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Alterations in retinal arteriolar microvascular structure associates with higher treatment burden in patients with diabetic macular edema: results from a 12-month prospective clinical trial

Blindbæk, S. L., Peto, T., & Grauslund, J. (2019). Alterations in retinal arteriolar microvascular structure associates with higher treatment burden in patients with diabetic macular edema: results from a 12-month prospective clinical trial. *Acta Ophthalmologica*. <https://doi.org/10.1111/aos.14278>

Published in:
Acta Ophthalmologica

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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1 Original paper

3 *Title:*

4 **Alterations in retinal arteriolar microvascular structure associates with higher treatment burden in**
5 **patients with diabetic macular edema: results from a 12-month prospective clinical trial.**

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22 **Word count**

23 Abstract: 250 (excluding headings)

24 Manuscript: 3703 (excluding headings, tables, figures and legend

25

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ABSTRACT

Purpose: This study was based on data from a 12-months prospective clinical trial and aimed to examine changes in retinal microvascular structure in eyes treated with intravitreal aflibercept in combination with focal/grid laser photocoagulation for diabetic macular edema (DME).

Methods: We included 32 treatment naïve eyes of 22 patients with center involving DME. The treatment algorithm comprised a loading phase of three monthly injections of aflibercept and focal/grid laser photocoagulation (baseline (BL)-month 3 (M3)) followed by a pro re nata (PRN) aflibercept phase until month 12 (M12). Eyes were divided in groups with and without need for PRN treatment after loading. Parameters of retinal microvascular structure were measured in 45° optic disc centered fundus images at BL, M3 and M12 using a semi-automated software (VAMPIRE-Web, Vessel Assessment and Measurement Platform for Images of the Retina, Universities of Dundee and Edinburgh, UK).

Results: A significant decrease in retinal arteriolar calibre was demonstrated at both M3 (-11.2 μm , $p=0.005$) and M12 (-11.5 μm , $p=0.04$) as compared to baseline in eyes that needed PRN treatment during follow up. In contrast, arteriolar calibre remained unchanged in eyes without need for PRN treatment (M3: -1.6 μm , $p=0.79$ and M12: -7.0 μm , $p=0.22$). For retinal venules, vessel caliber decreased both in eyes with and without need for PRN therapy at M3 (-9.5 μm , $p=0.01$ and -11.6 μm , $p=0.01$) as well as at M12 (-15.6 μm , $p=0.001$ and -11.0 μm , $p=0.04$).

Conclusion: Early changes in retinal arteriolar caliber are associated with an increased treatment burden during the first year of DME-treatment.

Keywords: diabetic macular edema, anti-VEGF, retinal imaging, prospective clinical trial

INTRODUCTION

The estimated number of patients with diabetes globally reached 425 million in 2017 (Cho et al. 2017). Diabetic retinopathy (DR) is the most common complication of diabetes and is almost universal in patients with long duration of the disease (Grauslund et al. 2009; Cheung et al. 2010). End stage DR comprises proliferative DR (PDR) and diabetic macula edema (DME) of which DME is the leading cause of preventable vision loss in the working age population of the developed countries and can occur at any stage of DR (Klein et al. 2010; Antonetti et al. 2012; Tan et al. 2017).

Vascular endothelial growth factor (VEGF) is a key mediator in the development and progression of diabetic macular edema (DME) and, hence, VEGF is currently a primary target in DME-treatment (Aiello et al. 1994). The release of VEGF is largely driven by retinal ischemia and tissue hypoxia as a consequence of hyperglycemic damage to the retinal microvascular structure (Cogan & Kuwabara 1963; Stitt et al. 2016). The upregulation of VEGF during disease progression ultimately causes vasodilation and increased permeability of the vessel wall leading to hyper-perfusion, increased hydrostatic pressure and ultimately fluid leakage and macular edema (Senger et al. 1986; Aiello et al. 1994; Ferrara 1999).

Changes in retinal microvascular structure have been associated with several systemic diseases as well as the development and progression of diabetic retinopathy (DR) in persons with diabetes in large population based studies. Thus, wider venular caliber have consistently been associated with more severe stages of DR while narrower arteriolar caliber as well as lower fractal dimension is associated with an increased risk of progression to vision threatening DR (Klein et al. 2004; Kifley et al. 2008; Klein et al. 2012; Broe et al. 2014).

