Short-term effect on the ocular circulation induced by unilateral intravitreal injection of aflibercept in age-related maculopathy

Anna Sophie Mursch-Edlmayr, Nikolaus Luft, Dominika Podkowinski, Michael Ring, Leopold Schmetterer and Matthias Bolz

1Department for Ophthalmology, Kepler University Hospital, Johannes Kepler University, Linz, Austria
2University Eye Hospital, Ludwig-Maximilians-University, Munich, Germany
3Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore
4Department of Ophthalmology, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore
5Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Medical School, Singapore, Singapore
6Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria
7Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria

ABSTRACT.

Purpose: Intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) is the standard treatment for neovascular age-related macular degeneration (AMD). As VEGF is a physiological key player for regulating retinal vascular tone, questions have been raised whether the application of anti-VEGF could induce alterations in ocular perfusion.

Methods: The study included 20 eyes from 20 Caucasian patients with unilateral neovascular AMD and 20 fellow eyes. All eyes were treated with standard intravitreal injection of aflibercept (IVA). Measurements of blood flow at the optic nerve head (ONH) and the choroid were performed with laser speckle flowgraphy (LSFG). The intraocular pressure (IOP), systolic and diastolic blood pressure, heart rate, mean arterial pressure (MAP) and ocular perfusion pressure (OPP) were analysed. Measurements were performed at baseline and repeated immediately after the injection and 30 and 45 min later.

Results: Mean time between injection of aflibercept and first follow-up was 8:56/4:25 min. The injection led to significant rise in IOP. In the injected eyes, mean blur rate (MBR, i.e. a relative measure of perfusion and the main outcome parameter of LSFG) within the major vessels of the ONH as well as at the entire ONH region decreased significantly ($p < 0.001$). No change in MBR was observed in the fellow eye. Choroidal blood flow was maintained stable in both eyes.

Conclusion: Intravitreal injection of aflibercept (IVA) led to a short-term reduction in perfusion only in the treated eye. This was independent from IOP, indicating a direct pharmacological effect. No changes in choroidal perfusion were observed during the first 45 min after the injection.

Key words: aflibercept – age-related macular degeneration – anti-VEGF – laser speckle flowgraphy – ocular perfusion

Introduction

Age-related macular degeneration (AMD) is one of the main causes of irreversible visual loss worldwide (Bourne et al. 2018). Neovascular (exudative or wet) AMD is characterized by angiogenesis originating from the choroidal or, less frequently, retinal circulation (Gupta et al. 2017). Vascular endothelial growth factor (VEGF) is known as a key player of choroidal neovascularization and plays a significant role in the pathologic upregulation of chorioretinal vascular permeability (McTigue et al. 1999). With the introduction of anti-VEGF agents, targeted treatment strategies for AMD were realized in the last two decades and have become the first-line therapy for neovascular AMD (Zhang et al. 2017). Currently, three anti-VEGF drugs are available for intravitreal injection: ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA, USA); bevacizumab (Avastin; Genentech, Inc.); and aflibercept (Eylea; Bayer HealthCare, Inc., Leverkusen, Germany, Europe and Regeneron Pharmaceutical, Inc., New York, US). Both ranibizumab and bevacizumab inhibit solely VEGF-A (Ferrara et al. 2006; Selid et al. 2014). In contrast, aflibercept is a recombinant fusion protein that inhibits VEGF-A,
VEGF-B and placent al growth factor (Stewart 2012). All three anti-VEGF agents are to be administered by injection into the vitreous cavity. Thus, their effect is not only limited to the choroidal neovascularization (CNV) lesion in the macular region but the entire retina and optic nerve head (ONH) area are exposed to the drug. On a physiological level, VEGF acts as a vasodilator as it activates endothelial nitric oxide (NO) synthase, which produces the potent vasodilator NO (McTigue et al. 1999). Hence, it can be hypothesized that therapeutic VEGF inhibition might induce vasoconstriction in retinal and ONH vessels with a subsequent decrease in ocular perfusion. A range of studies have indicated that both bevazucizumab (Ferrara et al. 2006; Sharei et al. 2010; Sugiyama et al. 2010; Stewart 2012; Selid et al. 2014; Zhang et al. 2017) and ranibizumab (Papadopoulou et al. 2009; Enaida et al. 2010; Sacu et al. 2011; Mendrinos et al. 2013; Kunikata et al. 2014; Okamoto et al. 2015; Sugimoto et al. 2017) induce alterations in ocular perfusion with sustained vasoconstrictive effects reported for up to 1 year after administration of the drug. The understanding of potential short- and long-term vasoconstrictive side-effects with consequent chronic ocular hypoperfusion and potentially harmful structural or functional changes to the neurosensory retina and/or the ONH is yet to be established. Moreover, potential short-term alterations induced by intravitreal anti-VEGF agents must not be disregarded. Injection of ranibizumab into the vitreous has been shown to transiently increase the intraocular pressure (IOP), most pronounced in the first 3 min after injection with rapid decline (Sharei et al. 2010).

