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*Original:*

Spini, A., Giometto, S., Donnini, S., Posarelli, M., Dotta, F., Ziche, M., et al. (2023). Risk of intraocular pressure increase with intravitreal injections of Vascular Endothelial Growth Factor Inhibitors: a cohort study. AMERICAN JOURNAL OF OPHTHALMOLOGY, 248, 45-50 [10.1016/j.ajo.2022.11.015].

*Availability:*

This version is availablehttp://hdl.handle.net/11365/1222775 since 2024-10-14T13:00:39Z

*Published:*

DOI:10.1016/j.ajo.2022.11.015

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27 March 2025

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PII: S0002-9394(22)00440-8
DOI: https://doi.org/10.1016/j.ajo.2022.11.015
Reference: AJOPHT 12402

To appear in: *American Journal of Ophthalmology*

Received date: August 4, 2022
Revised date: November 12, 2022
Accepted date: November 13, 2022

Please cite this article as: Andrea Spini , Sabrina Giometto , Sandra Donnini , Matteo Posarelli , Francesco Dotta , Marina Ziche , Gian Marco Tosi , Anna Girardi , Ersilia Lucenteforte , Rosa Gini , Mahyar Etminan , Gianni Virgili , Risk of intraocular pressure increase with intravitreal injections of Vascular Endothelial Growth Factor Inhibitors: a cohort study, *American Journal of Ophthalmology* (2022), doi: https://doi.org/10.1016/j.ajo.2022.11.015

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# Risk of intraocular pressure increase with intravitreal injections of Vascular Endothelial Growth Factor Inhibitors: a cohort study

Short title: Risk of intraocular pressure increase after intravitreal VEGF inhibitors

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**Keywords:** VEGF inhibitors; anti-VEGF; intraocular pressure; IOP; bevacizumab; ranibizumab; aflibercept; intravitreal injection; intravitreous injection; cohort study; real-word evidence; observational study; retrospective study.

**Abstract word count:** 250 (250 max)

**Text word count:** 2733 words

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## Abstract

**Purpose:** Intraocular pressure increase (IOPi) after intravitreal injections of vascular endothelial growth factor inhibitors (VEGFis) might be different between different VEGFi (bevacizumab, aflibercept, ranibizumab). The purpose of this study was to evaluate the risk of IOPi among new users of bevacizumab, ranibizumab, and aflibercept in non-diabetic patients in Tuscany, Italy.

**Design**: Retrospective cohort study.

**Methods:** Tuscan regional administrative database was used to identify subjects with a first VEGFis intravitreal injection between 2011 and 2020, followed to first incidence of IOPi. Diabetic subjects, those with pre-existing IOPi or previous use of dexamethasone implants were excluded. Multivariable Cox regression analyses (intention-to-treat and as treated) were conducted to evaluate risk of IOPi between aflibercept, bevacizumab and ranibizumab adjusting for potential confounding variables. IOPi was defined as the first record of ICD-9 code 365 or use of two glaucoma drugs dispensations within 180 days of each other.

**Results:** We identified 6,585 new users of VEGFis: 1,749 aflibercept, 1,112 bevacizumab and 3,724 ranibizumab. Women made up 60% of the cohort with a mean age of 73.6 years. In the intention-to-treat analysis, adjusted hazard ratio (HR) for incident IOPi compared with aflibercept was higher for bevacizumab (HR=2.20; CI95%:1.64-2.95) and ranibizumab users (HR=1.88; CI95:1.46-2.42) respectively. HRs remained robust after exclusion of patients with proxy of retinal vascular occlusion. As treated analysis confirmed such results (bevacizumab:HR=3.76; CI95%:2.30-6.17; ranibizumab:HR=2.49; CI95%:1.62-3.82).

**Conclusion:** This study found an increased risk of IOPi among non-diabetic patients with ranibizumab and bevacizumab compared with aflibercept. Future studies are needed to validate these findings.


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Table of content statement

The study compared the risk of IOP increase among three intravitreal VEGF-inhibitors in non-diabetic patients and demonstrated that the incidence of IOP increase was higher among bevacizumab and ranibizumab users compared with aflibercept users. Although there is evidence in the literature of an increase in IOP in patients using anti-VEGF, no study has compared the risk of incurring in IOP increase among the three drugs.

