Keywords
Fluocinolone acetonide implant · Ganglion cell layer · Diabetic macular edema · Vitrectomy

Abstract
Introduction: The objective of this study is to compare changes in ganglion cell layer (GCL) between vitrectomized and nonvitrectomized eyes with diabetic macular edema (DME) over a 2-year period following treatment with 0.2 µg/day fluocinolone acetonide (FAc) implant. Methods: Eighteen vitrectomized (group 1) and 8 nonvitrectomized (group 2) eyes were included in this cohort study. Changes in central macula GCL thickness were measured using the Spectralis spectral domain-optical coherence tomography at baseline and 6, 12, and 24 months of follow-up. Other parameters analyzed included best-corrected visual acuity (BCVA), central foveal thickness (CFT), and intraocular pressure (IOP). Results: Treatment with the FAc implant led to small reductions in mean global GCL thickness versus baseline and contrasts with the control group that was stable or slightly increased versus baseline. FAc therapy also led to improvements in mean BCVA and CFT that were observed at Month 6 and maintained to Month 24. For vitrectomized and nonvitrectomized eyes, no differences were observed between mean global GCL, BCVA, and CFT values during follow-up. Linear correlations revealed that in all groups mean BCVA at Month 24 positively correlated with mean GCL thickness at baseline and at Month 24. IOP remained stable throughout the 24 months. Conclusion: There was no evident retinal neurodegeneration in the 2-year period following treatment with FAc in both groups. GCL thickness may be a useful biomarker for assessing safety and effectiveness in patients with DME.

Changes in Ganglion Cell Layer Thickness after Treatment with the 0.2 µg/day Fluocinolone Acetonide Implant in Vitrectomized and Nonvitrectomized Eyes with Diabetic Macular Edema

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Introduction

Diabetic retinopathy (DR) is one of the most important microvascular complication of diabetes mellitus and is the leading cause of vision loss among working-age adults in the developed world. Diabetic macular edema (DME) is the main cause of the vision loss related to DR [1–3].

The concept of DR as a microvascular disease has evolved in recent years, and it is now considered a more complex entity in which neurodegeneration plays a significant role [4]. Retinal neurodegeneration is an early event in the pathogenesis of DR [4]. In the retina, glial, neural, and vascular cells are closely linked creating a “neurovascular unit” to maintain the homeostasis necessary for physiological neuroretinal function [5]. This neurovascular unit exists in health but also in disease. In addition, it has been demonstrated that there is a higher rate of thinning of the retinal nerve fiber layer and ganglion cell (GCL)/inner plexiform layer in patients with no to minimal nonproliferative DR [5] than that observed in healthy eyes related to aging [6, 7].

In diabetes, chronic hyperglycemia induces a process of low-grade inflammation, immune cell activation, and extracellular glutamate accumulation, which is responsible for retina neuronal degeneration and vascular lesions through the disruption of the blood-retinal barrier and diverse cell-type impairment, including neural cells [4]. Among them, retinal ganglion cells are the earliest cells affected and have the highest rate of apoptosis [8]. These key features of DR stress the importance of glucocorticoids in the treatment armamentarium of DME through their anti-inflammatory response, direct effects on tight junction proteins, and antiangiogenic and neuroprotective actions [9].

The multifactorial pathogenesis of DME may require different and complementary therapeutic approaches, such as metabolic control, laser therapy, antivascular endothelial growth factor (anti-VEGF), or corticosteroid intravitreal (IV) injections as well as vitrectomy [10, 11]. The most widely therapy approach for DME usually involves repeated pharmacological treatments, and, in vitrectomized eyes, some IV treatments, such as anti-VEGF and triamcinolone, had an increased vitreous clearance and a higher number of anti-VEGF injections is expected during the first 12 months of treatment [12–14]. ILUVIEN (0.2 µg/day fluocinolone acetonide [FAc]) IV implant is a long-acting steroid indicated for the treatment of vision impairment associated with chronic DME considered insufficiently responsive to available therapies (Alimera Sciences Ltd., Hampshire, UK).