Laboratory research has indicated that aflibercept exhibits an almost 100 times greater binding affinity for VEGF compared to bevacizumab and ranibizumab as well as substantially prolonged binding activity (Aizawa et al. 2011). Hence, enhanced and sustained effects on ocular perfusion might be anticipated. Lately, reduced retrobulbar blood flow in the ophthalmic artery, central retinal artery and posterior ciliary artery was described 1 week after Intravitreal injection of aflibercept (IVA) (Gök & Kapti 2018). Significant vasoconstriction of the retinal arterioles was observed by another group following three monthly injections of aflibercept (Tetikoglu et al. 2018).

Laser speckle flowgraphy (LSFG) is a promising technique for the measurement of ocular perfusion. This method enables two-dimensional, noninvasive measurements of perfusion at the ONH, the retina and the choroid (CHOR) using the laser speckle phenomenon (Sugiyama et al. 2010). Laser speckle flowgraphy (LSFG) has been used to assess the influence of intravitreal injection of bevacizumab and ranibizumab on ocular perfusion in patients with diabetic retinopathy, retinal vein occlusion or central serous chorioretinopathy (Enaida et al. 2010; Kunikata et al. 2014; Okamoto et al. 2015; Nagasato et al. 2016; Sugimoto et al. 2017).

The purpose of this study was to characterize for the first time the short-term changes in ocular perfusion induced by IVA in eyes with neovascular AMD by means of LSFG.

Materials and Methods

Patients

This prospective interventional study included 20 eyes of 20 Caucasian adult patients with unilateral neovascular AMD and 20 fellow eyes (FE). Patients were recruited consecutively from the medical retina clinic of the Kepler University Hospital Linz, Austria. The study protocol was reviewed and approved by the local ethics committee (Ethikkommission des Landes Oberösterreich; registration number B-119-16) and followed the guidelines set forth in the Declaration of Helsinki. Written informed consent was obtained before inclusion in the study. All subjects underwent a comprehensive screening examination, including a slit-lamp examination with indirect funduscopy and measurement of IOP using the Goldmann applanation tonometry.

Best-corrected visual acuity (BCVA) was assessed using the Jackson cross-illuminated method and the standard Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart.

The inclusion criteria were (1) age >50 years and (2) patients scheduled for three consecutive intravitreal injections (4-week intervals) of aflibercept for treatment of exudative AMD in one eye. The exclusion criteria included (1) active exudative AMD requiring treatment of both eyes, (2) ocular surgery (including intravitreal injection) during the 3 months preceding the study, (3) vitrectomized eyes, (4) ametropia ≥6 Dpt and (5) any relevant ophthalmic diseases/conditions that could interfere with LSFG measurements (e.g. glaucoma, ONH drusen, tilted disc). Subjects were instructed to abstain from alcohol and stimulating beverages containing xanthine derivatives (e.g. tea, coffee) 12 hr before the LSFG measurements, as these are known to potentially influence the results (Okuno et al. 2000). Measurements were performed in a quiet, dark room with the subject in sitting position.

Baseline measurements

Al subjects were evaluated carefully prior to the injection. Performed examinations included ETDRS BCVA and the Goldmann applanation tonometry. Optical coherence tomography (Spectralis; Heidelberg Engineering, Heidelberg, Germany) was employed to measure central retinal thickness (CRT). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the upper arm with a manometer in the sitting position after a resting period of 5 min. The mean arterial pressure (MAP) was calculated as MAP = DBP + 1/3 (SBP – DBP), and the ocular perfusion pressure (OPP) as OPP = 2/3 MAP – IOP.

