



**HAL**  
open science

## **Intraocular pressure changes and vascular endothelial growth factor inhibitor use in various retinal diseases: long-term outcomes in routine clinical practice: data from the fight retinal blindness! Registry**

Pierre-Henry Gabrielle, Vuong Nguyen, Benjamin Wolff, Rohan Essex, Stephanie Young, Adrian Hunt, Chui Ming Gemmy Cheung, Jennifer Arnold, Daniel Barthelmes, Catherine Creuzot-Garcher, et al.

### ► **To cite this version:**

Pierre-Henry Gabrielle, Vuong Nguyen, Benjamin Wolff, Rohan Essex, Stephanie Young, et al.. Intraocular pressure changes and vascular endothelial growth factor inhibitor use in various retinal diseases: long-term outcomes in routine clinical practice: data from the fight retinal blindness! Registry. *Ophthalmology Retina*, 2020, 4 (9), pp.861-870. 10.1016/j.oret.2020.06.020 . hal-02908021

**HAL Id: hal-02908021**

**<https://hal.inrae.fr/hal-02908021>**

Submitted on 5 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Intraocular Pressure Changes and VEGF Inhibitor Use in Various  
2 Retinal Diseases: Long-Term Outcomes in Routine Clinical  
3 Practice: Data from the Fight Retinal Blindness! Registry  
4

5 Pierre-Henry Gabrielle, MD<sup>1, 2</sup>; Vuong Nguyen, PhD<sup>2</sup>; Benjamin Wolff, MD<sup>3</sup>; Rohan  
6 Essex, MBBS<sup>4</sup>; Stephanie Young, MBBS<sup>5</sup>; Adrian Hunt, MBBS<sup>2</sup>; Chui Ming Gemmy  
7 Cheung, MBBS<sup>6</sup>; Jennifer J Arnold, MBBS<sup>7</sup>; Daniel Barthelmes, MD, PhD<sup>2, 8</sup>; Catherine  
8 Creuzot-Garcher, MD, PhD<sup>1</sup>; Mark Gillies, MBBS, PhD<sup>2</sup> for the Fight Retinal Blindness!  
9 Study Group

10 <sup>1</sup> Department of Ophthalmology, Dijon University Hospital, Dijon, France

11 <sup>2</sup> The University of Sydney, Sydney Medical School, Discipline of Ophthalmology, Save  
12 Sight Institute, New South Wales, Australia

13 <sup>3</sup> Private Clinic, Centre Ophtalmologique Maison Rouge, Strasbourg, France

14 <sup>4</sup> Academic Unit of Ophthalmology, Australian National University, Acton, Australian  
15 Capital Territory, Australia

16 <sup>5</sup> Gladesville Eye Specialists, New South Wales Australia

17 <sup>6</sup> Singapore National Eye Centre, Singapore, Singapore

18 <sup>7</sup> Marsden Eye Research, Sydney, Australia

19 <sup>8</sup> Department of Ophthalmology, University Hospital Zurich, University of Zurich, Zurich,  
20 Switzerland

21

22 **Financial Support:** The Fight Retinal Blindness! Project is supported by a grant from the  
23 Macular Disease Foundation Australia and unrestricted educational grants from Bayer and  
24 Novartis.

25 **Conflict of interest:** Gillies and Barthelmes are inventors of the software used to collect the  
26 data for this analysis

27 **Running head:** Intraocular Pressure Changes and VEGF Inhibitors in Retinal Diseases  
28

28

29 **Corresponding Author/Address for Reprints:**

30 Pierre-Henry Gabrielle, MSc, MD, FEBO

31 **Phone:** +61431360231

32 **Email:** phgabrielle@gmail.com  
33

33

34 This article contains additional online-only material. The following should appear online-  
35 only: Figures S1, S2 and S3, Tables S1 and S2 and the acknowledgements.  
36

36

37 **Abstract**

38 **Purpose:** To report long-term changes in intraocular pressure (IOP) in eyes receiving  
39 vascular endothelial growth factor (VEGF) inhibitors for various retinal conditions over  
40 12- and 24-months in routine clinical practice.

41 **Design:** Retrospective analysis of data from a prospectively designed observational  
42 outcomes registry: the Fight Retinal Blindness! Project.

43 **Participants:** Treatment-naïve eyes receiving monotherapy with VEGF inhibitors  
44 (ranibizumab [0.5mg], aflibercept [2mg] or bevacizumab [1 mg]) with at least 3  
45 injections, from December 2013 to 31 December 2018 and at least 12 months of follow-  
46 up.

47 **Methods:** IOP was measured at each clinical visit for all eyes as part of routine  
48 practice.

49 **Main Outcome measures:** The primary outcome was the mean change in IOP (mmHg)  
50 at 12-months. The following secondary IOP outcome measures were investigated at 12  
51 and 24 months: (1) mean change in IOP from baseline and (2) proportion of clinically  
52 significant IOP increase defined as an elevation of at least 6 mmHg to an IOP of more  
53 than 21 mmHg at any point during the follow-up.

54 **Results:** We identified 3429 treatment-naïve eyes (395 bevacizumab, 1138 aflibercept  
55 and 1896 ranibizumab) with complete IOP data from 3032 patients with 12 months of  
56 follow-up data, of which 2125 (62%) had 24 months of follow-up data. The overall mean  
57 [95%CI] IOP change was -0.5 [-0.6, -0.3] mmHg at 12 months and -0.4 [-0.6, -0.3]  
58 mmHg at 24 months while the proportion of clinically significant IOP increases were  
59 5.6% and 8.8%. A lower mean IOP change and fewer IOP elevations at 12- and 24-

60 months was observed in eyes receiving aflibercept than in those receiving bevacizumab  
61 and ranibizumab (for both comparison  $P \leq 0.01$  at each time point and outcomes). Eyes  
62 with pre-existing glaucoma had more IOP increases over 12- and 24-months (OR = 2.2  
63 [1.2, 3.8],  $P = 0.012$  and OR = 2.1 [1.1, 3.8],  $P = 0.025$ , respectively).

64 **Conclusions:** Mean IOP did not change significantly from baseline to 12 and 24  
65 months in eyes receiving VEGF inhibitors, while clinically significant IOP elevations  
66 occurred in a small proportion of eyes. Aflibercept was associated with fewer clinically  
67 significant IOP elevations, whereas eyes with pre-existing glaucoma were at a higher  
68 risk.