In accordance to previous studies of our group and other real-life studies, the FAc implant has demonstrated to be effective in both nonvitrectomized and vitrectomized eyes [15–19]. Furthermore, there is also evidence that IV FAc may decelerate diabetic retinal neurodegeneration, through a decrease in the rate of inner retinal thinning in patients with persistent DME [20]. As opposed to nonvitrectomized eyes, the influence of DME in retinal degeneration process in eyes without vitreous and submitted to internal limiting membrane (ILM) peeling is much less explored. Reductions in GCL thickness following ILM peeling have already been reported in several studies [21, 22], and a worse postoperative visual outcome has also been documented [21]. This study sought to compare the changes of GCL in DME patients between vitrectomized eyes with ILM peeling and nonvitrectomized eyes following treatment with the 0.2 µg/day FAc implant, aiming to better understand of the vitreous status influence in the neurodegeneration process and DME behavior, particularly after treatment with FAc implant.

Materials and Methods

Study Population

This study was designed as a retrospective, single-center observational cohort study conducted at Centro Hospitalar Universitário do Porto (CHUPorto), Porto, Portugal. Patient records from April 2015 to September 2016 were reviewed for cases with DME treated with a single 0.2 µg/day FAc implant and followed up for a minimum period of 24 months. The effect of FAc was assessed in two groups: vitrectomized-group 1, and nonvitrectomized eyes-group 2.

Ethics

The study was conducted according to the tenets of the Declaration of Helsinki in its latest amendment (Brazil, 2013) and was approved by the Ethics Committee of CHUPorto (2017.093 [084-DEFI/082-CES]). All patients signed a written informed consent form.

Inclusion Criteria

Patients with type 1 or type 2 diabetes mellitus, >18 years, and with center-involved DME, defined as central foveal thickness (CFT) of more than 300 µm on spectral domain-optical coherence tomography (SD-OCT) were eligible for the study. To be included in this analysis, patients in groups 1 and 2 had to have DME that was refractory to anti-VEGF and short-term steroid agents, defined as having persistent intraretinal and/or subretinal fluid on OCT (i.e., CFT >300 µm or ≤20% CFT decrease measured 1 month after at least 3 anti-VEGF injections that were given at monthly intervals or 6 months after 1 short-term steroid injection, regardless of visual acuity [VA]). For post vitrectomy cases, eyes were considered...
Table 1. Baseline demographics

| Parameter                                      | Full population | Vitrectomized Group 1 | Nonvitrectomized Group 2 | p value   |
|------------------------------------------------|-----------------|-----------------------|--------------------------|-----------|
| Patient eyes, n                                | 26              | 18                    | 8                        | N/A       |
| Mean age, years ± SD (median)                  | 67.9±7.2 (68)   | 66.4±7.0 (67)         | 71.3±6.8 (71)            | 0.1012    |
| Mean DME duration, years ± SD (median)         | 3.5±1.3 (3)     | 3.6±1.4 (3)           | 3.2±1.0 (3)              | 0.6567    |
| Mean GCL thickness, μm ± SD (median)           | 47.2±9.2 (50)   | 46.3±9.1 (46)         | 49.4±9.9 (51)            | 0.3640    |
| Mean BCVA, letters ± SD (median)               | 42.6±18.7 (40)  | 44.4±18.9 (40)        | 38.6±18.9 (38)           | 0.5229    |
| Mean CFT, μm ± SD (median)                     | 517.4±185.7 (477) | 516.8±179.0 (477)   | 518.9±212.9 (508)        | 0.8895    |
| Mean IOP, mm Hg ± SD (median)                  | 15.0±2.7 (15)   | 14.9±2.4 (15)         | 15.3±3.4 (16)            | 0.6974    |
| Mean age, years ± SD (median)                  | 67.9±7.2 (68)   | 66.4±7.0 (67)         | 71.3±6.8 (71)            | 0.1012    |
| Mean DME duration, years ± SD (median)         | 3.5±1.3 (3)     | 3.6±1.4 (3)           | 3.2±1.0 (3)              | 0.6567    |
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| Mean IOP, mm Hg ± SD (median)                  | 15.0±2.7 (15)   | 14.9±2.4 (15)         | 15.3±3.4 (16)            | 0.6974    |
| Pseudophakic eye, (%)                          | 77               | 89                    | 50                       | 0.0510    |
| Laser therapy (PRP and macular laser), n eyes (%)| 18 (100)       | 18 (100)              | 18 (100)                 | 1.0000    |
| PRP laser, n eye (%)                           | 8                | 10                    | 8                        | 1.0000    |
| Macular laser, n eyes (%)                      | 35               | 33                    | 38                       | 1.0000    |
| Anti-VEGF IV injections, n eyes (%)            | 77               | 78                    | 78                       | 1.0000    |
| Mean anti-VEGF IV injections, ± SD (median)    | 3.2±3.6 (3)     | 2.8±2.8 (2)           | 4.1±5.1 (3)              | 0.6567    |
| CCT IV injections, n eyes (%)                  | 92.6            | 100                   | 87.5                     | 0.3077    |
| Mean CCT IV injections, ± SD (median)          | 2.5±1.6 (2)     | 2.9±1.6 (3)           | 1.6±0.9 (2)              | 0.0668    |