## INTRODUCTION

In recent years the advent of intravitreal injections of vascular endothelial growth factor inhibitors (VEGFis) such as bevacizumab, ranibizumab, and aflibercept have revolutionized the treatment of retinal diseases especially age related macular degeneration (AMD) and diabetic macular edema.[1] Generally, few serious adverse events have been reported following intravitreal injections of VEGFis, but some large randomized clinical trials have suggested that intravitreal injections might lead to a sustained rise in intraocular pressure (IOP) increasing risk of glaucoma.[2–4] Published evidence has reported different explanations for the IOP increase following intravitreal injections of VEGFis including: 1) injection of a volume of fluid in the eye that increases aqueous humor,[5] 2) VEGFis might decrease nitric oxide levels through inhibition of nitric oxide synthase which can cause altered contractility of trabecular meshwork cells, thereby decreasing humor aqueous outflow through intercellular spaces,[5] and 3) alteration of VEGF and placental growth factor (PlGF) levels in the eye, inducing to pathological angiogenesis and inflammation.[6,7] More recently, an observational study conducted in Taiwan by Chang *et al.* compared the risk of glaucoma associated with the use of the different VEGFis and found a reduced risk of glaucoma in those patients receiving aflibercept when compared with ranibizumab users, although estimates were imprecise and they did not find a statistically significant association.[8] Moreover, the results of this study might not be generalizable to other ethnic groups and the authors did not exclude patients with diabetes (despite they adjusted analysis by indication of use) which is a risk factor for glaucoma. Another small observational study has shown that among patients with a history of glaucoma, aflibercept lowers IOP among patients whose IOP was elevated by intravitreal injection of bevacizumab or ranibizumab.[9]

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To complement evidence from the preceding studies we performed a pharmacoepidemiologic study to evaluate the risk of incidence IOPi in non-diabetic patients among users of three widely used VEGFis mainly intravitreal bevacizumab, ranibizumab, and aflibercept, in Tuscany (Italy).

## METHODS

This was a new user, retrospective cohort study that used the Tuscany regional administrative data source from 2011 year to 2020 year. A protocol was published in the ENCePP website (ID # EUPAS42993).

### *Datasource*

The Tuscan regional administrative data source is composed by the data banks of the Tuscany region of Italy, collected for the entire population of around 3.5 million residents who are residents and registered with a general practitioner in Tuscany. Each individual is assigned a pseudonym and data banks are linked one another at individual level. Specifically, the databanks included contained the following information: demographics (age, gender, vital status), diagnosis and procedures collected during hospitalization and emergency access and coded in the *International Classification of Diseases, Nineth Revision, Clinical Modification* (ICD-9-CM), secondary care encounters, diagnostic procedures, disease specific exemptions from copayment and data on drug dispensations.[10,11]

### *Cohort selection*

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We included all subjects with a first record of an intravitreal injection associated to a VEGFi (aflibercept, bevacizumab or ranibizumab) between January 1, 2011 and June 30, 2020. The date of the first injection was deemed the index date (cohort entry date). All subjects were required to have at least a 5 years look-back period and one year of follow-up. Incident VEGFi users were those who did not have any previous use of these drugs in the year prior to the index date.

The following patients were excluded from the cohort: 1) patients with a record of dexamethasone intravitreal implant in the look-back period; 2) patients with a previous diagnosis of diabetes including those with previous use of an antidiabetic medication any time prior to the first VEGFis use; 3) patients with a previous record of glaucoma including use of anti-glaucoma drugs in the 180 days prior to cohort entry.

***Outcome definition***

The outcome of interest was IOPi defined as the first record of ICD-9-CM code 365*(steroid induced glaucoma ICD-9-CM 365.3* or neovascular glaucoma codes ICD-9-CM 365.63 were not considered) or the second record of a glaucoma drug dispensation (ATC code S01E*) within 180 days.