Due to the association with both the severity and the progression of the disease, changes in retinal microvascular structure during treatment have been a subject of interest in several retinal vascular diseases. Thus, the diameter of macular vessels has been demonstrated to decrease following focal/grid laser treatment in patients with DME (Lundberg et al. 2013). Similar results have been demonstrated with inhibitors of VEGF (anti-VEGF) in the treatment of neovascular age related macular degeneration (nAMD) while results in regards of anti-VEGF treatment of DME are limited (Tatlipinar et al. 2012; Wickremasinghe et al. 2012; Consigli et al. 2018; Min et al. 2018; Tetikoglu et al. 2018).

Furthermore, only one study has addressed the association between retinal microvascular structure prior to treatment and treatment outcome in patients with DME (Moradi et al. 2014). They demonstrated that wider venular caliber at baseline was associated with better visual outcome after treatment with intravitreal ranibizumab. However, only vessel calibers were examined and without information on changes during treatment.

We recently demonstrated that during the first year of DME-treatment, approximately 40% of eyes did not need additional intravitreal therapy after combination therapy with three monthly injections of intravitreal aflibercept and focal/grid laser photocoagulation (Blindbaek et al. 2019).

Hence, in this ancillary analysis based on data from a 12-month randomized clinical trial we aimed to evaluate baseline parameters of retinal microvascular structure as predictors of need for additional therapy after combination treatment for DME. We hypothesize that parameters of retinal microvascular structure prior to treatment differ between eyes with different need for therapy. Furthermore, we wanted to examine whether changes in retinal microvascular structures can be used as a postoperative marker of need for therapy.

105

106 **METHODS**

107 This study was based on data from a previously reported 12-month randomized clinical trial and included
108 32 treatment naïve eyes of 22 patients with center involving DME. Patients were recruited at referral to
109 Odense University Hospital, Denmark, between 1 October 2015 and 31 December 2017.

110

111 Inclusion criteria were center involving DME, age 18-99 years, best corrected visual acuity (BCVA) 35-80
112 Early Treatment Diabetic Retinopathy Study (ETDRS) letters and central retinal thickness (CRT) >300 µm.
113 Patients who were pregnant, had active proliferative diabetic retinopathy, a history of panretinal
114 photocoagulation, had received any previous DME-treatment or had been subjected to intraocular surgery
115 within four months prior to inclusion were excluded.

116

117 At baseline (BL), all patients provided a full medical history and underwent ophthalmic evaluation including
118 BCVA using ETDRS charts (Precision Vision, Illinois, USA) at a starting distance of four meters followed by slit
119 lamp and fundus bio microscopy in mydriasis with tropicamide 10 mg/mL and phenylephrine 10%, fundus
120 photography and optical coherence tomography (OCT) (3D OCT-2000 Spectral domain OCT, Topcon, Tokyo,
121 Japan) (twelve-line radial scan) and 50 degrees macula-centered fundus fluorescein angiography (TRC-50DX
122 fundus camera, Topcon, Tokyo, Japan).

123 Furthermore, BL examination included measurement of brachial arterial blood pressure (Omron 705CP,
124 Hoofdrop, The Netherlands) and hemoglobin A1c (HbA1c) (Tosoh G8, Alere, Holstebro, Denmark). Mean
125 arterial pressure (MAP) was calculated as $BP_d + (BP_s - BP_d)/3$ where BP_d is the diastolic blood pressure and
126 BP_s is the systolic blood pressure and body mass index (BMI) as $\text{weight(kg)}/\text{height(m)}^2$.

127

128 Patients then entered a loading phase of three monthly injections of 2.0 mg aflibercept followed by
129 focal/grid laser photocoagulation using either Navilas® (OD-OS GmbH, Teltow, Germany) or PASCAL® laser
130 (Optimedica Corp., Santa Clara, CA, USA). After laser until month 12 (M12), patients were scheduled for
131 monthly follow-up and additional aflibercept was administered pro re nata (PRN) if CRT increased with
132 more than 20% as compared to the lowest measurement or if they experienced a loss in BCVA of more than
133 five ETDRS letters as compared to BL.