Values are presented as mean±standard deviation unless stated otherwise. Baseline defined as the day of FAc implant injection (preinjection). BCVA in ETDRS letters. p values were derived using a Mann-Whitney test for two samples and pertain to the comparison between groups. PRP, panretinal photocoagulation; VEGF, vascular endothelial growth factor; CCT, corticosteroids; SD, standard deviation. a 4 Eyes out of 30 were excluded due to inconsistent segmentation. b At Month 2. c At Month 0. d All vitrectomized eyes (17 of 18 with time to vitrectomy reported) had undergone ILM peeling. e These p values were derived from a Fischer’s exact test.

for inclusion provided the vitreectomy had been performed at least 6 months (range, 0.5–6.5 years) prior to IV injection of the FAc implant.

Patients were excluded if they had additional ocular diseases that could significantly affect the VA, history of ocular trauma, or surgery other than uncomplicated cataract surgery within the 3 months prior to FA therapy. A control group of 12 fellow eyes (disease-free, nonvitrectomized eyes from nondiabetic patients; a third group separated from groups 1 and 2), of eyes submitted to the previous vitrectomy for idiopathic epiretinal membrane, were included for comparison. For inclusion in the control group, the fellow eye (i.e., normal) was followed by one vitreoretinal surgeon, BP. These eyes were consecutively selected at a 24-month follow-up period after vitrectomy in the contralateral eye, between December 2020 and February 2021.

**Procedures**

Baseline patient demographic data, including previous treatments, were recorded. All patients, including control eyes, had a complete ophthalmological evaluation, including intraocular pressure (IOP) measurement (Goldmann Applanation Tonometry, Haag Streit GmbH, Wedel, Germany) along with a macular SD-OCT (dual-line scan) at baseline and at each follow-up visit (baseline, Month 1, Month 3, and then quarterly). For this analysis, the variation of GCL thickness (globally and by segmental quadrants: temporal, superior, nasal, and inferior) was assessed in comparison with baseline GCL thickness at baseline vs. BCVA at 24 months; and (c) mean BCVA at 24 months vs. the difference between the maximum and minimum values recorded between months 6 and 24; (d) mean GCL thickness at baseline vs. BCVA at 24 months; and (e) mean BCVA at 24 months versus mean GCL thickness at 24 months. For all vitrectomized eyes, a standard pars plana vitrectomy with ILM peeling was performed using trypan blue ophthalmic solution 0.15% (double-dye; Horus Pharma, Francheville, France).

**Optical Coherence Tomography**

OCT scans were obtained by an SD-OCT (dense line scan mode HR 20 × 20° Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany). A 3-mm area of the macular region centered on the fovea was examined with automated segmentation. For standardization, all examinations were performed by one well-trained technician to counteract a potential annotation bias. During the follow-up examinations, built-in automatic recognition system enabled scanning of the exact same location.

SD-OCT scans were evaluated for the presence of intraretinal fluid, subretinal fluid, epiretinal membrane, GCL thickness, and CFT. CFT and GCL thickness were analyzed using the retinal thickness map analysis protocol with nine ETDRS subfields.

The CFT was automatically measured by the software in the central 1 mm. The GCL thickness was measured globally (average) and by segmental quadrants: temporal, superior, nasal, and inferior, in the 3-mm region of the central macula. CFT and GCL thickness variation were evaluated in comparison with baseline OCT scans as well as OCT scans from the previous timepoint.