Intervention

After instillation of topical anaesthetic (0.4% oxybuprocaine hydrochloride and lidocaine; own production), sterilization of the eyelid (Betaisodona Lösung®, 11% povidone–iodine, Mundipharma, Limburg, Germany), and instillation of 1.25% povidone–iodine drops, 2.0 mg/0.05 ml of aflibercept (Eylea) was injected into the vitreous cavity through a standard pars plana approach (3.5 mm posterior to the limbus) under sterile conditions. Surgeons were asked to evaluate the amount of reflux (0 = no reflux, 1 = minimal reflux, 2 = moderate reflux, 3 = excessive reflux).

Laser speckle flowgraphy

Laser speckle flowgraphy measurements were performed with the LSFG RetFlow...
Table 1. Baseline characteristics of treated eyes (TEs) and fellow eyes (FEs) including biometric results (Student’s t-test).

| Characteristics | Male | Female | p     |
|-----------------|------|--------|-------|
| Sex             | 11   | 9      |       |
| Age (years)     | 75.1 | 8.2    |       |
| SBP (mmHg)      | 143.1| 18.8   |       |
| DBP (mmHg)      | 82.1 | 9.3    |       |
| HR (bpm)        | 71.7 | 9.8    |       |
| MAP (mmHg)      | 102.4| 10.6   |       |
| MRSE (dpt)      | -0.02| 2.11   | 0.967 |
| BCVA (LogMAR)   | 0.44 | 0.4    | <0.001|
| IOP (mmHg)      | 15.3 | 2.6    | 0.747 |
| OPP (mmHg)      | 15.0 | 2.3    |       |
| AL (mm)         | 53.0 | 7.2    | 0.94  |
| CRT (µm)        | 23.5 | 0.4    | 0.747 |

AL = axial length, BCVA = best-corrected visual acuity, Bpm = beats per minute, CRT = central retinal thickness, DBP = diastolic blood pressure, HR = heart rate, IOP = intraocular pressure, MRSE = manifest refraction spherical equivalent, OPP = ocular perfusion pressure, SBP = systolic blood pressure, SD = standard deviation.

* Marks indicate statistical significance.

Table 2. Changes in mean arterial pressure (MAP), intraocular pressure (IOP), ocular perfusion pressure (OPP) and mean blur rate at whole optic nerve head region (ONH-MA), at region of big vessels (ONH-MV) and at region of microvasculature (ONH-MT) and at mean blur rate at the choroid (CHOR).

|                     | 10 min – Baseline (%; mean ± SD) | 30 min – Baseline (%; mean ± SD) | 45 min – Baseline (%; mean ± SD) |
|---------------------|----------------------------------|----------------------------------|----------------------------------|
| MAP                 | 9.2 ± 7.4                        | 6.2 ± 13.4                       | 6.8 ± 7.0                        |
| IOP                 |                                  |                                 |                                 |
| TE                  | 26.2 ± 35.0                      | 5.1 ± 22.4                       | 0.0 ± 24.3                       |
| FE                  | 0.9 ± 18.1                       | 2.7 ± 19.1                       | -2.9 ± 22.7                      |
| OPP                 |                                  |                                 |                                 |
| TE                  | -4.5 ± 17.8                      | -0.5 ± 14.9                      | 1.0 ± 14.8                       |
| FE                  | 2.3 ± 12.0                       | -0.7 ± 13.0                      | 1.9 ± 11.7                       |
| ONH-MA              |                                  |                                 |                                 |
| TE                  | -10.47 ± 9.1                     | -16.3 ± 9.3                      | -18.7 ± 10.4                     |
| FE                  | 2.0 ± 10.6                       | -1.5 ± 15.6                      | -0.6 ± 12.8                      |
| ONH-MV              |                                  |                                 |                                 |
| TE                  | -14.1 ± 20.4                     | -23.2 ± 15.1                     | -27.4 ± 16.5                     |
| FE                  | -5.4 ± 13.1                      | -7.4 ± 20.5                      | -9.0 ± 17.7                      |
| ONH-MT              |                                  |                                 |                                 |
| TE                  | 8.1 ± 14.7                       | 6.5 ± 9.2                        | 3.4 ± 12.0                       |
| FE                  | 2.4 ± 14.3                       | -2.7 ± 18.8                      | -1.5 ± 17.4                      |
| CHOR                |                                  |                                 |                                 |
| TE                  | -3.8 ± 13.9                      | 3.8 ± 14.0                       | -4.5 ± 14.4                      |
| FE                  | 7.2 ± 10.9                       | 2.9 ± 17.4                       | -4.0 ± 19.4                      |
CRT was significantly larger in the TE group as compared to the FE group. In all TE, the intravitreal injection was successfully completed. No adverse events were observed during the injection or within the follow-up period. Mean time between injection and first LSFG measurement was 8:56/C6:4:25 min and ranged between 3:38 and 23:06 min.