134 As no difference in functional outcome or need for intravitreal therapy was identified
135 between treatment arms of Navilas® and Pascal® laser photocoagulation, data were pooled for further
136 analysis of retinal microvascular structure and grouped according to their need for PRN treatment. For the
137 rest of this paper, eyes without need for PRN treatment after loading and until follow-up at month 12 will
138 be referred to as "PRN÷" and eyes that did need PRN treatment as "PRN+".

139

140 **Retinal microvascular structure**

141 Image analysis was performed in 45° optic disc centered fundus images (3D OCT-2000 Spectral domain
142 OCT, Topcon, Tokyo, Japan) acquired at BL, month three (M3) prior to focal/grid laser photocoagulation
143 and M12 using VAMPIRE-Web (Vessel Assessment and Measurement Platform for Images of the Retina,
144 Universities of Dundee and Edinburgh, UK) (Fig. 1).

145 All image analyses were performed by a single trained grader (SLB) in accordance with a standard VAMPIRE
146 grading protocol. Image quality was assessed using a binary black and white vessel map demonstrating the
147 software's ability to delineate retinal vessels. A detailed description of the equipment and grading method

can be found elsewhere (Perez-Rovira et al. 2011; McGrory et al. 2018). In brief, a three-zoned grid is automatically placed around the optic disc by the VAMPIRE software. Zones A, B and C delineate retinal areas between 0-0.5, 0.5-1.0 and 0.5-2.0 disc diameters from the optic disc margin, respectively. Arterioles and venules were automatically labelled and color mapped in red and blue. Misidentified arterioles and venules were secondly manually re-labelled by the grader. Furthermore, if the software erroneously identified artefacts (e.g. hemorrhages and hard exudates) as vessels, if two vessels were detected as one, if a single vessel was registered as two separate vessels or if the delineation of a vessel was unsatisfactory the vessel section was excluded from the analysis (white on color map) (Fig. 1).

The following parameters were measured: vessel calibers are presented as the central retinal artery and vein equivalent, respectively, representing the six largest arterioles and venules coursing through zone B (Knudtson et al. 2003). To ease the interpretation of results, an image conversion factor, based on the assumption of an average disc diameter of 1800 μm , was used to translate vessel widths from pixels into μm . Tortuosity is a mathematical quantification of the curvature of a vessel segment as compared to a reference straight line of the same vessel segment. Vessel density is a simple measure of the total sum of pixels occupied by retinal vessels in a given retinal area and, thus, provides a combined measure of both vessel complexity and vessel width. The fractal dimension characterizes complex, repeating geometrical patterns in different spatial scales. It thus summarizes the branching complexity of the retinal vascular tree in a single non-integer value. Tortuosity, vessel density and fractal dimension are measured in zone C.

Fig. 1

Statistical analysis

Statistical calculations were performed using STATA version 15.1 (StataCorp LLC, College Station, TX, USA). Continuous data are presented as mean (with 95% confidence intervals) and categorical data as percent. P-values under 0.05 were considered statistically significant.

Differences between eyes in groups PRN $\div$ and PRN+, respectively, were tested employing cluster robust standard errors for linear regression models as patients were allowed to participate with both eyes.

Ethics

This study was carried out in accordance with the Helsinki Declaration and Good Clinical Practice and approved by the Regional Scientific Ethical Committee for Southern Denmark. Informed consent was obtained from all patients for being included in the study. Trial registration: <http://www.clinicaltrials.gov> (NCT02554747).

RESULTS

Thirty-nine eyes of 28 patients fulfilled the criteria of inclusion. Two patients were discontinued from the study at month five as eyes were not suitable for laser. One patient died during follow-up of reasons unrelated to the study and one patient dropped out due to hospitalization over other severe complications to diabetes. Finally, three eyes of two patients were excluded as images were deemed ungradable. Thus, 32 eyes of 22 patients were finally included.

