	2430
mean	36.20	35.63	36.36	28.19	27.84	28.49	1083	1038	1122
SD	2.64	2.33	2.85	2.61	2.73	2.49	429	510	347
median	36.00	35.78	36.71	28.43	28.29	28.71	1042	915	1120
p value		p=0.27			p=0.25			p=0.38	

Table 4. The predicted mean difference in retinal thickness (µm) between presence and absence of retinopathy of prematurity for foveal and parafoveal retinal layers at postmenstrual age: (A) 33, (B) 36, and (C) 39 weeks. Results are shown with 95% confidence intervals using **mean gestational age 27.5 weeks** at 5 locations (fovea 0, 1000 µm and 500 µm nasal and temporal to the fovea). RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OUTRETL – outer retinal layers, RPE – retinal pigment epithelium, padj – adjusted p-value.

A. 33 weeks PMA				***LOCATION***		

		nasal-1000µm	nasal-500µm	0µm	temporal-500µm	temporal-1000µm
RNFL	*padj*	**<0.001**	**<0.001**	0.33	**<0.001**	**<0.001**
	Mean (95% CI)	-2.73 (-3.61, -1.85)	-1.86 (-2.65, -1.08)	-1.02 (-1.8, -0.23)	-2.29 (-3.08, -1.5)	-3 .00 (-3.88, -2.12)
GCL	*padj*	0.060	1.0	1.0	0.51	**<0.001**
	Mean (95% CI)	-1.47 (-2.4, -0.54)	-0.6 (-1.44, 0.24)	0.25 (-0.6, 1.09)	-1.03 (-1.87, -0.18)	-1.74 (-2.67, -0.81)
IPL	*padj*	1.0	1.0	0.37	1.0	1.0
	Mean (95% CI)	-0.66 (-1.58, 0.26)	0.21 (-0.62, 1.04)	1.05 (0.23, 1.88)	-0.22 (-1.05, 0.61)	-0.93 (-1.86, 0.00)
INL	*padj*	**<0.001**	**0.005**	1.0	**<0.001**	**<0.001**
	Mean (95% CI)	-2.48 (-3.41, -1.55)	-1.61 (-2.45, -0.77)	-0.77 (-1.61, 0.08)	-2.04 (-2.88, -1.2)	-2.75 (-3.68, -1.82)
OUTR ETL	*padj*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.090
	Mean (95% CI)	1.62 (0.73, 2.51)	2.49 (1.7, 3.28)	3.33 (2.54, 4.13)	2.06 (1.27, 2.85)	1.35 (0.46, 2.24)
RPE	*padj*	**<0.001**	**0.008**	1.0	**<0.001**	**<0.001**
	Mean (95% CI)	-2.27 (-3.12, -1.42)	-1.4 (-2.16, -0.65)	-0.56 (-1.31, 0.2)	-1.83 (-2.59, -1.07)	-2.54 (-3.4, -1.69)
B. 36 weeks PMA				***LOCATION***		
		nasal-1000µm	**nasal-500µm**	**0µm**	**temporal-500µm**	**temporal-1000µm**

RNFL	*padj*	**<0.001**	0.27	1.0	**0.0025**	**<0.001**
	Mean (95% CI)	-1.7 (-2.44, -0.96)	-0.83 (-1.46, -0.21)	0.01 (-0.61, 0.64)	-1.26 (-1.89, -0.63)	-1.97 (-2.72, -1.23)
GCL	*padj*	0.12	1.0	1.0	1.0	**0.011**
	Mean (95% CI)	-1.12 (-1.88, -0.35)	-0.25 (-0.9, 0.4)	0.6 (-0.06, 1.25)	-0.68 (-1.33, -0.02)	-1.39 (-2.16, -0.62)
IPL	*padj*	1.0	0.34	**<0.001**	1.0	1.0
	Mean (95% CI)	-0.03 (-0.79, 0.73)	0.83 (0.19, 1.48)	1.68 (1.04, 2.33)	0.41 (-0.24, 1.05)	-0.31 (-1.07, 0.46)
INL	*padj*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Mean (95% CI)	-3.13 (-3.9, -2.36)	-2.26 (-2.92, -1.6)	-1.42 (-2.08, -0.75)	-2.69 (-3.35, -2.04)	-3.4 (-4.17, -2.63)
OUTR ETL	*padj*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Mean (95% CI)	2.09 (1.33, 2.85)	2.96 (2.32, 3.6)	3.8 (3.16, 4.44)	2.53 (1.88, 3.17)	1.82 (1.06, 2.58)
RPE	*padj*	**0.002**	1.0	1.0	**0.029**	**<0.001**
	Mean (95% CI)	-1.47 (-2.2, -0.74)	-0.6 (-1.21, 0.01)	0.24 (-0.37, 0.85)	-1.03 (-1.65, -0.42)	-1.74 (-2.47, -1.01)

C. 39 weeks PMA ***LOCATION***

		nasal-1000µm	nasal-500µm	0µm	temporal-500µm	temporal-1000µm
RNFL	*padj*	1.0	1.0	0.15	1.0	0.79
	Mean (95% CI)	-0.67 (-1.5, 0.16)	0.19 (-0.54, 0.93)	1.04 (0.31, 1.77)	-0.23 (-0.97, 0.5)	-0.95 (-1.78, -0.11)
GCL	*padj*	1.0	1.0	0.57	1.0	0.64
	Mean (95% CI)	-0.77 (-1.65, 0.12)	0.1 (-0.69, 0.89)	0.95 (0.16, 1.74)	-0.33 (-1.12, 0.46)	-1.04 (-1.93, -0.15)
IPL	*padj*	1.0	**0.007**	**<0.001**	0.28	1.0
	Mean (95% CI)	0.59 (-0.28, 1.46)	1.46 (0.69, 2.23)	2.31 (1.53, 3.08)	1.03 (0.25, 1.81)	0.32 (-0.55, 1.19)
INL	*padj*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Mean (95% CI)	-3.78 (-4.68, -2.89)	-2.92 (-3.72, -2.11)	-2.07 (-2.87, -1.26)	-3.34 (-4.15, -2.54)	-4.06 (-4.95, -3.16)
OUTR ETL	*padj*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Mean (95% CI)	2.56 (1.68, 3.44)	3.43 (2.64, 4.21)	4.27 (3.49, 5.06)	3 (2.21, 3.78)	2.29 (1.40, 3.17)
RPE	*padj*	1.0	1.0	0.12	1.0	0.70
	Mean (95% CI)	-0.67 (-1.48, 0.14)	0.2 (-0.51, 0.91)	1.04 (0.33, 1.75)	-0.23 (-0.94, 0.48)	-0.94 (-1.76, -0.13)