***Statistical analysis***

We used descriptive statistics to examine the baseline variables including age, gender, comorbidities (acute myocardial infarction and stroke[12]) and drug dispensations. We computed hazard ratios (HRs) for two separate models. The first was an intention-to-treat approach where subjects designated to each of the three VEGFis were followed

from the first injection to the first IOPi event and were censored when they 1) died, 2) end of study period, 3) emigration from the Tuscany region, and 4) insertion of dexamethasone intravitreal implant (which might be a proxy for possible IOP elevation or steroid induced glaucoma[13] not related to anti-VEGF therapies). Patients in the *intention to treat analysis* were considered exposed after receiving the first injection of any of the three study drugs. The Cox model was adjusted for the following covariates: age, gender, comorbidities, binocularity (3 injections in less than 55 days or 2 injections in less than 25 days), use of oral corticosteroids and use of anticoagulants. In the second analysis, to minimize exposure misclassification, patients who switched to a new VEGFis were considered exposed to the new drug (*as treated analysis*). Patients were censored at six months after the last administration of a VEGFi (discontinuation) as glaucoma events beyond this period are likely not due to an intravitreal injection. Crude and adjusted Hazard ratios (HRs) and corresponding 95% Confident Interval (CI) were be calculated. This dataset was also used to fit a Cox model.

In Italy the treatment of retinal vein occlusion (RVO), a potential confounder for this question, requires treatment with anticoagulants.[14] Thus to further control for confounding by RVO, we further stratified the patients in our study according to the use of anticoagulant drugs or presence of a code of RVO (ICD-9-CM 362.3x) in the six months before or after cohort entry.

## RESULTS

### *Cohort characteristics*

The characteristics of patients included in the study stratified by index drug are shown in Table 1. Of 6585 patients included, 1749, 1112 and 3724 had received aflibercept,



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bevacizumab and ranibizumab as index drug, respectively. Women were represented equally in the three groups (approximately 60%). Aflibercept users were slightly older (mean age: 75.9) than ranibizumab and bevacizumab users (mean age: 72.7 and 72.8, respectively). In the overall cohort 1,076 users (16.3%) were RVO patients: a slightly higher percentage of such patients was observed in ranibizumab users compared with aflibercept and bevacizumab users (17.5 vs 14.4% and 15.6%, respectively). The mean number of intravitreal injections before the event was 4.0 (standard deviation, SD 3.6), 2.2 (SD 1.5), and 3.1 (SD 2.5), for aflibercept, bevacizumab and ranibizumab, respectively.

**Outcome**

In the intention-to-treat analysis, 544 cases of IOPi were identified (see Table S1 in the supplementary material). The median time from first administration of the index drug to the first glaucoma diagnosis was 667 days [interquartile range (IQ): 293-1126] for patients who had received aflibercept, 564 [IQ: 162.5-1473.5] for bevacizumab, and 627 [IQ: 219.5-1289.5] for ranibizumab. The adjusted hazard ratios (HRs) resulting from this analysis for bevacizumab and ranibizumab use compared to aflibercept were 2.20 (CI95% 1.64-2.95) and 1.87 (CI95% 1.46-2.41) respectively (Table 2). In the analysis stratified by use of anticoagulants (proxy for patients with retinal vein occlusion), the HR was significant in non-users, and non-significant in anticoagulant users.

In the *as treated* analysis, the risk of IOPi was also higher in patients who received bevacizumab (adjusted HR 3.78; CI95% 2.30-6.19) and ranibizumab (adjusted HR 2.47; CI95% 1.60-3.80) compared with aflibercept. Similarly, to the previous analysis, the HR was significant in non-users and not significant in users of anticoagulants (Table 2).

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Similar results were obtained in the sensitivity analysis after censoring discontinuation at 4 months in the *as treated* analysis where the risk was similar to the analysis with the 6 months censoring criteria. (Table S2)**.**

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## DISCUSSION

This is the first large, epidemiologic study that compared the risk of IOPi among the three intravitreal VEGFis in non-diabetic patients without pre-existing glaucoma. In our study, the incidence of IOPi was higher among bevacizumab and ranibizumab users compared with aflibercept users. These results are in line with the results of other studies, which mainly focused on effects on IOP. An observational study from Taiwan by Chang *et al.* did not find a statistically significant increase in the risk of glaucoma among users of aflibercept when compared with those treated with ranibizumab (HR=0.63, 95%CI:0.37-1.06) although the very wide confidence intervals made these results inconclusive.[8] Moreover, our findings are also supported by results from the sub-analysis of two land-mark randomized controlled trials (VIEW1 and VIEW2) studies.[15] and also results of other observational studies reporting a lower incidence of IOP in eyes treated with aflibercept compared with those who received ranibizumab.[9,16,17] In particular, the study of Gabrielle *et al.* observed the increase in IOP of patients who had received aflibercept, bevacizumab and ranibizumab for retinal disease (study period: 2013-2018),[16] reported a significantly reduced risk of IOP elevations at 12 and 24 months in patients who received aflibercept compared with ranibizumab and bevacizumab.