## 69 **Introduction**

70 Despite the widespread use of vascular endothelial growth factor (VEGF) inhibitors for  
71 retinal conditions, such as neovascular age-related macular degeneration (AMD),  
72 diabetic macular edema (DME) and macular edema (ME) secondary to retinal vein  
73 occlusion (RVO), there are still only limited data on their effect on IOP. It is well known  
74 that there is a transient spike in intraocular pressure (IOP) immediately after an injection  
75 but this quickly decreases over the next 15 minutes for most patients.<sup>1-3</sup> The degree to  
76 which anti-VEGF agent injections contribute to long-term IOP changes is less clear. A  
77 large real-world study using the Intelligent Research in Sight (IRIS) registry reported a  
78 slight but significant decrease in IOP from baseline in eyes treated with anti-VEGF  
79 agents for more than one year compared with control eyes.<sup>4</sup> Moreover, 2.6% of the  
80 treated patients had clinically significant, sustained IOP elevations, confirming the  
81 results of previous studies.<sup>5-8</sup> Somewhat surprisingly, there were fewer cases of  
82 sustained IOP elevations in eyes treated with aflibercept (1.9%) than with those  
83 receiving ranibizumab (2.8%) or bevacizumab (2.8%).<sup>4,5</sup> A recent review highlighted the  
84 conflicting reports with regards to IOP changes and the risk factors for IOP rises with  
85 intravitreal injections of VEGF inhibitors, which may be partially due to variability in  
86 methodology and the definition of clinically significant IOP increase between studies.<sup>9</sup>  
87 More data are needed on long-term IOP change outcomes and the potential risk factors  
88 associated with IOP change in patients treated with VEGF inhibitors. This study aimed  
89 to explore changes in IOP in eyes receiving VEGF inhibitors for various retinal  
90 conditions over 12- and 24-months in routine clinical practice.

## 92 **Methods**

### 93 **Design and setting**

94 This was a retrospective analysis of treatment-naïve eyes that had received intravitreal  
95 anti-VEGF agents for various retinal diseases in routine clinical practice tracked in the  
96 prospectively designed observational database – The Fight Retinal Blindness!  
97 Registry.<sup>10</sup> Participants in this analysis included patients from practices in Australia,  
98 France, New Zealand, Singapore and Switzerland. Institutional approval was obtained  
99 from the Royal Australian and New Zealand College of Ophthalmologists Human  
100 Research Ethics Committee, the Southern Eastern Sydney Local Health District Human  
101 Research Ethics Committee, the French Institutional Review Board (IRB) (Société  
102 Française d’Ophtalmologie IRB), the SingHealth Singapore and the Cantonal Ethics  
103 Committee Zurich. All patients gave their informed consent. Informed consent (“opt-in  
104 consent”) was sought from patients in France, Singapore and Switzerland. Ethics  
105 committees in Australia and New Zealand approved the use of “opt-out” patient consent.  
106 This study adhered to the tenets of the Declaration of Helsinki and followed the  
107 STROBE statements for reporting observational studies.<sup>11</sup>

### 108 **Data Sources and Measurements**

109 The Fight Retinal Blindness! Registry has several modules that collect data from eyes  
110 being treated for neovascular AMD, DME and ME secondary to RVO.<sup>10</sup> Data were  
111 obtained prospectively from each clinical visit including IOP measurement (mmHg),  
112 treatment given, if any, and ocular adverse events. Demographic characteristics (age  
113 and gender), initial diagnosis (AMD, DME or RVO), history of any ocular condition (pre-

114 existing glaucoma status), and whether the eye received prior treatment (cataract  
115 surgery and vitrectomy) were recorded at baseline visit. Treatment decisions, including  
116 the choice of drug and injection frequency, were at the discretion of the physician in  
117 consultation with the patient, thereby reflecting real-world practice. Data on glaucoma  
118 treatment during follow-up such as introduction of IOP-lowering drop, laser or incisional  
119 glaucoma surgery were not systematically recorded in any of the three modules.

## 120 **Patient Selection and Groups**

121 Treatment-naïve eyes that received intravitreal monotherapy of VEGF inhibitors (eyes  
122 were excluded if switched or received any other type of intravitreal treatment) with either  
123 aflibercept (2mg Eylea, Regeneron Inc/Bayer), bevacizumab (1.25mg Avastin,  
124 Genetech Inc/Roche) or ranibizumab (0.5mg Lucentis, Genetech Inc/Novartis) for  
125 neovascular AMD, DME or ME secondary to RVO with a minimum of 3 injections and at  
126 least one year of follow-up including IOP measurement at least at baseline and 12-  
127 months after starting treatment from 1 December 2013 to 31 December 2018 were  
128 studied.

129 Prefilled syringes for administering ranibizumab were approved in early 2015 and global  
130 uptake of these prefilled syringes amongst clinicians using ranibizumab increased  
131 gradually over time. Prefilled packing was generally adopted in early 2017. To analyze  
132 the effect of prefilled vs. non-prefilled syringes on IOP, we analyzed the subset of eyes  
133 initially treated with intravitreal ranibizumab injection from 1 January 2017 (*prefilled*  
134 *group*) with those initially treated with intravitreal ranibizumab injection before 1 January

135 2014 and 1 January 2013 (*non-prefilled group*), thereby allowing 12 and 24 months of  
136 follow-up before the start of prefilled packaging, respectively.

137 Patients were also grouped by the total number of injections received: low (3-4 or 3-9  
138 injections), medium (5-8 or 10-14 injections) and high ( $\geq 10$  or  $\geq 15$  injections) during the  
139 12- and 24-month follow-up periods, respectively. Eyes with pre-existing glaucoma were  
140 identified at baseline.

## 141 **Outcomes**

142 The primary outcome was the adjusted mean change in IOP from baseline to 12 months  
143 using a regression model. Secondary outcomes were the proportion of eyes with  
144 clinically significant IOP elevation (defined as an IOP increase of at least 6 mmHg from  
145 baseline and resulting in an IOP of  $>21$  mmHg in a single event), and predictors of  
146 change in IOP and rate of clinically significant IOP elevations during 12- and 24-month  
147 follow-up (type of anti-VEGF agent, type of prefilled /non prefilled ranibizumab, number  
148 of injections, initial diagnosis, pre-existing glaucoma). Outcomes were analyzed by anti-  
149 VEGF agent, number of injections, prefilled/non-prefilled ranibizumab and pre-existing  
150 glaucoma status groups as defined above.

## 151 **Statistical analysis**

152 Descriptive data were summarized using the mean, standard deviation, median, first  
153 and third quartiles, and percentages where appropriate. Demographic characteristics  
154 were compared between anti-VEGF groups, prefilled groups, and glaucoma groups  
155 using ANOVA, Kruskal-Wallis test, t-tests, Wilcoxon rank sum tests, Chi-square tests, or



156 Fisher's exact tests where appropriate. The mean changes in IOP between subgroups  
157 were compared using linear mixed-effects regression models. The proportion of eyes  
158 with a clinically significant elevation in IOP was analyzed using logistic mixed-effects  
159 regression. The main predictors investigated were type of anti-VEGF agents, type of  
160 ranibizumab prefilled/non-prefilled syringes, number of injections, initial diagnosis, pre-  
161 existing glaucoma. Regression was adjusted for age, gender, baseline IOP, lens status,  
162 and cataract extraction during the follow-up as fixed effects, and nesting of outcomes  
163 within practitioners and patients with bilateral disease as random effects. Vitrectomy  
164 during the follow-up was not included in the model due to a very low incidence in our  
165 cohort. Since it was not mandatory to record IOP in the FRB registry, there was a  
166 possibility of bias due to selective reporting of IOP in high risk patients. A sensitivity  
167 analysis was done on the cohort of eyes followed by physicians who entered IOP  
168 measurement at least 50% of the time during follow-up visits.