Five were women (7 eyes) and 17 were men (25 eyes). Twenty-one had type 2 diabetes and one had type 1. Mean age and duration of diabetes was 59.5 (57.7-64.3) and 9.2 (4.6-13.7) years,

191 respectively, and mean HbA1c was 59.8 (52.2-67.4) mmol/mol. Mean MAP was 101.9 (96.0-107.8) mmHg
192 and mean BMI was 29.2 (26.5-32.0) kg/m². Mean BCVA was 71.7 (68.2-75.3) ETDRS letters and mean CRT
193 was 387.3 (352.0-422.6) μ m. Mean arteriolar and venular caliber was 139.9 (133.3-146.5) and 212.8 (206.1-
194 219.4) μ m. Mean tortuosity was -8.2 (-8.8-(-7.7)) and -8.4 (-8.5-(-8.2)) for arterioles and venules
195 respectively and likewise mean vessel density was 3961.5 (3514.2-4408.8) and 5532.1 (5108.2-5956.0)
196 pixels. Mean fractal dimension was 1.374 (1.361-1.388).

197 A more extensive thickening of the central retina was observed in eyes in the PRN+ group as
198 compared to eyes in the PRN÷ group (424.9 (360.6-489.2) μ m vs. 344.6 (325.5-363.7) μ m, p=0.03) whereas
199 no differences were found between groups in regards of vessel calibers, tortuosity, vessel density, fractal
200 dimension or any other BL parameters (Table 1).

201

202 Table 1

203

204 After laser until M12, 46.9% (15/32) of eyes did not need additional intravitreal therapy and were, thus,
205 categorized as PRN÷. According to treatment protocol, the PRN÷ group received 3.0 aflibercept injections
206 (loading) between BL and M12 whereas the mean number of injections was 5.2 (4.5-6.0) in the PRN+ group.

207

208 From BL to M3 BCVA improved by 8.2 (6.3-10.0) ETDRS letters and at M12 BCVA had improved by 9.2 (7.6-
209 10.8) ETDRS letters as compared to BL without differences between groups at either time point. Likewise
210 there was a CRT reduction of 104.8 (68.0-141.6) μ m between BL and M3 and 96.5 (61.4-131.6) μ m between
211 BL and M12. Between BL and M3, a numerically greater reduction in CRT was noted in the PRN+ group even
212 though the difference between groups did not reach statistical significance (72.3 (47.6-96.9) μ m vs. 133.4
213 (67.5-169.3) μ m, p=0.09). Hence, the difference in CRT between the groups demonstrated at BL was no
214 longer present either at M3 or M12 (272.3 (257.1-287.6) μ m vs. 291.5 (271.3-311.7) μ m, p=0.12 and 273.5
215 (253.8-293.3) μ m vs. 305.9 (269.5-342.4) μ m, p=0.11).

216

217 Table 2

218

219 For the entire cohort, retinal arteriolar caliber remained unchanged during the loading phase with
220 intravitreal aflibercept (BL-M3) (-6.7 (-14.0-0.62) μ m, p=0.07) whereas a decrease was seen between BL and
221 M12 (-9.4 (-17.4-(-1.4)), p=0.02). For retinal venules, vessel calibers decreased by 10.5 (-16.2-(-4.7)) μ m
222 (p=0.001) during the loading phase and remained constricted at M12 (-13.4 (-20.2-(-6.6)) μ m, p<0.001 as
223 compared to baseline).

224

225 For the groups separately, a significant decrease in retinal arteriolar caliber was demonstrated after loading
226 (-11.2 (-18.7-(-3.7)) μ m, p=0.005) in eyes that needed additional therapy during follow-up (PRN+). In
227 contrast, retinal arteriolar caliber of eyes in the PRN÷ group remained unchanged between BL and M3 (-1.6
228 (-13.8-10.7) μ m, p=0.79). A similar pattern was noted at M12, at which retinal arteriolar caliber significantly
229 decreased in the PRN+ group (-11.5 (-22.2-(-0.8)) μ m, p=0.04) as compared to baseline whereas it remained
230 unchanged in the PRN÷ group (-7.0 (-18.6-4.5) μ m, p=0.22).