Correlations between GCL thickness and BCVA in the overall population and per groups were also analyzed regarding: (a) VA at 24 months vs. the difference between the maximum and minimum values recorded between months 6 and 24; (b) mean GCL thickness at baseline vs. BCVA at 24 months; and (c) mean BCVA at 24 months versus mean GCL thickness at 24 months. For all vitrectomized eyes, a standard pars plana vitrectomy with ILM peeling was performed using trypan blue ophthalmic solution 0.15% (double-dye; Horus Pharma, Francheville, France).
Statistical Analysis

The demographics and clinical characteristics of this study cohort were evaluated using traditional descriptive methods. A two-sample Mann-Whitney test was used for comparisons between groups. A Fisher’s exact two-tailed test was used to assess categorical differences. Linear correlations were performed using a Spearman rank correlation. Multiple-group comparisons were performed using a Kruskal-Wallis test. A statistical difference was taken as a p value <0.05. Statistical analysis was performed using a mix of MS Excel (Microsoft, Redmond, WA, USA), SPC for Excel (BPI Consulting, Katy, TX, USA), and GraphPad Prism (GraphPad Software, San Diego, CA, USA). Values are reported as mean ± standard deviation unless otherwise stated.

Results

Patient Demographics

Overall, between April 2015 and September 2016, 26 eyes met the primary cohort criteria. The baseline characteristics of these 26 eyes are summarized in Table 1. This table also presents outcomes by their groupings (i.e., group 1 or 2, respectively).

Best-Corrected Visual Acuity and Central Foveal Thickness

At baseline (Table 1), mean BCVA was 44.4 ± 18.9 letters in group 1 and 38.6 ± 18.9 in group 2 (p > 0.05). A mean increase in BCVA was observed for both groups following injection of the FAc implant in the first 6 months (+16.1 letters in group 1 and +14.4 in group 2), and this was maintained to Month 24 (Fig. 1a). There were no significant differences (p > 0.05) between the two groups in absolute BCVA values at any of the timepoints measured.

In the whole population, by Month 6 mean BCVA increased by 15.6 ETDRS letters (median change of 13 letters; p = 0.0193 vs. baseline) and remained increased (15.2 ETDRS letters) to Month 24 (a median change of 13 letters; p = 0.0038 vs. baseline). Mean CFT decreased by 191.6 μm (a median change of 164 μm; p = 0.000001 vs.
baseline) and was stable ($-190.8 \, \mu m$) to Month 24 (a median change of $170 \, \mu m$; $p = 0.000003$ vs. baseline).

Mean CFT decreased from baseline in both groups 1 and 2 within the first 6 months ($-212.8 \pm 144.5 \, \mu m$ [$p = 0.00001$]) and $-140.0 \pm 120.8 \, \mu m$ [$p = 0.0177$], respectively, and mean CFT then remained stable in both groups with a significance difference from baseline being maintained to Month 24 (i.e., $-198.6 \pm 203.3 \, \mu m$ [$p = 0.000003$]).

Fig. 2. Mean changes from baseline in GCL thickness: global for all treated versus controls and vitrectomized versus nonvitrectomized eyes (a) and segmental regions for vitrectomized versus nonvitrectomized eyes (b-e). a Global GCL thickness: (i) all treated (open circles) versus controls (closed circles) The comparison between the overall population and the control group revealed significant differences in the changes at Months 6 ($p = 0.0082$), 12 ($p = 0.0016$), and 24 ($p = 0.0010$); (ii) vitrectomized versus nonvitrectomized $p > 0.05$ for the comparison of non-PPV and PPV values at each timepoint. b Nasal $p > 0.05$ for the comparison of non-PPV and PPV values at each timepoint. c Temporal $p > 0.05$ for the comparison of non-PPV and PPV values at each timepoint. d Superior $p > 0.05$ for the comparison of non-PPV and PPV values at each timepoint. e Inferior $p > 0.05$ for the comparison of non-PPV and PPV values at each timepoint.
GCL Thickness following the 0.2 µg/day Fluocinolone Acetonide Implant

Overall, there was no significant difference in absolute CFT between the groups at Months 6, 12, and 24 (p > 0.05). Also of note was a general tendency for a lower mean CFT in group 1 than in group 2 at Months 6 (281.2 ± 64.0 µm vs. 321.0 ± 82.5 µm; p = 0.7032) and 12 (268.8 ± 40.7 µm vs. 359.4 ± 132.7 µm; p = 0.3912), which was not evident at Month 24 (303.1 ± 99.5 µm vs. 289.1 ± 97.7 µm; p = 0.3912), respectively (Fig. 1b).