169 A *P*-value of 0.05 was considered statistically significant. *P*-values from pairwise  
170 comparisons between anti-VEGF groups were adjusted for using the Holm-Bonferroni  
171 correction method. All analyses were conducted using R software version 3.5.3  
172 (<http://www.R-project.org/>) with the *glmmTMB* package (V0.2.3) for linear mixed-effects  
173 and generalized linear mixed-effects regression and the *emmeans* package (V1.3.3) for  
174 pairwise comparison of adjusted means.

## 175 **Results**

### 176 **Study participants**

177 A total of 3429 treatment-naïve eyes (395 bevacizumab, 1138 aflibercept and 1896  
178 ranibizumab) from 3032 patients who received intravitreal monotherapy of VEGF  
179 inhibitors for neovascular AMD, DME or ME secondary to RVO with 12 months of IOP  
180 follow-up data after starting treatment from 1 December 2013 to 31 December 2018  
181 were identified (from an overall number of 10382 treatment naïve eyes entered in the  
182 registry that received anti-VEGF injections from the selected countries involved in this  
183 study), of which 2125 (62%) had 24 months of follow-up data. The mean (SD) age  
184 overall was 78.1 (10.4) years with 58.7% of women. There were 2901(84.6%) eyes  
185 treated for neovascular AMD, 221 (6.4%) eyes for DME and 307 (9%) for ME secondary  
186 to RVO. Table 1 summarizes the baseline characteristics in each of the groups. Eyes  
187 receiving ranibizumab were significantly older than those receiving aflibercept and  
188 bevacizumab (mean 74.3 vs. 76.4 vs. 79.9 years for bevacizumab, aflibercept and  
189 ranibizumab, respectively;  $P < 0.01$ ) and had a higher proportion of female patients  
190 (51.3% vs. 54.8% vs. 63.0% for bevacizumab, aflibercept and ranibizumab,  
191 respectively;  $P < 0.01$ ). The overall proportions of eyes in each group of total number of  
192 injections received were 10.2% (350 eyes) and 8.2% (175 eyes) in the low group, 46%  
193 (1578) and 44.8% (951) in the medium group and 43.8% (1501) and 47% (999) in the  
194 high group at 12 and 24 months of treatment, respectively. The proportion of eyes that  
195 had cataract surgery was 3.7% (107 eyes) and 6.3% (181 eyes) over 12- and 24-  
196 months of follow-up.

### 197 **Intraocular pressure change outcomes**

198 Baseline mean (SD) IOP was 14.4 (3.8) mmHg overall, 14.9 (3.6) for bevacizumab, 14.4  
199 (4.0) for aflibercept, and 14.4 (3.6) for ranibizumab. The overall mean [95%CI] IOP  
200 change at 12- and 24-months was -0.5 [-0.6, -0.3] mmHg ( $P < 0.01$ ) and -0.4 [-0.6, -0.3]  
201 mmHg ( $P < 0.01$ ), respectively. Figure 1 reports the adjusted mean IOP change at 12-  
202 and 24-months by type of VEGF inhibitors, number of injections, initial diagnosis and  
203 pre-existing glaucoma status. Eyes receiving aflibercept had significantly greater IOP  
204 reduction (adjusted mean [95% CI]) at 12- and 24-months (-1.3 [-1.6, -1.1] and -1.3 [-  
205 1.7, -1.0] mmHg, respectively) than bevacizumab (0.0 [-0.4, 0.4],  $P < 0.01$  and 0.0 [-0.6,  
206 0.6],  $P < 0.01$ , respectively) and ranibizumab (0.0 [-0.2, 0.2],  $P < 0.01$  and -0.1 [-0.4,  
207 0.2],  $P < 0.01$ , respectively). There was no difference between bevacizumab and  
208 ranibizumab at 12 and 24 months. There was also a significant trend for eyes with  
209 neovascular AMD (-0.5 [-0.7, 0.3]) and pre-existing glaucoma (-1 [-1.5, 0.4]) to be  
210 associated with a greater adjusted mean reduction in IOP at 12 months than eyes with  
211 RVO (+0.2 [-0.3, 0.6],  $P = 0.010$ ) or pre-existing glaucoma (-0.4 [-0.3, 0.6],  $P = 0.043$ ),  
212 respectively.

### 213 **Clinically significant intraocular pressure elevation outcomes**

214 The overall proportion of clinically significant IOP elevations was 5.6% (193 eyes) at 12  
215 months and 8.8% (186 eyes) at 24 months. Figure 2 describes the adjusted proportion  
216 of clinically significant IOP elevations during 12 and 24 months of follow-up by type of  
217 VEGF inhibitors, number of injections, initial diagnosis and pre-existing glaucoma  
218 status. Clinically significant IOP elevations during 12 and 24 months follow-up were less  
219 likely to occur in aflibercept-treated eyes (reference subgroup) than eyes treated with  
220 bevacizumab (OR = 2.2 [1.2, 4.0],  $P = 0.015$  and OR = 2.7 [1.4, 5.0],  $P < 0.01$ ,

221 respectively) or ranibizumab (OR = 2.8 [1.8, 4.1],  $P < 0.01$  and OR = 2.6 [1.7, 4.0],  $P <$   
222 0.01, respectively) (Table 2). The rates of clinically significant elevated IOP were 2.4%  
223 and 3.9% in the aflibercept group, 5.2% and 9.7% in the bevacizumab group and 6.4%  
224 and 9.4% in the ranibizumab group during 12- and 24-months follow-up, respectively  
225 (Figure 2). RVO treated eyes tended to be significantly more at risk of IOP elevations  
226 during the first 12 months of follow-up than eyes treated for AMD (OR = 1.9 [1.1, 3.1],  $P$   
227 = 0.039), though this association was not statistically significant at 24 months.  
228 Glaucomatous eyes were more likely to have an IOP elevation during both 12- and 24-  
229 months follow-up (OR = 2.2 [1.2, 3.8],  $P = 0.012$  and OR = 2.1 [1.1, 3.8],  $P = 0.025$ ,  
230 respectively) with an adjusted rate of 2.1% and 11.0% when treated with aflibercept,  
231 14.1% and 23% with bevacizumab, and 14.7% and 23.0% with ranibizumab. There was  
232 a trend for an increasing rate of IOP elevations with more injections during both one and  
233 two-years of follow-up, however, this was not statistically significant (Figure 2).

