Noninfectious Inflammation After Intravitreal Injection of Aflibercept: Clinical Characteristics and Visual Outcomes

ROGER A. GOLDBERG, CHIRAG P. SHAH, TORSTEN W. WIEGAND, AND JEFFREY S. HEIER

* PURPOSE: To report the presenting features and clinical outcomes of a series of patients with noninfectious inflammation after intravitreal aflibercept injection.

* DESIGN: Noncomparative consecutive case series.

* METHODS: Medical records of patients who presented with noninfectious inflammation after intravitreal aflibercept injection between November 18, 2011 and June 30, 2013 were retrospectively reviewed.

* RESULTS: A total of 20 cases of postinjection inflammation were identified in 5356 aflibercept injections. The patients presented 1–13 days after aflibercept injection (median 3 days); all noted decreased vision, while 3 of 20 (15%) had pain and 2 of 20 (10%) had conjunctival injection. One patient had a hypopyon (0.5 mm), and the average anterior chamber cell was 1.8+ (range 0 to 4+). All eyes had some degree of vitritis (average 1.8+; range 0.5+ to 4+). Patients on average had received 6 prior aflibercept injections (range 0–16). Only 1 patient—the first to present with inflammation in this series—received an intravitreal tap (culture negative) and injection of antibiotics. All patients were managed with frequent topical steroids and were followed closely for signs of improvement. All but 1 patient regained their preinjection visual acuity (average: 33 days; range: 7–73 days). Four patients were subsequently rechallenged with aflibercept, and 1 developed inflammation again after 5 additional aflibercept injections. The overall incidence of inflammation after intravitreal aflibercept injection was 20 of 5356 injections (0.37%) or 19 of 844 patients (2.25%). However, a disproportionate number of cases clustered around 1 physician (17/20, 85%; P < .001 vs all other providers) and around the 2 office locations where this physician primarily worked (16/20, 75%; P < .001 vs 5 other offices).

* CONCLUSIONS: Noninfectious inflammation after intravitreal aflibercept injection typically presents without pain, conjunctival injection, or hypopyon, and responds to topical steroid therapy. The visual outcomes are generally favorable, though the return to baseline acuity can take many weeks. (Am J Ophthalmol 2014;158: 733–737. © 2014 by Elsevier Inc. All rights reserved.)

APPROVED IN NOVEMBER 2011, AFLIBERCEPT (EYLEA; Regeneron, Tarrytown, New York, USA) has been widely adopted for the treatment of neovascular age-related macular degeneration (AMD) and, more recently, retinal vein occlusion (RVO). In the pivotal phase III studies leading to US Food & Drug Administration approval, intravitreal aflibercept injection was well tolerated and associated with a low overall complication rate. Since then, however, cases of noninfectious inflammation after aflibercept injection have surfaced, prompting a pharmaceutical surveillance committee of the American Society of Retina Specialists to investigate further. Differentiating inflammation from infectious endophthalmitis, a feared complication of intravitreal injection, is critical for patients and clinicians alike. To that end, we herein report a large series of noninfectious inflammation after aflibercept injection, including a description of its clinical presentation and course. We also estimate its incidence and describe investigative efforts into its cause.

INSTITUTIONAL REVIEW BOARD EXEMPTION WAS GRANTED from Chesapeake Research Review, Inc, Columbia, Maryland, USA, which waived the requirement for informed consent. This report is compliant with the Health Insurance Portability and Accountability Act requirements, the Declaration of Helsinki, and all state and federal laws. The medical records of all patients treated by the retina service at Ophthalmic Consultants of Boston (Boston, Massachusetts, USA) who developed noninfectious inflammation after intravitreal aflibercept injection between November 18, 2011 and June 30, 2013 were retrospectively reviewed.

The data collected include the age and sex of each patient, the affected eye and its lens status, the date of the inciting intravitreal aflibercept injection, the number of prior aflibercept injections and other anti–vascular endothelial growth factor (VEGF) injections, the underlying diagnosis and clinical indication for intravitreal injection, and the preinjection visual acuity. The lot number, office location, injecting physician, and preparation technique, as well as use of postinjection antibiotics, were recorded. Presentation and diagnostic data were collected as well, including the date of presentation, the visual acuity and
intraocular pressure, the pain score, the presenting symptoms and signs, and the initial management. The patients’ clinical courses, including the visual acuity over time and response to additional intravitreal aflibercept injections, were documented.

An estimate of incidence was obtained by determining the total number of aflibercept injections between November 18, 2011, and June 30, 2013. All eyes were prepared for injection with subconjunctival or topical anesthesia and povidone-iodine antisepsis. The practice includes 6 retina providers and 7 office locations.

### RESULTS

A total of 20 cases in 19 eyes of 19 patients were included in this study. The average age at the time of presentation with noninfectious inflammation after intravitreal aflibercept injection was 79.3 (range 70–93) years; 19 of 20 cases were being treated for neovascular AMD, the other for RVO. Preinjection visual acuities ranged from 20/25 to count fingers. All but 1 patient had received prior aflibercept injection without incident (average 6 prior injections, range 0–16). No patient had a history of uveitis or inflammation after intravitreal injection with another agent.