Grouping the data based on lens status revealed no overall differences (p > 0.05) between mean BCVA and CFT values at any timepoint for eyes with a phakic or pseudophakic lens. There was one small difference between mean CFT values at Month 6 (323.8 ± 60.2 µm vs. 282.4 ± 72.1 µm [phakic vs. pseudophakic]; p = 0.3912).

GCL Thickness

In the overall population treated with FAc implant, there was a decrease in mean global GCL thickness from 47.2 ± 9.2 µm at baseline to 43.0 ± 9.2 µm at Month 6 (i.e., −4.8 µm; p = 0.1183 absolute values vs. baseline values) and then remained stable at Months 12 (40.7 ± 9.9 µm; p = 0.0344 vs. baseline) and 24 (42.6 ± 10.2 µm; p = 0.1242 vs. baseline) (Fig. 2a[i]). In the control group of patients, i.e., eyes without diabetes and included as a reference in the current study (group 3), GCL thickness remained relatively stable at baseline (49.5 ± 5.9 µm), Months 6 (49.8 ± 6.5 µm), 12 (50.6 ± 5.6 µm), and 24 (50.0 ± 5.4 µm) (Fig. 2a[i]) with statistical changes observed between Month 6 and baseline (p = 0.0359).

A comparison between the overall population and the control group revealed no statistical differences at baseline (i.e., 47.2 ± 9.2 µm vs. 49.5 ± 5.9 µm [p = 0.4878]) and Month 6 (43.0 ± 9.2 µm vs. 49.8 ± 6.5 µm [p = 0.2271]), but there were statistical differences at Months 12 (40.7 ± 9.9 µm vs. 50.6 ± 5.6 µm [p = 0.0069]) and 24 (42.6 ± 10.2 µm vs. 50.0 ± 5.4 µm [p = 0.0437]), respectively (Fig. 2a[i]). Similar changes were obtained in group 1 (−5.0 ± 4.8 µm at Month 6 [p = 0.0875 for absolute values vs. baseline values], −6.8 ± 8.7 µm at Month 12 [p = 0.0279], and −5.1 ± 4.6 µm at Month 24 [p = 0.1169]) and group 2 (−4.0 ± 5.2 µm at Month 6, −4.4 ± 6.5 µm at Month 12, and −6.5 ± 10.1 µm at Month 24 [p > 0.05 for all absolute values vs. baseline values]). The comparison between groups 1 and 2 at the follow-up timepoints revealed no statistical differences (p > 0.05; Fig. 2a[ii]).

In group 1, the smallest mean change from baseline was observed in the nasal segment at each timepoint (i.e., −3.7 µm at Month 6 [p = 0.2482 absolute values vs. baseline values], −5.6 at Month 12 [p = 0.1737], and −3.2 at Month 24 [p = 0.3554]), and the largest mean change, again at each timepoint, was observed in the superior segment (−5.8 µm at Month 6 [p = 0.1173], −8.5 µm at Month 12 [p = 0.0227], and −6.3 µm at Month 24 [p = 0.1813]) (Fig. 2b–e). In group 2, the smallest mean changes from baseline were seen in nasal (0.0 µm at Month 6 [p = 0.9491 absolute values vs. baseline values], −0.8 µm at Month 12 [p = 0.4822], and +1.8 µm at Month 24 [p = 0.9491]). In the superior segmental region, there was a smaller variation at Month 12 in comparison to the other time points

GCL Thickness following the 0.2 µg/day Fluocinolone Acetonide Implant

Fig. 3. Plots showing mean global GCL thickness in all eyes, vitrectomized (PPV), nonvitrectomized (non-PPV), and control eyes. The comparison between groups using a Kruskal-Wallis test revealed a difference between groups at Month 12 (χ² = 10.06, p = 0.018, df = 3). A two-sample Mann-Whitney test revealed differences between control and all eyes groups (p = 0.007), and between control and PPV eyes (p = 0.002).