#### 234 **Intraocular pressure change outcomes by type of ranibizumab syringe**

235 There was no statistically significant difference in the adjusted mean (95% CI) IOP  
236 change at 12- and 24-months between the two types of ranibizumab syringe (-0.1 [-0.4,  
237 0.2] and -0.2 [-0.7, 0.4] for non-prefilled syringe vs. 0.1 [-0.3, 0.5] and 0.7 [-0.3, 1.6] for  
238 prefilled syringe,  $P = 0.34$  and  $P = 0.11$ , respectively) (Figure 3A and 3B). Nor did we  
239 find a significant association with the type of syringe and clinically significant IOP  
240 elevation (Table 2; Figure 3C). There was no significant variation at 12 and 24 months  
241 in the adjusted mean IOP change from baseline ( $P = 0.39$  and  $0.87$ , respectively) and  
242 rate of IOP elevations ( $P = 0.81$  and  $0.75$ , respectively) by year of treatment initiation.

243

## 244 **Discussion**

245 We used the FRB! observational outcomes database to explore the effect of intravitreal  
246 VEGF inhibitors on long-term IOP change in treatment naïve eyes for various exudative  
247 retinal diseases in routine clinical practice. The overall mean IOP at 12- and 24-months  
248 slightly decreased in our cohort, which corroborates recent results.<sup>4,8</sup> Interestingly, eyes  
249 treated with aflibercept had significantly greater adjusted mean reduction in IOP than  
250 bevacizumab and ranibizumab at 12 and 24 months. This finding was previously  
251 reported in a secondary analysis of intraocular pressure outcomes in the VIEW 1 and 2  
252 study that compared aflibercept (2mg q4, 2mg q8, 0.5mg q4) with ranibizumab (0.3mg  
253 q4) for neovascular AMD.<sup>5</sup> This statistically significant difference seen in our study is  
254 minor and may not be clinically relevant.

255         The outcomes of clinically significant IOP elevation (increase of at least 6 mmHg  
256 from baseline and resulting in an IOP of > 21 mmHg in a single event) are of more  
257 clinical interest. Our overall rate of 5.6% at 12 months is higher than the 2.8% rate of  
258 elevated IOP of the IRIS registry study and 3.4% by the study of Adelman *et al.*<sup>4,7</sup> This  
259 difference may be explained by the stricter definition of IOP elevation (proportion of  
260 eyes with a baseline IOP ≤ 21 mmHg that had an IOP rise of at least 6 mmHg which  
261 resulted in an IOP greater than 21 mmHg at 2 consecutive visits) for the former study  
262 and the exclusion of glaucoma patients at baseline for the latter, who are possibly more  
263 at risk of IOP elevations. An exploratory *ad hoc* analysis of DRCRnet studies assessing  
264 IOP change between ranibizumab versus focal/grid laser for DME through 3 years  
265 found similar rates of IOP elevations with 5.7% at one year and a cumulative incidence  
266 of 9.5% at 3 years in the ranibizumab group, though their definition was also different

267 (same criteria at 2 consecutive visits or initiation/augmentation of IOP-lowering drug).  
268 The rate of elevated IOP reported in VIEW 1 and 2, defined as IOP > 21 mmHg at 2  
269 consecutive visits, was in keeping with our results with 5.2% vs. 2.5 % vs. 2.4% vs.  
270 1.5% at one year and 8.4% vs 3.2% vs. 4.2% vs. 2.7% at 2 years for ranibizumab,  
271 aflibercept 2q4, 2q8 and 0.5q4, respectively.<sup>5</sup>

272 The pathophysiological process underlying sustained IOP elevations following  
273 treatment with VEGF inhibitors has not been identified, although several etiologies have  
274 been proposed. It may be related to a decrease in aqueous outflow by chronic  
275 mechanical damage to the trabecular meshwork from repeated injection-related IOP  
276 spikes or direct toxicity of VEGF inhibitors itself or obstruction due to accumulation of  
277 protein aggregates or silicone droplets, or even trabecular meshwork constriction  
278 mediated by inhibition of nitric oxide synthesis.<sup>6,12-14</sup>

279 We found that aflibercept-treated eyes were consistently less likely to have IOP  
280 elevations than bevacizumab- and ranibizumab-treated eyes during the follow-up, which  
281 is consistent with the analyses in VIEW 1 and 2.<sup>5</sup> Freund *et al.* proposed that  
282 accumulation of protein aggregates might be greater with ranibizumab compared to  
283 aflibercept or that repeated ranibizumab injections lead to progressive trabeculitis  
284 secondary to an endotoxin inflammatory response caused by a different manufacturing  
285 process involving *Escherichia coli* bacteria compared with Chinese hamster ovary cells  
286 for aflibercept.<sup>5</sup> However, bevacizumab is produced with similar production processes  
287 as aflibercept, and we found a similar consistent rate of IOP elevations between  
288 bevacizumab and ranibizumab, which does not support this theory. Ranibizumab and  
289 bevacizumab bind to all VEGF-A isoforms, while aflibercept can trap VEGF-A, VEGF-B

290 and placental growth factor (PIGF).<sup>15</sup> PIGF only acts on pathological angiogenesis and  
291 inflammation and is not involved in physiological angiogenic processes.<sup>16-18</sup> Anti-VEGF  
292 agents may lead to drug resistance due to an angiogenic rescue program with up-  
293 regulation of other growth factors such as PIGF.<sup>17-19</sup> Repeated injections of ranibizumab  
294 and bevacizumab may induce a progressive inflammatory response secondary to  
295 upregulation of intraocular PIGF levels, which make those eyes more at risk of  
296 inflammatory-related IOP elevations than aflibercept, which possibly controls this  
297 deleterious upregulation.

298 No previous studies in the literature have assessed the impact of syringe  
299 packaging on IOP outcomes. Our study tried to address this question with a sub-  
300 analysis of the ranibizumab cohort. Our finding reported no significant difference in IOP  
301 outcomes between the prefilled and non-prefilled syringe period. This is not consistent  
302 with the theory that droplets of silicone oil, applied to lubricate the components of insulin  
303 syringe used for bevacizumab or non-prefilled ranibizumab packing, play a role in IOP  
304 elevations.<sup>12,14</sup> This finding needs to be confirmed in further studies.