231

232 For retinal venules, both groups demonstrated a significant decrease in vessel calibers at
both M3 (PRN+: -9.5 (-17.0-(-2.0)) μ m, p=0.01 and PRN÷: -11.6 (-20.1-(-3.1)) μ m, p=0.01 as compared to BL)

233 and M12 (PRN+: -11.5 (-22.2-(-0.8)) μ m, p=0.001 and PRN $\div$: -11.0 (-22.1-(-0.19)) μ m, p=0.04 as compared to
234 BL).

235

236 A transient decrease in vessel density of retinal arterioles was noted between BL and M3 in the PRN+ group
237 (-367.3 (-720.7-(-13.9)) pixels, p=0.04). Thus, the decrease was no longer statistical significant at month 12
238 as compared to baseline. Vessel density remained unchanged at both M3 and M12 as compared to BL for
239 eyes without need for PRN treatment. For the remaining measured parameters of retinal microvascular
240 structure there were no change between BL and M3 and M12, respectively (Table 2).

241

242 **DISCUSSION**

243 In this explorative study of changes in retinal microvascular parameters during DME-treatment, early
244 changes in retinal arteriolar caliber after loading with intravitreal aflibercept associated with an increased
245 treatment burden during the first year of therapy. Furthermore, our study verifies previous reports of
246 vasoconstrictive effects of anti-VEGF therapy and extends the results till 12 months follow-up.

247

248 While we hypothesized that the pathophysiological association between vessel dilation/hyper-perfusion
249 and disease severity and edema formation would reflect treatment load, our data suggest the opposite
250 (Klein et al. 2004; Kifley et al. 2008; Klein et al. 2012). Thus, our results did not demonstrate any difference
251 in BL vessel calibers between eyes with and without need for PRN treatment after loading with intravitreal
252 aflibercept and macular laser. The only baseline parameter that differed between eyes with and without
253 need for PRN therapy was CRT which was maybe expected even though the difference in CRT was no longer
254 present after loading with aflibercept.

255 Interestingly, only eyes that needed PRN treatment demonstrated a decrease in retinal
256 arteriolar caliber after loading with aflibercept even though there was no difference in BL arteriolar caliber
257 between eyes with and without need for PRN treatment. This may, however, seem counterintuitive
258 according to the discussion above. We speculate that the VEGF load may be higher in eyes with a greater
259 need for therapy and, hence, the effects of blocking its physiological effects may be more pronounced as
260 compared to in eyes with a smaller burden of treatment. It must, however, be emphasized that no
261 between-group difference was demonstrated.

262

263 For the entire cohort, this study demonstrated that both retinal arteriolar and venular calibers decreased
264 during DME-treatment with intravitreal anti-VEGF and furthermore, that the decrease is maintained even
265 after switching from monthly therapy during loading to a PRN regimen. Even eyes that did not receive PRN
266 aflibercept after loading exhibited lasting venular constriction at M12. We assume that the beneficial effect
267 of anti-VEGF therapy on retinal thickness in DME-treatment is at least partly due to reduced hydrostatic
268 pressure as a consequence of a decrease in the pathologically increased vessel calibers towards normal
269 levels. However, it must be emphasized that a similar response to treatment has been reported in
270 neovascular age related macular degeneration (nAMD) even though nAMD is not associated with increased
271 vessel calibers (Micieli et al. 2012; Mendrinos et al. 2013; Tetikoglu et al. 2018).

272

273 At M3, a decrease in arteriolar vessel density was demonstrated in eyes that needed PRN treatment. As
274 vessel density is the sum of pixels occupied by retinal vessels in a given retinal area it is expected to
275 fluctuate with vessel calibers. Hence, our results may simply reflect the decrease in retinal arteriolar caliber

276 in eyes that needed PRN treatment. However, venular vessel density remained unchanged even though a
277 decrease in venular caliber was demonstrated in both groups. The usage of measuring vessel density is yet
278 not fully understood and is an experimental parameter provided in the VAMPIRE software. We speculate
279 that measuring vessel density may be more useful in predicting development and/or progression of DR
280 than treatment outcome in a similar manner as fractal dimension (Cheung et al. 2009; Broe et al. 2014).
281 Thus, there were no association between BL fractal dimension and treatment outcome in our study despite
282 a well-documented association between fractal dimension and long term risk of vision threatening DR.
283 Neither did the fractal dimension change during treatment in either group.

