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Original Article

A cluster of presumed, noninfectious endophthalmitis after intravitreal injection of bevacizumab: long-term follow-up

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Abstract

Purpose—To report the outcome of 5 consecutive cases of presumed, noninfectious endopththalmitis following intravitreal injection of bevacizumab (IVB).

Methods—Ten pre-loaded syringes of bevacizumab (1.25 mg/50 µL) furnished by a compounding pharmacy were injected intravitreally. Treatments were performed in the operating room by the same surgeon on 2 consecutive days.

Results—Of 10 eyes, 5 showed moderate to severe ocular inflammation within a few days of injection. All patients were treated in the same surgical session. Vitreous tap performed in the patient presenting with the most severe grade of inflammation was negative for bacteria and fungi. At the time of the vitreous biopsy, this patient was injected with vancomycin 1 mg/100 µL in the vitreous cavity. Other eyes with moderate inflammation received topical and systemic antibiotics and topical steroid treatment. Visual acuity returned to pre-endophthalmitis or better levels in all eyes within 1 month. The other 5 patients treated with IVB from the same batch in the other surgical session did not develop inflammation.

Conclusions—IVB can induce noninfectious endophthalmitis. The use of compounded syringes can explain clustering of the inflammation. We were unable to identify the reasons for the variable grade of inflammation we observed in our patients.

Bevacizumab (Avastin; Roche, Basel, Switzerland) is a humanized monoclonal antibody binding vascular endothelial growth factor (VEGF) that was originally developed to treat metastatic carcinoma of the colon and rectum. Michels et al[1] used intravenous bevacizumab infusions as an off-label treatment for neovascular age-related macular degeneration (n-AMD). Its efficacy in this context led to off-label intravitreal bevacizumab (IVB) administration and eventually widespread use as a treatment for ocular diseases associated with retinal neovascularization and edema, including macular edema secondary to retinal vein occlusion (RVO), n-AMD myopic choroidal neovascularization (m-CNV), CNV secondary to angioid streaks, diabetic macular edema (DME), and neovascular glaucoma.[2]

IVB can cause procedure-related complications (traumatic cataract, retinal detachment, hemovitreous, and infectious endophthalmitis) and drug-related systemic and local side effects, including noninfectious endopththalmitis,[3] which must be distinguished from infectious endopththalmitis,[4–16] because management and prognosis of these two entities differs greatly. We report a cluster of noninfectious endophthalmitis ranging in severity from mild inflammation to severe vitritis with or without retinal involvement after IVB. In our patients, we employed single-use syringes of bevacizumab from the same batch, prepared by a single compounding pharmacy.

Subjects and Methods

This retrospective study adhered to the tenets of the Declaration of Helsinki; formal approval was waived by

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Digital Journal of Ophthalmology, Vol. 22

the Ethics Committee of the Fondazione Policlinico Tor Vergata, Rome, Italy. Commercially available bevacizumab (100 mg/4 mL) was fractioned into smaller doses (2.5 mg/0.1 mL) under sterile conditions by an Italian compounding pharmacy in single-use, 1 mL, sterile, Luer-Lock plastic syringes (BD, Franklin Lakes, NJ). These were stored at 4° C and protected from light until use. The compounding pharmacy furnished the preloaded syringes packed in double sterile envelopes and set a 60-day shelf life. The syringes had a sterile stopper that was then exchanged for a new 30-gauge needle. Bevacizumab doses were prepared on December 23, 2010.

At that time, despite the availability of ranibizumab (Lucentis, Novartis Pharmaceuticals, Basel, Switzerland), the Agenzia Italiana del Farmaco authorized the reimbursement and off-label use of bevacizumab in some eye diseases for which the ranibizumab had not yet obtained authorization (DME, RVO, m-CNV) or for which reimbursement was not allowed (n-AMD, presenting with best-corrected visual acuity of <0.8 logMAR).

On January 24 and 25, 2011, bevacizumab 1.25 mg/50 µL was injected each day in 5 patients: 2 eyes of 2 patients with macular edema following RVO; 5 eyes of 5 patients with DME; 2 eyes of 2 patients with n-AMD; 1 eye of 1 patient with m-CNV. All patients were treated with one or more anti-VEGF injection in the past without problems. In the same operative sessions, ranibizumab was injected in ten more patients; 5 patients per day.

All injections were performed in the operating room, and the same procedure was always followed. As endophthalmitis prevention, 0.3% tobramycin eyedrops were prescribed 3 times daily for 3 days before intravitreal administration. Povidone-iodine 5% and sterile, single dose 4% lidocaine eyedrops were applied to the ocular surface 3 times just prior to transferring the patient into the operative theater. The eyelids were disinfected with povidone-iodine 10%. Sterile drapes and lid speculums were used.

Bevacizumab was injected into the vitreous cavity at a distance of 3.5–4.0 mm posterior to the corneal limbus using a 30-gauge needle. Patients were directed to use antibiotic and steroid eye drops (tobramycin 0.3% and dexamethasone 0.1% 3 times daily) for an additional week after intravitreal injection.