Table 2 shows the calculated delta values between baseline and follow-up. A repeated-measures ANOVA with Bonferroni correction was conducted to analyse the effect of the intravitreal injection on the IOP, MAP, OPP and LSFG parameters (Table 3). In the TEs, there was a significant effect on IOP \[ F(1.957,37.187) = 7.911, p = 0.001, \text{partial } \eta^2 = 0.294 \]; ONH-MA \[ F(3,57) = 36.251, p < 0.001, \text{partial } \eta^2 = 0.656 \]; ONH-MV \[ F(2.214,57) = 25.542, p < 0.001, \text{partial } \eta^2 = 0.573 \]; and ONH-MT \[ F(3,57) = 2.906, p = 0.042, \text{partial } \eta^2 = 0.133 \]. No significant effects were observed on choroidal LSFG measurements \[ F(3,54) = 0.907, p = 0.44, \text{partial } \eta^2 = 0.048 \].

In the FE, no significant alterations in ONH or choroidal perfusion were observed (see Table 3).

Figure 1 demonstrates changes throughout follow-up of IOP, OPP and ONH-MA, ONH-MV, ONH-MT and CHOR. Changes in ONH-MA, ONH-MV, ONH-MT and CHOR showed no significant correlation with the change in IOP immediately after injection (ONH-MA \( r = -0.182, p = 0.443 \); ONH-MV \( r = -0.65, p = 0.786 \); ONH-MT \( r = 0.096, p = 0.688 \); CHOR \( r = 0.002, p = 0.993 \)).

In 50% (10 eyes) of the injections, no reflux was observed. In six cases, minimal reflux was observed (30%) and in four eyes moderate (20%). The amount of reflux did not correlate with the IOP 15 min after the injection (Pearson’s correlation, \( p = 0.677 \)).

Discussion

The present study is the first to evaluate the immediate influence of aflibercept on ocular perfusion in AMD. Our data indicate that IVA rapidly leads to reduced perfusion in the whole ONH (ONH-MA) and the central retinal vessels (ONH-MV) with no significant correlation to the short-term rise in IOP. Intraocular pressure (IOP) increased significantly in the TE after the injection but declined to baseline values after 30 min, whereas MA and MV were significantly reduced up to 60 min after IVA. Alteration of ONH-MT, a parameter for the perfusion of the microvasculature, showed borderline level of significance. An in vitro model has shown that aflibercept binds to VEGF with a maximum response after about 100 seconds (Sivertsen...
et al. 2018); thus, our results could indicate a direct pharmacological effect on the perfusion independent of the IOP increase. Previous studies using colour Doppler imaging on eyes with AMD showed that the retrobulbar circulation was decreased 1 week after a bevacizumab injection (Toklu et al. 2011). More recently, this was also shown for intravitreal aflibercept (Gok & Kapti 2018). Lately, another paper reported significant vasoconstriction of the retinal arterioles after three monthly injections of aflibercept using a computer-based approach (Tetikoglu et al. 2018).

Laser speckle flowgraphy (LSFG) data on ocular perfusion after anti-VEGF treatment were published for patients with macular oedema after branch retinal vein occlusion (BRVOME) or diabetic macular oedema (DME). Intravitreal injection of bevacizumab significantly decreased the blood flow in the ONH, retinal artery and vein and the CHOR in patients with DME 1 week and 1 month after injection (Kunikata et al. 2014).

Optic nerve head (ONH)-MA, ONH-MV and ONH-MT in the FE maintained stable throughout the whole follow-up of our protocol, indicating that aflibercept does not enter the systemic circulation, at least not within the first hour after intravitreal injection. This result is in agreement with results published on ranibizumab in patients with DME or BRVOME (Sugimoto et al. 2017).

Choroidal perfusion was maintained stable in both, the treated and the FE up to 1 hr after the injection. Studies on the blood flow autoregulation in the human CHOR have been published before. In agreement with these previous studies, our data indicate that the CHOR has some autoregulatory capacity in response to small changes in IOP (Riva et al. 1997; Simader et al. 2009; Schmidl et al. 2012). It has been also shown that choroidal autoregulation depends on pressure levels at both the arterial and the venous sides, indicating its complexity (Longo et al. 2004; Schmidl et al. 2016). In patients with DME, it has been shown that the choroidal MBR decreases significantly 1 week and 1 month after injecting bevacizumab (Kunikata et al. 2014). Whether this is related to the breakdown of autoregulation in patients with diabetes remains to be shown (Movaffagh et al. 2002).