Some mechanisms have been hypothesized by which bevacizumab and ranibizumab might increase the risk of glaucoma respect to aflibercept. Aflibercept binds to VEGF-B and PlGF in addition to VEGF-A (the target of bevacizumab and ranibizumab), and therefore has a different pharmacodynamic effects than the other two drugs. It is known that VEGFis can promote up-regulation of other growth factors such as PlGF as (PIGF acts sustaining pathological angiogenesis and inflammation and is not involved in

physiological angiogenic processes) stimulating compensatory local response.[7,18,19] Repeated intravitreal injections of ranibizumab and bevacizumab may promote an inflammatory response due to increased PlGF levels in the eye: this could lead treated eyes to a higher risk of inflammatory-related increases in IOP than aflibercept.[16] Moreover, in both the *intention-to-treat* and *as treated* analysis, a slightly higher incidence of IOPi was observed in patients who received bevacizumab compared with ranibizumab. These findings are also in line with other evidence.[20,21] In the study by Good *et al.* the prevalence rates of prolonged IOP elevation with bevacizumab and ranibizumab were 9.9% and 3.1%, respectively.[20] However, given the same drug target, the possible mechanism behind this slight difference between bevacizumab and ranibizumab in terms of glaucoma diagnosis or IOPi is still unclear. Moreover, two hypotheses can be made. The first is the different half-life of the ranibizumab and bevacizumab at the intra-ocular level.[22] The second concern might be the different packaging and compounding practices and formulations between the two drugs, with ranibizumab formulated in smaller doses for intravitreal use. As reported, bevacizumab repackaged in plastic syringes could contain protein aggregates raising the concern for obstruction of aqueous outflow and increase intraocular pressure.[23]

Our study has strengths and limitations. The main strength of the study is its large sample size, with adequate numbers of users for the three main VEGFis. The main limitation of the study is the lack of data on glaucoma location (right *vs* left eye). In addition, we did not have information on indication for which VEGFis were used for. As such, to ensure that the patients included in the study were mainly users of VEGFis for AMD, patients with previous history of diabetes were excluded although it is possible a small number of these patients remained in the cohort that had undiagnosed diabetes.

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Thus, we also stratified our risk analysis by patients with a diagnosis of retinal vein occlusion code or with anti-coagulant drug use (proxy).[14] Finally, the results of our study has to be interpreted within the setting in which it was conducted and with the current clinical practice of these patients in Italy, where VEGFis for intravitreal use are rarely used on a regular basis according to the product summary but rather as a 'treat-and-extend regimen' (progressive extension of treatment intervals up to 12 weeks depending on clinical findings) or "pro-re-nata" regimen (monthly injection only in case of active disease).[24,25] Finally, IOPi could only be identified through ICD-9-CM coding or drug use. As such more specific clinical information on glaucoma for the study participants could not be ascertained. Nevertheless, we did not include events with codes referring to steroid-induced and neovascular glaucoma, despite their use might be limited in the Tuscany setting.

**Conclusions**

The results of our study are suggestive of an increased risk of IOPi in non-diabetic patients who use bevacizumab and ranibizumab compared with users of aflibercept. Future studies are needed to confirm these results.

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**Acknowledgments and financial disclosure**

This study is founded by Regione Toscana: Bando Ricerca Salute 2018 (DD n. 15397 of 26/9/18), project code 181 – Acronym: INTACT. No financial disclosures.

RG and AG are employed by ARS Toscana, a public research center participating in pharmacoepidemiology studies funded by public or private institutions and compliant

with the ENCePP Code of Conduct. The budget of ARS is partially sustained by such studies.