Prior to injection in all patients, best-corrected visual acuity was measured using Early Treatment Diabetic Retinopathy Study (ETDRS) charts. All patients received anterior segment, vitreous, and fundus examinations.

After injection control visits were scheduled at day 1, week 1, and week 4. As standard routine all the patients were informed about self-assessment of severe signs or symptoms of procedural side effects.

Results

Four days after IVB, a 79-year-old patient with n-AMD contacted us to report blurred vision and floaters in the injected eye, without pain. Slit-lamp examination (1 mm slit-beam field) revealed >50 anterior chamber cells and corneal edema, with Descemet's folds but without hypopyon. The anterior vitreous contained cells and debris. The central vitreous opacity was so dense and diffuse that the posterior pole details were barely visible. Peripheral retina examination showed the presence of intraretinal hemorrhages. Visual acuity dropped to 0.9 logMAR (preoperative visual acuity, 0.2 logMAR).

Due to the presence of a severe intraocular inflammation mimicking an infectious endophthalmitis, the patient was hospitalized. A vitreous tap was performed and at the same time intravitreal vancomycin was also injected (1 mg/100 µL). Intravenous broad-spectrum systemic antibiotic therapy (piperacillin 2 g, tazobactam 0.25 g intravenous infusion 3 times daily) was also started.

According to our safety protocol, we contacted all the other patients treated in the same surgical session (N = 10) and 5 more patients treated with IVB of the same batch the day before. A control visit was scheduled for the same day. We confirmed that all patients had executed preand postoperative treatment as prescribed by our protocol.

All of the other sterile syringes (N = 17) of the same batch number were delivered to the National Institute of Health, as recommended by Italian legislation, for security checks: cultures and molecular techniques (polymerase chain reaction for universal primers and toxins) were used.

All 4 other patients treated at the same IVB surgical session showed signs of intraocular inflammation in the injected eye. In 3 patients, a moderate intraocular inflammation was observed, with anterior chamber cells ranging from 5 to 50 per 1 mm slit-beam field, without hypopyon or fibrin, and mild vitritis localized at the site of injection (inferotemporal quadrant). These patients experienced a complete clinical resolution with topical antibiotic-steroid therapy (0.5% levofloxacin plus 0.3%

Digital Journal of Ophthalmology, Vol. 22

Table 1. Demographic and clinical data of the patients affected of presumed noninfectious endoptththalmitis

					BCVA, logMAR			
Patient	Age, years	Disease	Prior PDT	Prior IVB	Baseline	Day 1	Month 24	Ocular inflammation
1	79	n-AMD	0	5	0.2	0.9	0.2	Severe
2	84	ME-RVO	0	2	0.7	1.0	0.7	Moderate
3	71	DME	0	1	0.4	0.5	0.3	Moderate
4	64	M-CNV	2	6	0.4	0.5	0.4	Moderate
5	55	DME	0	0	1.0	0.6	0.8	Mild

BCVA, best-corrected visual acuity; *DME*, diabetic macular edema; *IVB*, intravitreal Bevacizumab; *LogMAR*, logarithm of the minimum angle of resolution; *M-CNV*, myopic choroidal neovascularization; *ME-RVO*, macular edema–retinal vein occlusion; *n-AMD*, neovascular age-related macular degeneration; *PDT* photodynamic therapy.

tobramycin and 0.1% dexamethasone 4 times daily) combined with systemic antibiotic therapy (piperacillin 2 g, tazobactam 0.25 g intravenous infusion twice daily for 5 days). In 1 patient presenting with mild inflammation (cells in the anterior chamber and localized vitreous cells), only topical antibiotic-steroid therapy was prescribed.

Laboratory vitreous cultures in the patients who underwent vitreous tap were negative for bacteria and fungi, and these cases were diagnosed as sterile endophthalmitis.

None of the patients treated with IVB of the same batch the day before, nor the patients treated with ranibizumab on the same day presented with signs of ocular inflammation. The patient with a severe intraocular inflammation recovered preoperative best-corrected visual acuity, and the inflammatory response was resolved completely within 4 weeks. None of the other patients with inflammation had visual acuity loss compared to preoperative best-corrected visual acuity. Inflammation in these other patients resolved in 2 weeks.

After the complete resolution of the intraocular inflammation, all patients were switched to ranibizumab therapy, and none of them showed an inflammatory response with this drug at 24 months' follow-up. Furthermore, visual function was mostly preserved in all cases (see Table 1).

Two months after this outbreak of presumed, noninfectious endophthalmitis, we obtained the microbiological results from the National Institute of Health revealing neither infectious contamination nor toxins in the examined samples, confirming the noninfectious nature of the intraocular inflammation.

Discussion

This study describes 5 consecutive cases of presumed, noninfectious endophthalmitis after IVB from a single batch prepared in a compounding pharmacy. Four days after IVB, 1 patient reported blurred vision without pain. We performed a vitreous tap for culture due to the presence of a severe intraocular inflammation and retina hemorrhages.