284
285 Our result align with previous reports on the effects of both aflibercept and ranibizumab on retinal vascular
286 calibers in the treatment of DME as well as other retinal vascular diseases (Tatlipinar et al. 2012;
287 Wickremasinghe et al. 2012; Min et al. 2018; Tetikoglu et al. 2018). We speculate that the vasoconstriction
288 of retinal vessels demonstrated in this study following intravitreal injection of aflibercept is probably in
289 truth a mere reversal of the vasodilative effect of VEGF. Thus, animal studies have demonstrated that VEGF
290 induces vasodilation in a dose-dependent fashion to produce transient tachycardia, hypotension and
291 decreased cardiac output (Ferrara 1999). However, studies on retinal vessels in human subject are limited.

292 Only one study using bevacizumab did not demonstrate any changes in retinal vessel calibers
293 after treatment (Wickremasinghe et al. 2017). Whether this explains any of the difference in functional
294 outcome between aflibercept and ranibizumab over bevacizumab is, however, unknown (Wells et al. 2016).

295
296 In addition, our study presents long term outcome on changes in vascular calibers, also in patients that did
297 not receive additional intravitreal therapy after loading. Interestingly, eyes without need for PRN treatment
298 demonstrated lasting venular constriction similar to eyes that continued intravitreal PRN therapy. While
299 retinal images at BL and M3 were captured prior to focal/grid laser photocoagulation, images at M12 were
300 captured afterwards. Hence, the measured changes in retinal microvasculature between BL and M3
301 represent the isolated effect of anti-VEGF, whereas the vessel analyses conducted on images captured at
302 M12 would be affected by both aflibercept and focal/grid laser photocoagulation.

303 Lundberg et al. demonstrated a narrowing of macular vessels after focal/grid laser
304 photocoagulation. The authors argued that the vessel constriction should be explained by a lowered
305 metabolic demand after destruction of photoreceptors and improved oxygenation by diffusion through
306 laser scars in accordance with the oxygen theory presented by Stefansson (Stefansson 2006). Likewise,
307 vasoconstriction has been readily demonstrated after panretinal photocoagulation and is thought to be
308 explained by the same theory. Thus, it can only be speculated whether the lasting venular constriction
309 demonstrated at M12 can be attributed to adjunctive use of focal/grid laser photocoagulation.

310 Also, our data does not allow us to test whether eyes without need for PRN treatment
311 demonstrated a more stable decrease in venular calibers during the course of this study as compared to
312 eyes with a continuous need for intravitreal therapy.

313
314 Large population-based studies have demonstrated that both retinal arteriolar and venular calibers
315 correlate with age in persons with type 2 diabetes. Thus Klein et al. demonstrated that the caliber of retinal
316 arterioles decrease by approximately 2.0 $\mu\text{m}/10$ years and for retinal venules by approximately 2.5 $\mu\text{m}/10$
317 years. Given the extend of vascular constriction as well as the relatively short period of follow-up in our

318 study, we do, thus, not believe the demonstrated decrease in both retinal arteriolar and venular caliber to
319 be a simple effect of time.

320

321 We only measured BMI, HbA1c and blood pressure at BL. Thus we cannot account for whether any these
322 parameters may have changed during the follow-up period and whether potential changes may be
323 different between the groups with and without need for PRN treatment. However, a previous study has
324 demonstrated that glycemic control does not improve even after major events in diabetes as e.g. stroke,
325 blindness or limb amputation (Jorgensen et al. 2009). We may, thus, anticipate that the same will apply in
326 our study even though the lack of repeated measurements is a potential limitation to our study.

327 Also, no sample size calculation for this specific study was conducted. Instead, sample size
328 was determined for the original randomized controlled trial which may be a limitation to this current study.
329 However, no relevant data could be identified in terms of estimating the expected effect size on retinal
330 microvascular parameters of the intervention described in this study. Consequently, this current study
331 should be considered as explorative.