305 It has been reported that increased frequency and number of injections may raise  
306 the risk of IOP elevations.<sup>4,20,21</sup> There was a trend for an increasing rate of elevated IOP  
307 with a higher number of injections in the present study, although this was not statistically  
308 significant; there are conflicting reports on this topic.<sup>22,23</sup>

309 Eyes with pre-existing glaucoma were more at risk of clinically significant IOP  
310 elevation during the follow-up, which is also consistent with previous reports.<sup>4,24,25</sup>  
311 Bergen *et al.* recently reported that PIGF aqueous levels were elevated in glaucomatous  
312 patients.<sup>17</sup> The consistently increased rate of IOP elevations in bevacizumab and

313 ranibizumab treated eyes compared to aflibercept in the glaucomatous subgroup  
314 emphasizes a possible involvement of PIGF in the pathophysiology of IOP elevation in  
315 eyes treated with VEGF inhibitors.

316         This study has several strengths and limitations. Observational studies provide  
317 data that represent the ability of a drug to achieve its intended purpose in the real world.  
318 Our data are representative of a wide variety of real-world international practices.  
319 Though there is variability in the quality of data in observational studies, the FRB!  
320 system includes quality assurance measures that eliminate out of range and missing  
321 data.<sup>10</sup> Well-designed observational studies may not systematically overestimate the  
322 effect of treatment as randomized clinical trials may.<sup>26</sup> The limitations of the study are,  
323 first, no specific protocol for IOP measurements was defined so there may be variability  
324 in methodology, frequency and timing of IOP measurements between practitioners and  
325 over time. We did not record which instrument was used to record the IOP, so we were  
326 unable to adjust for this in our analysis. We included nesting of outcomes within  
327 practitioners in our models to help control for these effects but there may still be biases,  
328 particularly if practitioners used different instruments within the same clinic. We also  
329 performed a sensitivity analysis on the cohort of eyes followed by physicians who  
330 entered IOP measurements at  $\geq 50\%$  follow-up visits to account for possible bias  
331 caused by doctors who might only enter IOP measurements for at-risk cases. This did  
332 not modify the main findings of the primary analysis (see supplemental material)  
333 (available at <http://www.aaojournal.org>). Second, the baseline diagnosis of glaucoma  
334 may vary between practitioners, however our data are consistent with previous reports  
335 showing that eyes with pre-existing glaucoma are more at risk of IOP elevations.<sup>4</sup> Third,



336 pseudo-phakic status at baseline may have been underreported since the rate reported  
337 seemed to be relatively low. We have attempted to control for the influence of lens  
338 status on IOP outcomes by adjusting the statistical analysis for baseline lens status and  
339 cataract extraction during the follow-up. Fourth, data on baseline subtype and severity  
340 of glaucoma and management of elevated IOP during follow-up such as addition or  
341 introduction of IOP-lowering drops, laser or incisional glaucoma surgery, were not  
342 monitored. We were unable to address the influence of these factors on the results.  
343 Fifth, the definition of clinically significant IOP elevation was based on a single event.  
344 While it may have overestimated the results, we preferred a single event definition due  
345 to the absence of data on glaucoma treatment during follow-up. Sixth, we did not  
346 assess the influence of IOP change outcomes on the onset of glaucoma and the  
347 anatomical and functional progression of glaucoma. Seventh, we were not able to  
348 include fellow eye data as a control for our results. Fellow eyes have a basic rate of IOP  
349 rises.<sup>4,8</sup> However, our results are in keeping with the literature and the absence of  
350 control does not influence our finding regarding predictors of IOP changes and  
351 elevations.

352 In conclusion, our study found aflibercept reduces IOP slightly from baseline to  
353 12 and 24 months compared to bevacizumab and ranibizumab, though this difference  
354 was not clinically significant. Of more clinical interest, clinically significant IOP elevations  
355 occurred in a small proportion of eyes receiving VEGF inhibitors. Aflibercept was  
356 associated with fewer clinically significant IOP elevations, whereas eyes with pre-  
357 existing glaucoma were at a higher risk. Aflibercept may be safer than bevacizumab or

358 ranibizumab in eyes with glaucomatous optic neuropathy or ocular hypertension that  
359 develop exudative retinal disease.

360 **References**

- 361 1. Singer MA, Awh CC, Sadda S, et al. HORIZON: an open-label extension trial of ranibizumab  
362 for choroidal neovascularization secondary to age-related macular degeneration. *Ophthalmology*  
363 2012;119:1175–1183.
- 364 2. Kim JE, Mantravadi AV, Hur EY, Covert DJ. Short-term intraocular pressure changes  
365 immediately after intravitreal injections of anti-vascular endothelial growth factor agents. *Am J*  
366 *Ophthalmol* 2008;146:930-934.e1.
- 367 3. Lee JW, Park H, Choi JH, et al. Short-term changes of intraocular pressure and ocular  
368 perfusion pressure after intravitreal injection of bevacizumab or ranibizumab. *BMC Ophthalmol*  
369 2016;16:69.
- 370 4. Atchison EA, Wood KM, Mattox CG, et al. The Real-World Effect of Intravitreal Anti-  
371 Vascular Endothelial Growth Factor Drugs on Intraocular Pressure: An Analysis Using the IRIS  
372 Registry. *Ophthalmology* 2018;125:676–682.
- 373 5. Freund KB, Hoang QV, Saroj N, Thompson D. Intraocular Pressure in Patients with  
374 Neovascular Age-Related Macular Degeneration Receiving Intravitreal Aflibercept or  
375 Ranibizumab. *Ophthalmology* 2015;122:1802–1810.
- 376 6. Bakri SJ, Moshfeghi DM, Francom S, et al. Intraocular pressure in eyes receiving monthly  
377 ranibizumab in 2 pivotal age-related macular degeneration clinical trials. *Ophthalmology*  
378 2014;121:1102–1108.
- 379 7. Adelman RA, Zheng Q, Mayer HR. Persistent ocular hypertension following intravitreal  
380 bevacizumab and ranibizumab injections. *J Ocul Pharmacol Ther* 2010;26:105–110.
- 381 8. Bressler SB, Almkhatar T, Bhorade A, et al. Repeated intravitreal ranibizumab injections for  
382 diabetic macular edema and the risk of sustained elevation of intraocular pressure or the need for  
383 ocular hypotensive treatment. *JAMA Ophthalmol* 2015;133:589–597.
- 384 9. Hogue A, Chen PP, Junk AK, et al. The Effect of Anti-Vascular Endothelial Growth Factor  
385 Agents on Intraocular Pressure and Glaucoma: A Report by the American Academy of  
386 Ophthalmology. *Ophthalmology* 2019;126:611–622.
- 387 11. Gillies MC, Walton R, Liang J, et al. Efficient capture of high-quality data on outcomes of  
388 treatment for macular diseases: the fight retinal blindness! Project. *Retina* 2014;34:188–195.
- 389 11. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational  
390 Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J*  
391 *Clin Epidemiol* 2008;61:344–349.
- 392 13. Bakri SJ, Ekdawi NS. Intravitreal silicone oil droplets after intravitreal drug injections.  
393 *Retina* 2008;28:996–1001.
- 394 13. Morshedi RG, Ricca AM, Wirostko BM. Ocular Hypertension Following Intravitreal  
395 Antivascular Endothelial Growth Factor Therapy: Review of the Literature and Possible Role of  
396 Nitric Oxide. *J Glaucoma* 2016;25:291–300.
- 397 14. Liu L, Ammar DA, Ross LA, et al. Silicone oil microdroplets and protein aggregates in  
398 repackaged bevacizumab and ranibizumab: effects of long-term storage and product  
399 mishandling. *Invest Ophthalmol Vis Sci* 2011;52:1023–1034.
- 400 1. Fogli S, Del Re M, Rofi E, et al. Clinical pharmacology of intravitreal anti-VEGF drugs. *Eye*  
401 2018;32:1010–1020.
- 402 16. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth  
403 factor and placental growth factor contributes to angiogenesis and plasma extravasation in  
404 pathological conditions. *Nat Med* 2001;7:575–583.
- 405 17. Bergen T, Jonckx B, Hollanders K, et al. Inhibition of placental growth factor improves