Fig. 3. Plots showing mean global GCL thickness in all eyes, vitrectomized (PPV), nonvitrectomized (non-PPV), and control eyes. The comparison between groups using a Kruskal-Wallis test revealed a difference between groups at Month 12 (χ² = 10.06, p = 0.018, df = 3). A two-sample Mann-Whitney test revealed differences between control and all eyes groups (p = 0.007), and between control and PPV eyes (p = 0.002).
−0.3 μm at Month 12 \( (p = 0.6093) \), and −4.2 μm at Month 24 \( (p = 0.7015) \) (Fig. 2b–e). Figure 3 plots global GCL thickness for control eyes, all eyes, PPV, and non-PPV eyes.

### Correlation between GCL Thickness and BCVA in Global Treated Population

For all eyes treated with the FAc implant, BCVA at 24 months was not correlated with the difference between the maximum and minimum values recorded between Months 6 and 24 (Rho = −0.22, \( p > 0.05 \)) (Fig. 4a). However, a positive correlation was observed between BCVA at Month 24 and the mean GCL thickness at Month 24 (Rho = 0.64, \( p = 0.006 \); Fig. 4b) and the mean GCL thickness at baseline and VA at 24 months (Rho = 0.44, \( p = 0.036 \)) (Fig. 4c).

### Correlation between GCL Thickness and BCVA in Group 1 and Group 2

BCVA at 24 months was not correlated (Rho = −0.09, \( p > 0.05 \)) with the difference between the maximum and minimum GCL values recorded between Months 6 and 24 in vitrectomized eyes (Fig. 5a); however, there was a strongly negative correlation that was not statistically significant (Rho = −0.70, \( p = 0.105 \)) observed in nonvitrectomized eyes (Fig. 5b). BCVA at 24 months was correlated with mean GCL thickness at 24 months in vitrectomized eyes (Rho = 0.64, \( p = 0.006 \); Fig. 5c) and nonvitrectomized eyes (Rho = 0.37, \( p = 0.003 \)) (Fig. 5d). Mean GCL thickness at baseline reaches a borderline significance (Rho = 0.47, \( p = 0.051 \)) and a statistical significance (Rho = 0.54, \( p = 0.003 \)) when correlated with BCVA at 24 months in vitrectomized and nonvitrectomized eyes, respectively (Fig. 5e, f).
Fig. 5. Correlation of GCL thickness with VA for Group 1 (a, c, e) and Group 2 (b, d, f) eyes. 

**a** Group 1: mean ETDRS VA at 24 months versus the difference between the maximum and minimum values recorded between months 6 and 24. ETDRS VA at 24 months versus the difference between the maximum and minimum values recorded between Months 6 and 24.

**b** Group 2: mean ETDRS VA at 24 months versus the difference between the maximum and minimum values recorded between months 6 and 24.

**c** Group 1: mean BCVA at 24 months versus mean GCL thickness at 24 months.

**d** Group 2: mean BCVA at 24 months versus mean GCL thickness at 24 months.

**e** Group 1: mean GCL thickness at baseline versus BCVA at 24 months.

**f** Group 2: mean GCL thickness at baseline versus BCVA at 24 months.

ETDRS VA at 24 months versus the difference between the maximum and minimum values recorded between Months 6 and 24.
Safety

No statistically significant differences were observed between groups at any timepoints in terms of the absolute values or changes in mean IOP ($p > 0.05$; Fig. 1c). Mean IOP values remain below 21 mm Hg throughout. Compared with 50% at baseline, 61.5% of patients required IOP-lowering medication ($p > 0.05$, Fisher’s exact test) during the follow-up period. IOP-lowering surgery was performed on one eye in the vitrectomized group during this period. All phakic eyes ($n = 6$) underwent cataract surgery between Month 9 and Month 12.

Supplemental Therapies

Regarding additional therapy in the 24 month post-FAc implantation, 26.9% ($n = 7$) required IV therapy and 19.2% ($n = 5$) required laser therapy.