332

333 Despite extensive knowledge about the associations between parameters of retinal microvascular structure
334 and ocular as well as systemic diseases, quantification of retinal microvasculature has currently very limited
335 use in clinical practice. This is largely due to difficulties with translation of results of cross-sectional studies
336 into clinical practice. Hence, the prospective design was a considerable strength of this study as well as the
337 well-characterized study population and pre-specified treatment algorithm. Furthermore, while previous
338 studies have predominantly addressed potential predictors of functional outcome in DME
339 treatment, this study is unique by addressing potential predictors of treatment load which is of great
340 interest from a clinical point of view. Thus, both distinct features of retinal morphology as determined by
341 OCT (disruption of retinal inner layers) as well as retinal oximetry parameters have been suggested as
342 predictors of functional outcome in DME treatment whereas no previous studies have addressed
343 parameters of retinal microvascular structures as predictors of treatment load (Radwan et al. 2015; Sun et
344 al. 2015; Bek & Jorgensen 2016). This study was, however, limited especially by the number of
345 participants. Furthermore, our study did not include an untreated control group which was, in our case, not
346 possible due to ethical reasons.

347

348 In conclusion, our results suggest that early changes in retinal arteriolar caliber are associated with an
349 increased treatment burden during the first year of DME-treatment. Further studies with larger populations
350 are needed to assess fluctuations in retinal vessel calibers between intravitreal injections.

351

352 **ACKNOWLEDGEMENT**

353 This study was supported by grants from the University of Southern Denmark, grants from Odense
354 University Hospital, grants from Fight for Sight Denmark, grants from The Synoptik Foundation and grants
355 from The Danish Diabetes Academy supported by the Novo Nordisk Foundation, during the conduct of the
356 study. The funding organizations had no involvement in study design, data collection, data analysis,
357 manuscript preparation and/or publication decision.

358 We thank all funding organizations for the financial support received for this study and the Department of
359 Ophthalmology, Odense University Hospital for providing the facilities and equipment necessary.

360

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459 **Fig. 1**
460 Illustration of vessel analysis using VAMPIRE web (Vessel Assessment and Measurement Platform for
461 Images of the Retina, Universities of Dundee and Edinburgh, UK). Vessel calibers are measured zone B (0.5-
462 1.0 disc diameters from the optic disc margin) and tortuosity, vessel density and fractal dimension in zone C
463 (0.5-2.0 disc diameters from the optic disc margin). Arterioles and venules are labelled in red and blue,
464 respectively, and segments excluded from analysis are labelled in white.
465
466

Table 1 Baseline characteristics of eyes with and without need for intravitreal aflibercept after laser

	Total (n=32)	PRN÷ (n=15)	PRN+ (n=17)	<i>p</i>
Demographics				
Age (years)	59.5 (57.7-64.3)	61.6 (55.7-67.5)	57.7 (51.6-63.9)	0.28
Sex (% women)	21.9	13.3	29.4	0.40
Duration of diabetes (years)	9.2 (4.6-13.7)	9.9 (2.7-17.0)	8.5 (3.3-13.7)	0.74
Hemoglobin A1c (mmol/mol)	59.8 (52.2-67.4)	60.9 (47.7-74.2)	58.9 (51.8-65.9)	0.76
Body mass index (kg/m ²)	29.2 (26.5-32.0)	29.6 (26.9-32.4)	28.9 (24.7-33.1)	0.74
MAP (mmHg)	101.9 (96.0-107.8)	101.5 (95.3-107.8)	102.2 (92.8-111.6)	0.90
BCVA (ETDRS letters)	71.7 (68.2-75.3)	73.3 (70.2-76.4)	70.4 (63.7-77.0)	0.44
CRT (μm)	387.3 (352.0-422.6)	344.6 (325.5-363.7)	424.9 (360.6-489.2)	0.03*
Microvascular parameters				
Caliber (μm)				
Arterioles	139.9 (133.3-146.5)	141.6 (131.5-151.7)	138.4 (129.6-147.1)	0.62
Venules	212.8 (206.1-219.4)	208.2 (202.8-213.7)	216.8 (205.4-228.3)	0.17
Torquosity				
Arterioles	-8.2 (-8.8-(-7.7))	-8.0 (-9.2-(-6.8))	-8.4 (-8.7-(-8.2))	0.48
Venules	-8.4 (-8.5-(-8.2))	-8.4 (-8.6-(-8.2))	-8.3 (-8.6-(-8.1))	0.58
Vessel density (pixels)				
Arterioles	3961.5 (3514.2-4408.8)	3953.9 (3426.5-4481.3)	3968.2 (3248.9-4687.6)	0.97
Venules	5532.1 (5108.2-5956.0)	5410.8 (4799.7-6021.9)	5639.1 (5069.6-6208.6)	0.58
Fractal dimension				
Arterioles+venules	1.374 (1.361-1.388)	1.369 (1.346-1.391)	1.379 (1.363-1.396)	0.46