However, choroidal thickness has lately been shown to decline in patients with AMD following three injections of aflibercept (Tetikoglu et al. 2018). As expected, we observed transient rise in IOP, but no correlation with the amount of reflux was shown. It has been previously speculated that reflux led to decreased IOP rise; however, other studies did not quantify reflux (Sharei et al. 2010; Lemos-Reis et al. 2014).

Our study was limited by a small sample size. Also, due to logistic factors as well as patient factors, in many cases we did not manage to perform the LSFG measurements within 5 min after the injection. However, statistical results indicate significant differences with high power.

In conclusion, this study adds information about the short-term effects of aflibercept on ocular perfusion. Results indicate that aflibercept has an immediate direct and significant effect on the retinal perfusion of the TE but not on that of the FE. Choroidal blood flow on the other side is not significantly altered in the treated or in the FE up to 1 hr after IVA.

References

Aizawa N, Yokoyama Y, Chiba N et al. (2011): Reproducibility of retinal circulation measurements obtained using laser speckle flowgraphy-NAVI in patients with glaucoma. Clin Ophthalmol 5: 1171–1176.

Bourne RRA, Jonas JB, Bron AM et al. (2018): Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe in 2015: magnitude, temporal trends and projections. Br J Ophthalmol 102: 575–585.

Enaide H, Okamoto K, Fujii H & Ishibashi T (2010): LSFG findings of proliferative diabetic retinopathy after intravitreal injection of bevacizumab. Ophthalmic Surg Lasers Imaging 41 Online: e1–e3.

Ferrara N, Damico L, Shams N, Lowman H & Kim R (2006): Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. Structure 14: 119–130.

Gok M & Kapti HB (2018): Effect of intravitreal aflibercept (Eylea®) on retrobulbar hemodynamics in patients with neovascular age-related macular degeneration. Int Ophthalmol 38: 713–719.

Gupta P, Ting DSW, Thakku SG et al. (2017): Detailed characterization of choroidal morphologic and vascular features in age-related macular degeneration and polypoidal choroidal vasculopathy. Retina 37: 2269–2280.

Kunikata H, Nitta F, Aizawa N, Omodaka K, Shiga Y, Yasuda M & Nakazawa T (2014): The effect of intravitreal bevacizumab on ocular blood flow in diabetic retinopathy and branch retinal vein occlusion as measured by laser speckle flowgraphy. Clin Ophthalmol 8: 1119–1127.

Lemos-Reis R, Moreira-Gonzalves N, Melo A, Carneiro A & Falcão-Reis F (2014): Immediate effect of intravitreal injection of bevacizumab on intraocular pressure. Clin Ophthalmol 23: 1383–1388.

Longo A, Geiser MH & Riva CE (2004): Posture changes and subfoveal choroidal blood flow. Invest Ophthalmol Vis Sci 45: 546–551.

Luft N, Wozniak PA, Aschinger GC et al. (2016): Ocular blood flow measurements in healthy white subjects using laser speckle flowgraphy. PLoS ONE 11: e0168190.

McTigue MA, Wickersham JA, Pinko C et al. (1999): Crystal structure of the kinase domain of human vascular endothelial growth factor receptor 2: a key enzyme in angiogenesis. Structure 7: 319–330.

Mendrinos E, Mangioris G, Papadopoulo DN, Donati G & Pournaras CJ (2013): Long-term results of the effect of intravitreal ranibizumab on the retinal arteriolar diameter in patients with neovascular age-related macular degeneration. Acta Ophthalmol 91: 184–190.

Movaffagh A, Chamot SR, Dozzo A, Pournaras CJ, Sommerhalder JR & Riva CE (2002): Effect of isometric exercise on choroidal blood flow in type I diabetic patients. Klin Monbl Augenheilkd 219: 299–301.

Nagassato D, Mitamura Y, Sembu K, Akaiwa K, Nagasawa T, Yoshizumi Y, Tabuchi H & Kiiuchi Y (2016): Correlation between optic nerve head circulation and visual function before and after anti-VEGF therapy for central retinal vein occlusion: prospective, interventional case series. BMC Ophthalmol 16: 1–10.