Discussion

Overall, there was no statistically significant difference in absolute CFT between vitrectomized and nonvitrectomized eyes groups between Months 6 and 24. There were overall decreases in global GCL that were observed for all treated patients from around Month 6, before stabilizing, and this compares with a stabilization/slight increase in control eyes (without diabetes), which is in line with what was expected. Bonnin et al. [23] showed that GCL thickness in eyes treated for DME, following DME resolution, is reduced in comparison with diabetic eyes without DME, although their central macular thickness is within a normal range.

These results suggest that the FAc implant contributes to stabilization of GCL. While the effect of the FAc implant can start to appear within the first month, this usually peaks at 3–6 months and then stabilizes up to 36 months. This may explain the small decrease in the GCL thickness within the first 6 months, even though the possible stabilization on GCL thickness by FAc after Month 24 or until Month 36 needs still to be addressed in future studies.

The total retinal thickening in DME seen on OCT may represent edematous or degenerative changes and that is expected to occur also for the GCL layer. We observed a significant positive mild correlation between baseline global GCL thickness and BCVA at Month 24 for all eyes. This result may mean that GCL against a background DME may be a predictive biomarker, although this needs further research. Under an effective DME therapy towards a dry macula, as demonstrated by a significant DME reduction, globally and separately in both groups following FAc therapy during the 2 years period analyzed in our study, a positive correlation between GCL thickness and VA observed at that timepoint can be expected to occur. The effect and negative impact of DME in the inner retinal layers on functional outcome have been objectively demonstrated by Prager et al. [24] who found a positive correlation between a reduction of inner retinal layers thickness, particularly in the nasal quadrant, and a visual gain, both with ranibizumab and triamcinolone applied to DME treatment [24].

Our study intended to analyze the effect of the FAc implant on GCL layer thickness preservation, which has an eminent importance in visual function, as demonstrated with ranibizumab or triamcinolone [24]. What we believe is that the common effect among different therapeutic options is the achievement of an efficient edema reduction, which is continuous and longstanding (i.e., predictable) with the FAc implant. To the best of our knowledge, there are no studies reporting the effect of the FAc implant on GCL thickness.

The observation of a BCVA at 24 months with no correlation with difference between the maximum and minimum GCL thickness values recorded between Months 6 and 24 in vitrectomized and nonvitrectomized eyes, even though a negative correlation was observed with no statistical significance in nonvitrectomized, may indicate that the presence of vitreous plays a role in the response pattern to the FAc implant, with a more constant, predictable, and stable effect observed in vitrectomized eyes, which is also in line with another previous publication of our group [25]. In addition, it may also reflect the negative effect of edema in the GCL layer and its negative correlation with VA, as demonstrated by Bonnin et al. [23] besides the value of FAc in the management of DME and achieving the goal of retina neuroprotection in vitrectomized and nonvitrectomized eyes, particularly in vitrectomized eyes [25].

The above finding reinforces the importance of a stable, effective therapy for DME and particularly in vitrectomized eyes that have a characteristic lower GCL thickness reservoir (see Fig. 3). This is something that is related to the ILM peeling procedure during vitrectomy which, in our study, had been performed in all vitrectomized eyes included. Several studies have reported reductions in GCL thickness following ILM peeling [21, 22], which was associated with worse postoperative BCVA [21]. However, other studies [26, 27] have assessed postoperative changes in GCL thickness in the eyes with or without ILM peeling during pars plana vitrectomy.
Hence, in vitrectomized eyes with or without ILM peeled, the same effort toward an effective DME therapy should actively be pursued.

Regarding the differences between groups in the amount of GCL reduction by quadrants (superior, inferior, nasal, or temporal), it is interesting to verify that FAc therapy induced the greatest change in the superior quadrant of vitrectomized eyes and the least affected was the nasal quadrant, in both subgroups. A possible explanation may be both the easier access to superior quadrant for the ILM peeling approach and the intention to preserve, as much as possible, the nasal part where the papillomacular bundle is located. This leaves the remaining sectors to be removed clockwise with a minimal interruption [28]. The traumatic pinches in the superior quadrant may be a cause of some inflammatory retina response with a secondary reactive edema and a more pronounced response to IV corticosteroid therapy in the most traumatized tissue. Furthermore, the smaller reduction in the nasal quadrant, also seen in nonvitrectomized eyes, may be a sign of a more pronounced blood-retinal barrier affected location in DME patients.