All 10 patients treated with IVB of the same batch were recalled in order to detect inflammatory signs in consideration of a possible cluster of endophthalmitis, as reported by other authors.[7–11] Inflammation was found in 4 other patients, treated in the same surgical session but not in the other 5 patients treated with IVB from the same batch not the other 10 patients treated with ranibizumab in the same operative sessions.

These data, as well as the negative cultures from the NIH, suggest a noninfectious nature of the inflammation.

Noninfectious endophthalmitis (also known as "pseudoendophthalmitis") is described as any acute intraocular inflammation without infection that resolves without antibiotic treatment, unlike true endophthalmitis.[17] Because the positive culture rate of vitreous tap can be quite low even in infectious cases and because these patients were treated with antibiotics, we refer to these cases as "presumed noninfectious."

In the literature, noninfectious, or *presumed* noninfectious, endoptththalmitis cases have been reported after intravitreal injection of all anti-VEGF agents including bevacizumab, ranibizumab, aflibercept and pegaptanib.

Digital Journal of Ophthalmology, Vol. 22

The incidence of noninfectious endopththalmitis after IVB ranges between 0.03% and 1.49%. Noninfectious endophthalmitis has been widely reported both as sporadic events and as clusters; in most cases inflammation regressed with no treatment or with topical treatment.[17]

Multiple case series have described clusters of presumed, noninfectious endophthalmitis in patients treated with injections from the same batch. Sato et al[7] reported that 5 of 35 patients (14.3%) injected with the same lot of bevacizumab developed severe intraocular inflammation. Yamashiro et al described 14 consecutive cases (73.7%) of noninfectious endophthalmitis after injection of IVB prepared from a single batch (N = 19), some of which required pars plana vitrectomy.[8] Fukami et al9 reported 6 cases of 12 treated patients who presented with noninfectious endophthalmitis in Japan. Sinha et al[10] reported 4 cases of noninfectious endophthalmitis in India. And in China, Wang et al[11] reported 80 patients of 116 (69%) who developed a noninfectious endophthalmitis after intravitreal injection of counterfeit bevacizumab. This study implicated endotoxins as the cause of the inflammation.

A variable incidence of presumed noninfectious endophthalmitis has been reported after intravitreal injections of all anti-VEGF agents. There are several hypotheses concerning the etiology, relating either to the molecule itself or to compounding.[17–20]

First, according to the manufacturer's guidelines (http://www.gene.com/download/xml/avastin.xml#s2.2), bevacizumab must be stored in the original sterile glass vials at 2°–8° C (36°–46° F) and protected from light. Diluted bevacizumab solution may be stored at 2°–8°C for up to 8 hours. Any variance from storage and handling protocol (eg, post-packaging storage time, type of syringes, freeze-thawing, mechanical shock during shipping, exposure to light) could result in degradation of the agent, with reduced activity and increased immunogenicity.

Second, inflammation has been associated with excess silicone oil droplets and particulates (protein aggregates) in commercially available bevacizumab vials. Silicone oil is used to lubricate the barrel of the plastic syringe. The US Pharmacopeia (USP) manufacturing requirements for intravenous drug formulations permit higher subvisible particulate counts than that for ophthalmic solutions.[21,22] Mishandling (freezing and mechanical shock) could further increase levels of particle contaminants.

Third, in comparison to ranibizumab, which is an antibody fragment, bevacizumab has an Fc fragment that may make it more immunogenic or proinflammatory. Larger molecules with Fc constant fragments and antibody-binding Fab fragments are more immunogenic than those with the antibody-binding fragment alone.

Fourth, bacterial endotoxin contamination has been reported in the pharmaceutical production phase of antibody preparation.

Finally, the eye may develop an immune response to the antibody molecule after repeated exposure to the drug.

In our case series, we were unable to confirm a correlation between the number of previous IVB injections and the rate and grade of intraocular inflammation. No patient in this series had a history of uveitis. It is not possible to fully explain such a variable intraocular inflammation following intraocular injection of bevacizumab obtained from the same vial, as observed in our patients.

It could, perhaps, result from the combination of two factors: first, the individual immune-mediated response to the drug injected; second, the different state (presence of particulates, degradation, etc) of the drug molecule. Patients injected with the same lot of bevacizumab in the first operative session did not develop any problems. These facts support the hypothesis that the inflammatory reaction may have been induced by a factor related to the storage or handling of the medication.

In conclusion, noninfectious inflammation is an adverse event that should be included in the patient informed consent procedure. This is of particular importance because the clinical distinction between infectious and noninfectious endophthalmitis may be challenging. All of our patients had primarily vitreous and anterior chamber cells, without hypopyon and/or anterior chamber fibrin. Hypopyon or fibrin is very common in infectious endopththalmitis but uncommon in the setting of noninfectious endopththalmitis. Thus, they are very strong predictors of an infectious process. Not all cases of infectious endopththalmitis present with hypopyon or fibrin. On the contrary, if an eye presents with hypopyon and fibrin, one must assume an infectious etiology until proven otherwise. Nonetheless, in cases of doubt vitreous tap with intravitreal antibiotic injection should always be considered. Noninfectious endopththalmitis has been found in all anti-VEGF agents.

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Digital Journal of Ophthalmology, Vol. 22