406 surgical outcome of glaucoma surgery. *J Cell Mol Med* 2013;17:1632–1643.

407 18. Van Bergen T, Etienne I, Cunningham F, et al. The role of placental growth factor (PlGF)

408 and its receptor system in retinal vascular diseases. *Progress in Retinal and Eye Research*

409 2019;69:116–136.

410 19. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine

411 kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients.

412 *Cancer Cell* 2007;11:83–95.

413 20. Mathalone N, Arodi-Golan A, Sar S, et al. Sustained elevation of intraocular pressure after

414 intravitreal injections of bevacizumab in eyes with neovascular age-related macular

415 degeneration. *Graefes Arch Clin Exp Ophthalmol* 2012;250:1435–1440.

416 21. Hoang QV, Mendonca LS, Della Torre KE, et al. Effect on intraocular pressure in patients

417 receiving unilateral intravitreal anti-vascular endothelial growth factor injections.

418 *Ophthalmology* 2012;119:321–326.

419 22. Choi DY, Ortube MC, McCannel CA, et al. Sustained elevated intraocular pressures after

420 intravitreal injection of bevacizumab, ranibizumab, and pegaptanib. *Retina* 2011;31:1028–1035.

421 23. Knip MM, Välimäki J. Effects of pegaptanib injections on intraocular pressure with and

422 without anterior chamber paracentesis: a prospective study. *Acta Ophthalmol* 2012;90:254–258.

423 24. Boyer DS, Goldbaum M, Leys AM, et al. Effect of pegaptanib sodium 0.3 mg intravitreal

424 injections (Macugen) in intraocular pressure: posthoc analysis from V.I.S.I.O.N. study. *Br J*

425 *Ophthalmol* 2014;98:1543–1546.

426 25. Kim YJ, Sung KR, Lee KS, et al. Long-term effects of multiple intravitreal antivascular

427 endothelial growth factor injections on intraocular pressure. *Am J Ophthalmol* 2014;157:1266-

428 1271.e1.

429 26. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the

430 hierarchy of research designs. *N Engl J Med* 2000;342:1887–1892.

431

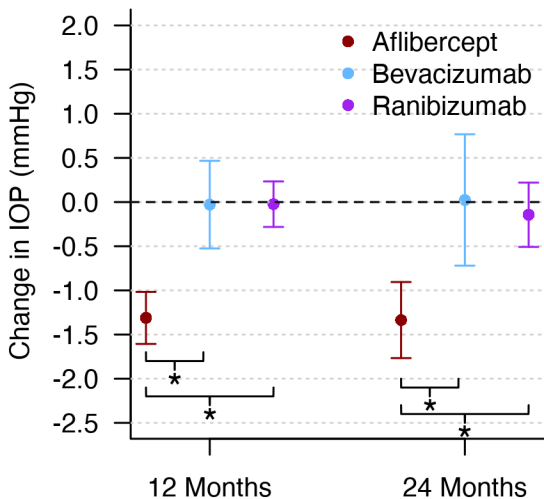
**Figures legends**

**Figure 1.** Adjusted change in intraocular pressure from baseline until 12- and 24-month follow-up visits by A) type of VEGF inhibitors, B) number of injections, C) initial diagnosis, and D) pre-existing glaucoma status.

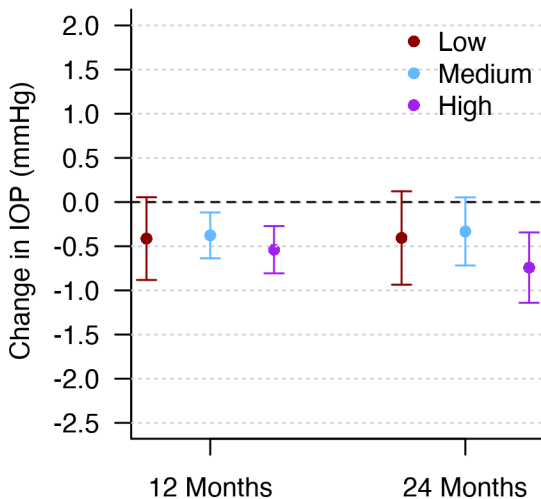
**Figure 2.** Adjusted proportion of clinically significant intraocular pressure elevations during the 12- and 24-month follow-up by A) type of VEGF inhibitors, B) number of injections, C) initial diagnosis, and D) pre-existing glaucoma status.

**Figure 3.** Adjusted change in intraocular pressure from baseline (A and B) and adjusted proportion of clinically significant intraocular pressure elevations (C) by year of treatment initiation at 12- and 24-months for eyes treated with ranibizumab