In these cases, all patients complained of decreased vision and were seen on average 3.25 days after aflibercept injection (median 3 days, range 1–13 days). Lines of visual acuity lost between injection and presentation averaged 3.5 (median 3 lines, range 0–9 lines lost). Pain was not a common presenting symptom—noted in 3 of 20 cases (15%)—and was mild in nature when present. The conjunctiva was rarely injected (2/20, 10%), and hypopyon was seen in only 1 patient (Case 14; 0.5 mm in height). The degree of anterior chamber inflammation ranged from 0 to +4 cell, and vitreous inflammation was noted in all 20 cases (median +2 cell, range 0.5+ to +4 cell). The Table summarizes the pertinent clinical findings for each case.

All patients were treated with frequent topical steroid eye drops (prednisolone acetate 1% dosed every hour while awake) and were followed closely for signs of

### Table

#### TABLE. Summary of Patients With Noninfectious Inflammation After Intravitreal Aflibercept Injection

<table>
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<th>Case</th>
<th>Age (y)</th>
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<th>Needle Gauge</th>
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<th>Pain</th>
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AC = anterior chamber; AMD = age-related macular degeneration; Conj = conjunctival injection (0–4+); CRVO = central retinal vein occlusion; Dx = diagnosis; HM = hand motions vision; IAI = intravitreal aflibercept injection; InjVA = Snellen visual acuity on day of IAI; MD = physician; Pres = presentation; Pres VA = Snellen visual acuity on the day of presentation; T&I = tap and inject; VA return = time (in days) for return of vision to baseline VA on the day of IAI.

*Seven office locations (A–G) injected aflibercept during the study time period (November 18, 2011 - June 30, 2013).

Six physicians (1–6) injected aflibercept during the study time period.

Case 14 involved the only patient to present with a hypopyon (0.5 mm height).

C Culture-negative.

Case 13 involved resolution of inflammation by day 28 after IAI, though the visual acuity remained at 20/200.
improvement. Though suspicion of infection was low given the lack of pain, conjunctival injection, and anterior chamber hypopyon, the first patient to present with inflammation after aflibercept injection, in Case 1, received an intravitreal tap and injection of antibiotics; culture results were negative. Nineteen of 20 cases regained their preinjection visual acuity, on average 33 days after intravitreal aflibercept injection (median 28 days; range 7–73 days). The 1 patient (Case 13) whose vision did not return to baseline had resolution of inflammation by day 28, though the visual acuity remained at 20/200 from a baseline of 20/60; he was lost to further follow-up.

Four patients were treated subsequently with aflibercept, and 1 patient (Cases 16 and 17) developed noninfectious inflammation a second time after 5 uneventful aflibercept injections. Four patients received bilateral injections with aflibercept from the same manufacturing lot number, and in all cases only 1 eye was affected (2 right eyes, 2 left eyes).

During the study period, the incidence of noninfectious inflammation after aflibercept injection was 0.37% (20 cases in 5356 aflibercept injections during the study period). Alternatively, 19 patients out of 844 who received at least 1 aflibercept injection during the study period were affected (2.25% of patients).

However, 17 of 20 cases (85%) were from one of the practice’s 6 retinal specialists. This represents a 0.94% incidence for this provider vs 0.08% for all others (P < .001). This provider disproportionately practices in 2 office locations, where 16 of 20 cases originated (80%, P < .001 vs 5 other office locations).

The 20 cases developed from 10 different manufacturing lots of aflibercept, including lot 8073400023, which was involved in 6 of 20 cases (30%). An investigation conducted by the practice into the drug handling and storage, needle type used, and injection technique did not reveal a cause for these cases of noninfectious inflammation. The cases were reported to the manufacturer as they occurred.

**DISCUSSION**

**NONINFECTIOUS INFLAMMATION HAS BEEN REPORTED after intravitreal injections of bevacizumab, ranibizumab, and triamcinolone acetonide,** as well as aflibercept. Speculation regarding the cause of these cases of noninfectious inflammation has largely focused on contaminants associated with syringe preparation and storage in the cases of bevacizumab, which is aliquoted from a 4 mL or 16 mL vial by a compounding pharmacy, or on preservatives in the cases associated with triamcinolone. However, both ranibizumab and aflibercept are delivered in single-dose, sterile glass vials without preservatives, and are drawn into an individual patient syringe at the time of the injection.

Consistent with prior reports of noninfectious inflammation after intravitreal injections, the visual outcomes in this series were generally good. All but 1 case returned to baseline visual acuity, on average within 1 month; one-third of patients were substantially better by 2 weeks post injection. Although the patients in this series responded promptly to topical steroid eye drops, other therapies, such as topical nonsteroidal antiinflammatory drugs, may also be effective.

Differentiating noninfectious inflammation from infectious endophthalmitis is important, as infectious endophthalmitis requires early intravitreal antibiotic therapy and can result in poor visual outcomes. Clues to the noninfectious nature of these cases included lack of pain, lack of conjunctival injection, and lack of hypopyon, though none of these alone should be considered diagnostic. Patients were managed with aggressive topical corticosteroids and were followed closely initially (often later the same day or the next morning) for signs of stabilization and improvement.