*Indicates p-value<0.05 and calculated for the comparison between groups of treatment success and treatment failure.

PRN÷: eyes without need for additional intravitreal therapy (pro re nata) after loading, PRN+: eyes with need for additional intravitreal therapy (pro re nata) after loading, MAP: mean arterial blood pressure, BCVA: best corrected visual acuity, CRT: central retinal thickness.

Table 2 Changes in retinal microvasculature at three and twelve months follow-up in patients with, respectively, success and failure of DME-treatment with 3 monthly injections of 2.0 mg aflibercept and focal/grid laser photocoagulation.

Microvascular parameters		BL-M3			BL-M12		
		n	Δ -value	p_{β}	n	Δ -value	p_{β}
Caliber (μ m)							
Arterioles	PRN÷	15	-1.6 (-13.8-10.7)	0.79	15	-7.0 (-18.6-4.5)	0.22
	PRN+	15	-11.2 (-18.7-(-3.7))	0.005*	17	-11.5 (-22.2-(-0.8))	0.04*
	p_{α}		0.17			0.55	
Venules	PRN÷	15	-11.6 (-20.1-(-3.1))	0.01*	15	-11.0 (-22.1-(-0.19))	0.04*
	PRN+	15	-9.5 (-17.0-(-2.0))	0.01*	17	-15.6 (-24.0-(-7.2))	0.001*
	p_{α}		0.70			0.50	
Tortuosity							
Arterioles	PRN÷	15	1.5 (-0.3-3.2)	0.10	15	0.2 (-1.7-2.0)	0.86
	PRN+	15	1.6 (-0.1-3.3)	0.07	17	1.9 (-0.2-4.1)	0.07
	p_{α}		0.90			0.18	
Venules	PRN÷	15	-0.2 (-0.4-0.1)	0.14	15	0.5 (-0.8-1.7)	0.45
	PRN+	15	-0.2 (-0.4-0.0)	0.11	17	0.3 (-0.8-1.3)	0.57
	p_{α}		0.95			0.83	
Vessel density (pixels)							
Arterioles	PRN÷	15	-382.9 (-828.9-61.2)	0.09	15	17.3 (-572.9-607.4)	0.95
	PRN+	15	-367.3 (-720.7-(-13.9))	0.04*	17	-200.6 (-710.8-309.6)	0.42
	p_{α}		0.95			0.57	
Venules	PRN÷	15	-334.5 (-809.7-140.7)	0.16	15	-170.6 (-745.6-404.4)	0.55
	PRN+	15	-142.0 (-493.7-209.7)	0.41	17	-202.8 (-546.6-141.1)	0.24
	p_{α}		0.49			0.92	
Fractal Dimension							
Arterioles+venules	PRN÷	15	0.001 (-0.023-0.025)	0.91	15	0.010 (-0.010-0.034)	0.31
	PRN+	15	0.001 (-0.014-0.016)	0.85	17	-0.001 (-0.017-0.016)	0.92
	p_{α}		>0.99			0.37	

*Indicates p-value<0.05.

PRN÷: eyes without need for additional intravitreal therapy (pro re nata) after loading, PRN+: eyes with need for additional intravitreal therapy (pro re nata) after loading. BL: baseline, M3: month 3, M12: month 12, Δ : change from baseline to specified time for follow-up, p_{α} : difference between groups of treatment success and treatment failure, p_{β} : difference between BL and follow-up at M3 and M12, respectively.