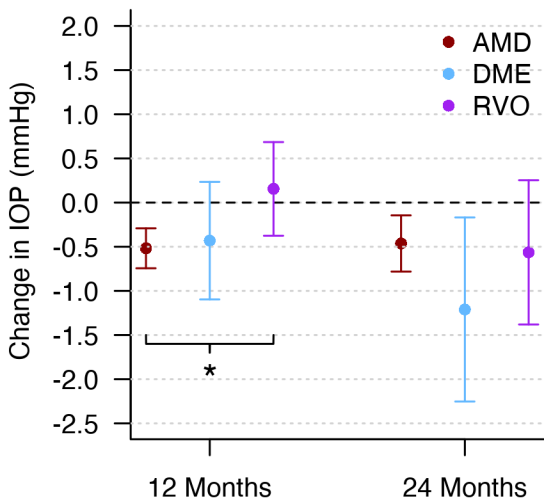
### A) Type of anti-VEGF agent



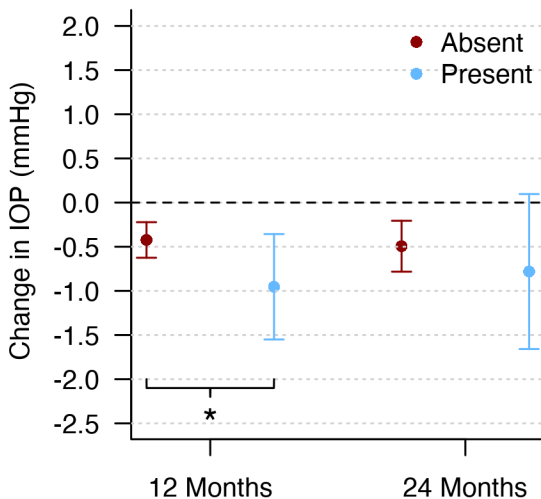
### B) Number of injections



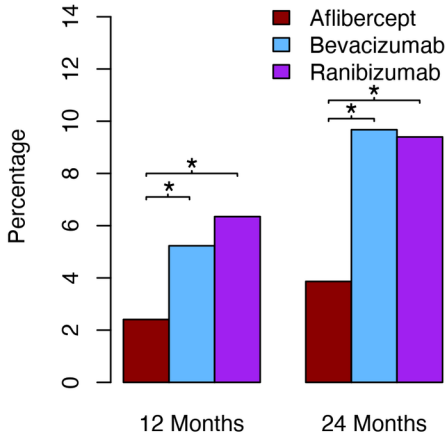
### C) Initial diagnosis



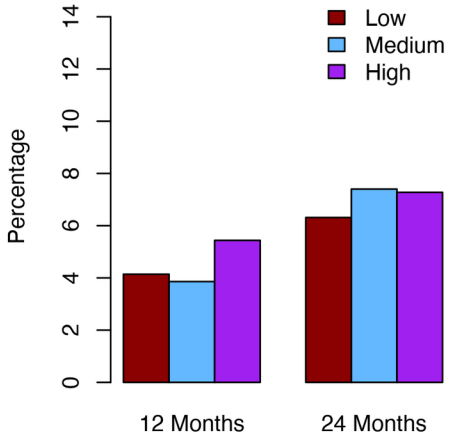
### D) Pre-existing glaucoma status



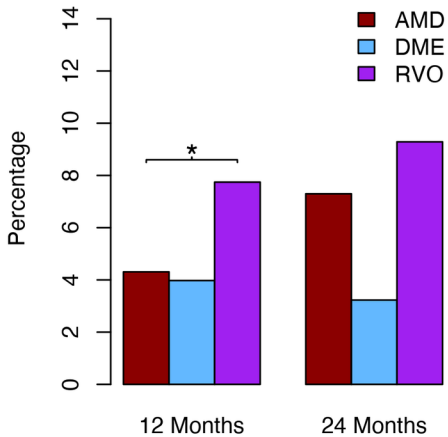
### Type of anti-VEGF agent



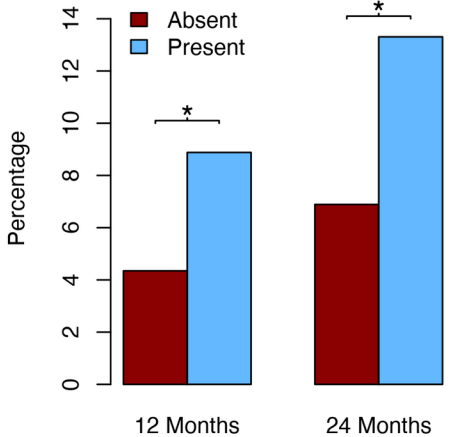
### Number of injections



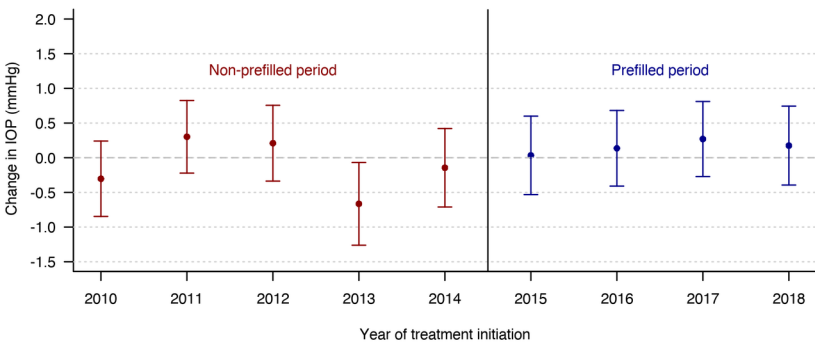
### Initial diagnosis



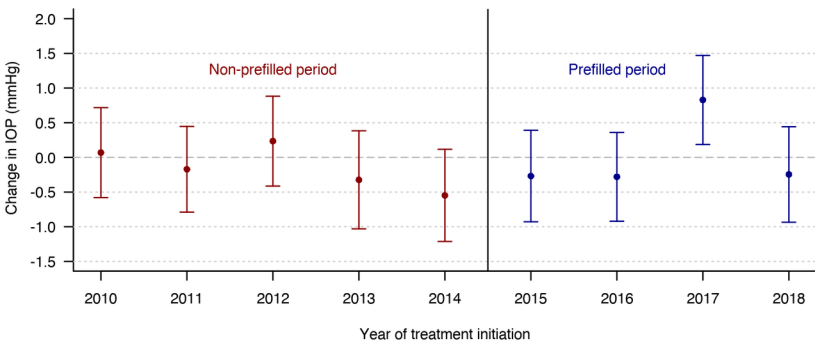
### Pre-existing glaucoma status



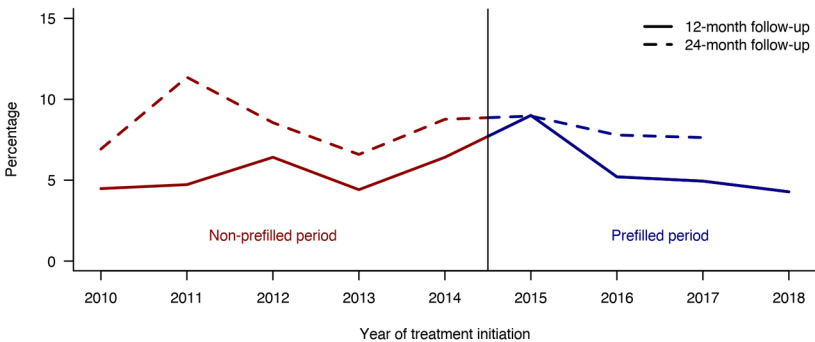
**A) IOP Change at 12 months**



**B) IOP Change at 24 months**



**C) Proportion of clinically significant IOP elevations**





**Table 1.** Baseline demographic characteristics of eyes grouped by type of VEGF inhibitor, type of syringe and pre-existing glaucoma status.