The estimated incidence of noninfectious inflammation after intravitreal aflibercept injection was 0.37% in this series (2.25% of patients). This is consistent with a previous report from another retina practice, which estimated an incidence of 0.28% (Koji M, et al. IOVS 2013; 54: E-Abstract 1104). However, given the clustering of cases seen in this series, the estimates of incidence should be approached with caution. In the 8 months since the study period ended (July 2013 through February 2014), 3222 additional injections of aflibercept have been performed in the practice, with only 1 additional case of noninfectious inflammation (0.03%). This is consistent with Regeneron’s postmarketing surveillance data: between November 2011 and December 2013, during which time over 1 million doses of aflibercept were administered, the reported rate of postinjection inflammation was 0.04% (personal communication, Andrea Gibson, Director, Ophthalmology Medical Affairs, Regeneron, March 27, 2014).

Interestingly, these cases clustered around 1 provider and the 2 office locations where this doctor practiced primarily. A previous report of noninfectious inflammation after aflibercept injection also demonstrated this clustering effect, with 9 of 11 cases from 1 provider within a Connecticut-based practice. **This clustering effect may further skew estimates of incidence, but does raise interesting questions regarding etiology.**

The cause of these and other cases of noninfectious inflammation after aflibercept injection has not been determined. Speculation regarding protein denaturation of aflibercept, perhaps owing to the more viscous nature of the molecule compared to bevacizumab or ranibizumab, has been implicated as a possible etiology. When passed through the provided 19 gauge filter needle, aflibercept often forms small bubbles (Figure), which are difficult to remove from the syringe barrel prior to injection. Denaturation can occur at these hydrophobic–hydrophilic
interfaces, as the protein tries to orient its hydrophobic groups in the nonaqueous layer.

The process of denaturation may be accelerated by sheer stress exerted when injecting through a 32 gauge vs a 30 gauge needle, which has a 33% smaller internal lumen diameter. However, the manufacturer reports that aflibercept has a high degree of molecular stability across a range of tested conditions, including multiple freeze–thaw cycles, numerous passes through the filter needle, and injection through 30 gauge and 32 gauge needles (personal communication, Andrea Gibson, Director, Ophthalmology Medical Affairs, Regeneron, March 27, 2014). Additionally, whereas the first 7 cases in this series occurred with a 32 gauge needle, the remaining 13 used the provided 30 gauge needle.

Investigative efforts conducted by the practice managers and physicians did not reveal a specific cause for these cases.

The supply chain within the practice from delivery, internal distribution and storage, and day of use was intact; refrigeration conditions were assessed at each stage and for each office and found to be within the manufacturer's recommended range (36–46 F). Injection preparation technique, including choice of anesthesia, use of povidone-iodine, and use of postinjection antibiotics, also does not appear to be a factor in these cases: the same technique was used by each provider for all intravitreal injections, and no cases of inflammation developed during this period associated with other anti-VEGF agents.

No patients in this series had a history of uveitis, which might predispose an eye to developing inflammation. Additionally, although 1 patient (Case 9) developed inflammation after the first aflibercept injection, most patients had received multiple prior aflibercept injections without incident. Additionally, 4 patients were rechallenged with aflibercept subsequently, and only 1 patient developed recurrent inflammation, after 5 uneventful injections.

Though impurities may have stimulated these inflammatory reactions, it seems unlikely that they are related to the manufacturing process. The 20 cases in this series arose from 10 different manufacturing lot numbers, and 4 patients received bilateral same-day injections with drug from the same lot number, with only 1 eye developing inflammation.

An impurity may be associated with the provided 5-μm filter needle, perhaps entering the syringe when the more viscous aflibercept is drawn rapidly through the filter into the syringe. The physician in this series associated with 17 of the cases had the same senior technician preparing the aflibercept syringes for injection. Since drawing up the aflibercept syringes personally, with attention to the speed with which the medication is pulled into the syringe barrel, this physician has not had any additional cases of inflammation after aflibercept injection. Further testing, however, is required to evaluate this hypothesis in a controlled setting.

In conclusion, noninfectious inflammation after intravitreal aflibercept injection has a generally favorable visual outcome, but it can be carefully distinguished from infectious endophthalmitis. Suspected cases should be reported to the manufacturer for postmarketing surveillance. Further investigations to elucidate the cause—and ultimately to prevent the occurrence—of postinjection inflammation is warranted.
REFERENCES

Biosketch

Roger A. Goldberg, MD, MBA is a vitreoretinal specialist at Bay Area Retina Associates in California. He completed his residency at the Bascom Palmer Eye Institute, and his fellowship at Tufts/Ophthalmic Consultants of Boston. A graduate of Yale College, he spent a year as a Rotary Scholar at the University of Leiden, The Netherlands. After working as a healthcare consultant at McKinsey & Co., he obtained an MD and MBA from Yale. His interests include vitreoretinal diseases and surgery, innovation and technology development, and healthcare policy.