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**Table 1. Characteristics of aflibercept, bevacizumab and ranibizumab users**

| | Aflibercept | Bevacizumab | Ranibizumab | Overall |
|---|---|---|---|---|
| N | 1,749 | 1,112 | 3,724 | 6,585 |
| Women, n (%) | 1,053 (60.2) | 661 (59.4) | 2,272 (61.0) | 3,986 (60.5) |
| Age, mean (standard deviation) | 75.9 (10.7) | 72.8 (13.2) | 72.7 (12.6) | 73.6 (12.3) |
| Comorbidities | | | | |
| Stroke, n (%) | 31 (1.8) | 23 (2.1) | 96 (2.6) | 150 (2.3) |
| Acute myocardial infarction, n (%) | 33 (1.9) | 20 (1.8) | 55 (1.5) | 108 (1.6) |
| RVO, n (%) | 251 (14.4) | 173 (15.6) | 652 (17.5) | 1,076 (16.3) |
| Suspected binocularity, n (%) | 209 (11.9) | 109 (9.8) | 314 (8.4) | 632 (9.6) |
| Mean number of injections during follow up, n (standard deviation) * | 4.0 (3.3) | 2.1 (1.5) | 3.1 (2.0) | 3.2 (2.2) |

*before event occurrence; RVO: retinal vein occlusion

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**Table 2. Results of the intention-to-treat and as treated analyses**

| **Intention-to-treat analysis** | | | | | |
|---|---|---|---|---|---|
| **Drug** | **Events** | **Person-years** | **Incidence** | **Crude hazard ratio (95% CI)** | **Adjusted hazard ratio (95% CI) *** |
| Aflibercept | 75 | 6596.1 | 1.1 | reference | reference |
| Bevacizumab | 123 | 5351.0 | 2.3 | **2.28 (1.70-3.04)** | **2.20 (1.64-2.95)** |
| Ranibizumab | 346 | 17499.3 | 2.0 | **1.91 (1.48-2.45)** | **1.87 (1.46-2.41)** |
| RVO patients | | | | | |
| Aflibercept | 15 | 851.9 | 1.8 | reference | reference |
| Bevacizumab | 20 | 823.9 | 2.4 | 1.57 (0.79-3.11) | 1.50 (0.76-2.99) |
| Ranibizumab | 66 | 2696.9 | 2.4 | 1.56 (0.89-2.74) | 1.49 (0.84-2.63) |
| Non RVO patients | | | | | |
| Aflibercept | 60 | 5744.2 | 1.0 | reference | reference |
| Bevacizumab | 103 | 4527.1 | 2.3 | **2.45 (1.78-3.38)** | **2.40 (1.73-3.32)** |
| Ranibizumab | 280 | 14802.4 | 1.9 | **1.98 (1.50-2.62)** | **1.97 (1.49-2.61)** |
| **As treated analysis ¶** | | | | | |
| **Drug** | **Events** | **Person-years** | **Incidence** | **Crude hazard ratio (95% CI)** | **Adjusted hazard ratio (95% CI) *** |
| Aflibercept | 27 | 1961.4 | 1.4 | reference | reference |
| Bevacizumab | 43 | 739.9 | 5.8 | **3.94 (2.41-6.46)** | **3.78 (2.30-6.19)** |
| Ranibizumab | 110 | 2967.6 | 3.7 | **2.56 (1.67-3.93)** | **2.47 (1.60-3.80)** |
| RVO patients | | | | | |
| Aflibercept | 8 | 259.4 | 3.1 | reference | reference |
| Bevacizumab | 5 | 122.2 | 4.1 | 1.06 (0.34-3.25) | 1.04 (0.34-3.19) |
| Ranibizumab | 27 | 493.9 | 5.5 | 1.49 (0.67-3.30) | 1.46 (0.65-3.25) |
| Non-RVO patients | | | | | |
| Aflibercept | 19 | 1702.0 | 1.1 | reference | reference |
| Bevacizumab | 38 | 617.7 | 6.2 | **5.45 (3.09-9.61)** | **5.25 (2.97-9.27)** |
| Ranibizumab | 83 | 2473.6 | 3.4 | **2.98 (1.79-4.94)** | **2.90 (1.74-4.82)** |

*Analyses were adjusted for the following covariates: age, gender, comorbidities, binocularity, use of oral corticosteroids and anticoagulants (the last variable was used also for additional stratification); ¶ in the *as exposed* analysis patients were censored for discontinuation (6 months). RVO: Retinal vein occlusion

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