Okamoto M, Matsuura T & Ogata N (2015): Choroidal thickness and choroidal blood flow after intravitreal bevacizumab injection in eyes with central serous chorioretinopathy. Ophthalmic Surg Lasers Imaging Retina 46: 25–32.

Okuno T, Sugiyama T, Tominaga M, Kojima S & Ikeda T (2000): Effects of caffeine on microcirculation of the human ocular fundus. Jpn J Ophthalmol 46: 170–176.

Papadopoulo DN, Mendrinos E, Mangioris G, Donati G & Pournaras CJ (2009): Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. Ophthalmology 116: 1755–1761.

Riva CE, Titz P, Hero M & Petrig BL (1997): Effect of acute decreases of perfusion pressure on choroidal blood flow in humans. Invest Ophthalmol Vis Sci 38: 1752–1760.

Sauci S, Pemp B, Weigert G, Matt G, Garhofer G, Prunte C, Schmetterer L & Schmidt-Erfurth U (2011): Response of retinal vessels and retrobulbar hemodynamics to...
intravitreal anti-vegf treatment in eyes with branch retinal vein occlusion. Invest Ophthalmol Vis Sci 52: 3046–3050.

Schmidl D, Boltz A, Kaya S et al. (2012): Comparison of choroidal and optic nerve head blood flow regulation during changes in ocular perfusion pressure. Invest Ophthalmol Vis Sci 53: 4337–4346.

Schmidl D, Schmetterer L, Witkowska KJ, Rauch A, Werkmeister RM, Garhofer G & Popa-Cherecheanu A (2016): Factors associated with choroidal blood flow regulation in healthy young subjects. Invest Ophthalmol Vis Sci 57: 5705.

Selig PD, Jundt MC, Fortney AC & Beal JR (2014): Intravitreal bevacizumab and aflibercept for the treatment of exudative age-related macular degeneration. Ophthalmic Surg Lasers Imaging Retina 45: 275–281.

Sharei V, Höhn F, Hattenbach L-O & Mirshahi A (2010): Course of intraocular pressure after intravitreal injection of 0.005 mL ranibizumab (Lucentis). Eur J Ophthalmol 20: 174–179.

Simader C, Lung S, Weigert G, Kolodjaschna J, Fuchsjäger-Mayrl G, Schmetterer L & Polska E (2009): Role of NO in the control of choroidal blood flow during a decrease in ocular perfusion pressure. Invest Ophthalmol Vis Sci 50: 372–377.

Sivertsen MS, Jørstad ØK, Grevys A, Foss S, Moe MC & Andersen JT (2018): Pharmaceutical compounding of aflibercept in pre-filled syringes does not affect structural integrity, stability or VEGF and Fc binding properties. Sci Rep 8: 1–9.

Stewart MW (2012): Aflibercept (VEGF trap-eye): the newest anti-VEGF drug. Br J Ophthalmol 96: 1157–1158.

Sugimoto M, Nunome T, Sakamoto R, Kobayashi M & Kondo M (2017): Effect of intravitreal ranibizumab on the ocular circulation of the untreated fellow eye. Graefes Arch Clin Exp Ophthalmol 255: 1543–1550.

Sugiyama T, Araie M, Riva CE, Schmetterer L & Orgul S (2010): Use of laser speckle flowgraphy in ocular blood flow research. Acta Ophthalmol 88: 723–729.

Tetikoglu M, Kurt M, Sagdısk H, Aktas S, Yildirim M & Ozeura F (2018): Retrospective analysis of the effect of aflibercept loading dose on the retinal vessel diameters in patients with treatment-naive neovascular AMD. Cutan Ocul Toxicol 37: 84–89.

Toklu Y, Cakmak HB, Raza S, Anayol A & Asik E (2011): Short-term effects of intravitreal bevacizumab (Avastin) on retrobulbar hemodynamics in patients with neovascular age-related macular degeneration. Acta Ophthalmol 89: 41–45.

Zhang Y, Chioreso C, Schweizer ML & Abramoff MD (2017): Effects of aflibercept for neovascular age-related macular degeneration: a systematic review and meta-analysis of observational comparative studies. Invest Ophthalmol Vis Sci 58: 5616–5627.

Received on August 13th, 2018.
Accepted on March 3rd, 2019.

Correspondence:
Matthias Bolz
Department of Ophthalmology
Kepler University Hospital
Krankenhausstraße 9
4020 Linz
Austria
Tel: +43576808378414
Fax: +4357680741945
Email: matthias.bolz@kepleruniklinikum.at