These results suggest that there was no evident neurodegeneration in the 2-year period following treatment with the 0.2 μg/day FAc implant in both vitrectomized and nonvitrectomized eyes, even though the possible stabilization on GCL thickness by FAc after month 24 or until month 36 needs still to be addressed in future studies. The sustained long-standing action of this intracocular delivery implant reduces the burden on the patient and allows the achievement of a sustained drying of the macula and improvement in vision. In addition, post hoc analysis of the FAME A and B trials also found that more subjects who received FAc experienced 2-or-more or 3-or-more step improvements in DR severity than subjects who received sham [29].

The current study has a number of limitations including its retrospective design, the small number of patients included in the primary groups analyzed, and no treated control arm, which would be useful in comparing the effect of different IV therapies. Hence, further research and trials are required at the current time to complement this research.

**Conclusion**

Overall, these results suggest that GCL thickness may be a useful biomarker for assessing the effectiveness of the FAc implant therapy in patients with DME.

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**Statement of Ethics**

The study was conducted according to the tenets of the Declaration of Helsinki in its latest amendment (Brazil, 2013) and was approved by the Ethics Committee of CHU Porto (2017.093 [084-DEFI/082-CES]). All patients signed a written informed consent form.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

B.P. was responsible for the study conception and design. Data collection was performed by B.P. and C.C. All authors contributed to data analysis and interpretation. The first draft of the manuscript was written by B.P., and all authors reviewed on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data Availability Statement**

Data are available from the corresponding author upon request.

**References**

1. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy: IV. Diabetic macular edema. *Ophthalmology*. 1984;91:1464–74.
2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35:556–64.
3. Ding J, Wong TY. Current epidemiology of diabetic retinopathy and diabetic macular edema. *Curr Diab Rep*. 2012;12:346–54.
4. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61:1902–12.
Lee SS, Ghosn C, Yu Z, Zacharias LC, Kao H, Lanni C, et al. Vitreous VEGF clearance is increased after vitrectomy. Invest Ophthalmol Vis Sci. 2010;51:2135–8.

Bressler SB, Melia M, Glassman AR, Almukhtar T, Jampol LM, Shami M, et al. Ranibizumab plus prompt or deferred laser for diabetic macular edema in eyes with vitrectomy before anti-vascular endothelial growth factor therapy. Retina. 2015;35:2516–28.

Alfawadi F, Lip PL, Elsherbyny S, Chavan R, Mitra A, Mushtaq B. Report of 12-months efficacy and safety of intravitreal fluocinolone acetonide implant for the treatment of chronic diabetic macular oedema: a real-world result in the United Kingdom. Eye. 2017;31:650–6.

El-Ghrably I, Steel DHW, Habib M, Vaideanu-Collins D, Manvirk S, Hillier RJ. Diabetic macular edema outcomes in eyes treated with fluocinolone acetonide 0.2 µg/d intravitreal implant and in people with diabetic macular edema. Curr Med Res Opin. 2017;33:5–17.

Meireles A, Goldsmith C, El-Ghrably I, Erginay A, Meireles A, Beirão JNM. Optical coherence tomography biomarkers: vitreous status influence in outcomes for diabetic macular edema therapy with 0.19 mg fluocinolone acetonide implant. Ophthalmic Res. 2021;64(4):639–47.

Akino K, Nagai N, Watanabe K, Ban N, Kurihara T, Uchida A, et al. Risk of newly developing visual field defect and neurodegeneration after pars plana vitrectomy for idiopathic epiretinal membrane. Br J Ophthalmol. 2021;105(12):1683–7.

Lee CH, Lee MW, Choi EY, Byeon SH, Kim SS, Koh HJ, et al. Comparison of individual retinal layer thicknesses after epiretinal membrane surgery with or without internal limiting membrane peeling. J Ophthalmol. 2018;2018:1256781.

Won JY, Kim M, Park YH. Postoperative changes in the retinal thickness and volume after vitrectomy for epiretinal membrane and internal limiting membrane peeling. Medicine. 2017;96:e6709.

Wykowski CC, Chakravarthy U, Campochiaro PA, Bailey C, Green K, Cunha-Vaz J. Long-term effects of intravitreal 0.19 mg fluocinolone acetonide implant on progression and regression of diabetic retinopathy. Ophthalmology. 2017;124:440–9.