	Overall	Type of anti-VEGF inhibitors			Type of syringe <sup>a</sup>		Pre-existing glaucoma status	
		Bevacizumab	Aflibercept	Ranibizumab	Prefilled	Non-prefilled	Present	Absent
Eyes	3429	395	1138	1896	318	1104	184	3245
Patients	3032	380	1015	1688	293	990	177	2871
Females, n (%)	1779 (58.7%)	195 (51.3%)	556 (54.8%)	1064 (63%)	199 (67.9%)	617 (62.3%)	98 (55.4%)	1691 (58.9%)
Age, mean y (SD)	78.1 (10.4)	74.3 (11.2)	76.4 (11.1)	79.9 (9.4)	80.2 (10.3)	79.6 (8.7)	80.1 (9.1)	78 (10.4)
Intraocular pressure, mean mmHg (SD)	14.4 (3.8)	14.9 (3.6)	14.4 (4.0)	14.4 (3.6)	13.5 (3.4)	14.8 (3.7)	15.2 (5.2)	14.4 (3.7)
Lens status, n phakic (%)	2895 (84.4%)	357 (90.4%)	917 (80.6%)	1621 (85.5%)	230 (72.3%)	1002 (90.8%)	116 (63%)	2779 (85.6%)
Initial diagnosis, n (%)								
<i>Neovascular age-related macular degeneration</i>	2901 (84.6%)	284 (71.9%)	898 (78.9%)	1719 (90.7%)	288 (90.6%)	1026 (92.9%)	132 (71.7%)	2769 (85.3%)
<i>Diabetic macular edema</i>	221 (6.4%)	24 (6.1%)	130 (11.4%)	67 (3.5%)	11 (3.5%)	34 (3.1%)	6 (3.3%)	215 (6.6%)
<i>Macular edema secondary to RVO</i>	307 (9.0%)	87 (22.0%)	110 (9.7%)	110 (5.8%)	19 (6%)	44 (4%)	46 (25.0%)	261 (8.0%)
History of glaucoma, n (%)	184 (5.4%)	12 (3.0%)	70 (6.2%)	102 (5.4%)	18 (5.7%)	61 (5.5%)	184 (100.0%)	0 (0%)

VEGF, Vascular endothelial growth factor; RVO, Retinal vein occlusion.

<sup>a</sup> Cohort of eyes treated with ranibizumab: eyes initially treated from 1 January 2017 were defined as the prefilled syringe group and those initially treated before 1 January 2014 and 1 January 2013 were defined as the prefilled syringe group, thereby allowing 12 and 24 months of follow-up before the start of prefilled packaging in January 2015, respectively.

**Table 2.** Odds ratios from univariate and multivariate logistic regression for clinically significant IOP elevation at 12 and 24 months.

Subgroup (Reference subgroup)	12 months follow-up				24 months follow-up			
	Clinically significant IOP elevation				Clinically significant IOP elevation			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
By type of anti-VEGF drug (aflibercept) <sup>a</sup>								
<i>Bevacizumab</i>	2.37 (1.34, 4.18)	<b>&lt;0.01</b>	2.24 (1.24, 4.04)	<b>&lt;0.01<sup>c</sup></b>	2.81 (1.54, 5.12)	<b>&lt;0.01</b>	2.66 (1.42, 4.98)	<b>&lt;0.01<sup>d</sup></b>
<i>Ranibizumab</i>	2.30 (1.56, 3.39)		2.75 (1.82, 4.14)		2.38 (1.57, 3.60)		2.58 (1.68, 3.96)	
By type of ranibizumab syringe (non-prefilled) <sup>b</sup>								
<i>Prefilled</i>	0.76 (0.41, 1.42)	0.39	0.93 (0.49, 1.79)	0.83	0.61 (0.27, 1.37)	0.23	0.79 (0.34, 1.87)	0.60
By number of injections (low) <sup>a</sup>								
<i>Medium</i>	0.83 (0.49, 1.41)	0.07	0.93 (0.54, 1.60)	0.09	0.99 (0.64, 1.54)	0.95	1.19 (0.75, 1.88)	0.75
<i>High</i>	1.21 (0.72, 2.03)		1.33 (0.77, 2.28)		1.05 (0.67, 1.63)		1.16 (0.73, 1.85)	
By initial diagnosis (neovascular AMD) <sup>a</sup>								
<i>Diabetic macular edema</i>	1.19 (0.65, 2.19)	<b>&lt;0.01</b>	0.92 (0.45, 1.87)	<b>0.039<sup>e</sup></b>	0.65 (0.28, 1.54)	<b>0.034</b>	0.42 (0.16, 1.11)	0.07
<i>Macular edema secondary to RVO</i>	2.39 (1.53, 3.74)		1.86 (1.13, 3.09)		1.85 (1.11, 3.09)		1.30 (0.73, 2.31)	
By pre-existing glaucoma status (absent) <sup>a</sup>								
<i>Glaucoma</i>	2.25 (1.34, 3.78)	<b>&lt;0.01</b>	2.15 (1.23, 3.75)	<b>0.012</b>	2.21 (1.26, 3.88)	<b>&lt;0.01</b>	2.07 (1.13, 3.81)	<b>0.025</b>

OR, odds ratio; AMD, age-related macular degeneration; RVO, retinal vein occlusion; VEGF, vascular endothelial growth factor.

Number of injections groups were defined as low if 3-4 injections during the first year or 3-9 injections during two years of follow-up, medium if 5-8 injections during first year or 10-14 injections during two years of follow-up and high if at least 10 injections during the first year or at least 15 injections during two years of follow-up.

<sup>a</sup> Multivariate model included type of anti-VEGF agent, age, gender, lens status, cataract extraction during the follow-up, baseline IOP, frequency of injection, history of glaucoma.

<sup>b</sup> Multivariate model included type of ranibizumab syringe, age, gender, lens status, cataract extraction during the follow-up, baseline IOP, frequency of injection, history of glaucoma.

Pairwise comparison with Holm-Bonferroni adjustment for multiple comparisons

<sup>c</sup> Aflibercept vs. Bevacizumab ( $P = 0.015$ ); Aflibercept vs. Ranibizumab ( $P < 0.01$ ); Bevacizumab vs. Ranibizumab ( $P = 0.44$ )

<sup>d</sup> Aflibercept vs. Bevacizumab ( $P < 0.01$ ); Aflibercept vs. Ranibizumab ( $P < 0.01$ ); Bevacizumab vs. Ranibizumab ( $P = 0.91$ )

<sup>e</sup> neovascular AMD vs. DME ( $P = 0.82$ ); neovascular AMD vs. RVO ( $P = 0.046$ ); DME vs. RVO ( $P = 0.13$ ).