The Impact of Prefilled Syringes on Endophthalmitis Following Intravitreal Injection of Ranibizumab

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• PURPOSE: To compare the rates of infectious endophthalmitis following intravitreal injection of ranibizumab using prefilled syringes vs conventional preparation.

• DESIGN: Multicenter retrospective cohort study.

• METHODS: All eyes receiving intravitreal injection of 0.5 mg ranibizumab for retinal vascular diseases at 10 retina practices across the United States (2016 to 2017) and Japan (2009 to 2017) were included. The total numbers of eyes and injections were determined from billing codes. Endophthalmitis cases were determined from billing records and evaluated with chart review. Primary outcome was the rate of postinjection acute endophthalmitis. Secondary outcomes were visual acuity and microbial spectrum.

• RESULTS: A total of 243 754 intravitreal 0.5 mg ranibizumab injections (165 347 conventional and 78 407 prefilled) were administered to 43 132 unique patients during the study period. In the conventional ranibizumab group, a total of 43 cases of suspected endophthalmitis occurred (0.026%; 1 in 3845 injections) and 22 cases of culture-positive endophthalmitis occurred (0.013%; 1 in 7516 injections). In the prefilled ranibizumab group, 12 cases of suspected endophthalmitis occurred (0.015%; 1 in 6534 injections) and 2 cases of culture-positive endophthalmitis occurred (0.0026%; 1 in 39 204 injections). Prefilled syringes were associated with a trend toward decreased risk of suspected endophthalmitis (odds ratio 0.59; 95% confidence interval 0.31-1.12; $P = .10$) and a statistically significant decreased risk of culture-positive endophthalmitis (odds ratio 0.19; 95% confidence interval 0.045-0.82; $P = .025$). Average logMAR vision loss at final follow-up was significantly worse for eyes that developed endophthalmitis from the conventional ranibizumab preparation compared to the prefilled syringe group (4.45 lines lost from baseline acuity vs 0.38 lines lost; $P = .0062$). Oral-associated flora was found in 27.3% (6/22) of conventional ranibizumab culture-positive endophthalmitis cases (3 cases of *Streptococcus viridans*, 3 cases of *Enterococcus faecalis*) compared to 0 cases in the prefilled ranibizumab group.

• CONCLUSION: In a large, multicenter, retrospective study the use of prefilled syringes during intravitreal injection of ranibizumab was associated with a reduced rate of culture-positive endophthalmitis, including from oral flora, as well as with improved visual acuity outcomes. (Am J Ophthalmol 2019;199:200–208. © 2018 Elsevier Inc. All rights reserved.)

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VASCULAR ENDOTHELIAL GROWTH FACTOR INHIBITORS (anti-VEGF) remain the standard of care to treat several common retinal diseases, primarily neovascular age-related macular degeneration (AMD), retinal vein occlusion (RVO), and diabetic macular edema (DME). Since the advent of anti-VEGF therapy, intravitreal injection use has become one of the most commonly performed procedures in all of medicine. In 2000, fewer than 2000 injections were performed across the United States; in 2016, over 3.2 million injections were administered.[1]

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While uncommon, acute bacterial endophthalmitis following intravitreal injection can be visually devastating.[2] Reported incidence rates have ranged from as high as 1 case in approximately 500 injections to as low as 1 in 19 000 injections, with the majority of large studies reporting an incidence rate of approximately 1 in 2000 injections.[1,3–9] While certain measures such as topical povidone-iodine[10,11] or aqueous chlorhexidine[12] and reducing dispersion of oral flora by minimizing speaking or use of a face mask may reduce endophthalmitis incidence,[13] other measures such as postinjection antibiotics,[6,14] operating room setting,[15] and lid scrubbing[16] do not seem to have an effect on the risk of postinjection endophthalmitis.

Traditionally, anti-VEGF medication is packaged in a glass vial and in order to prepare the medicine for injection, multiple steps must be taken, including aspiration with a large bore needle. The transfer of medication often occurs in a clinic or procedure room. Endophthalmitis likely occurs when bacteria are introduced into the eye at the time of a procedure. It is possible that transferring medication from a glass vial to a syringe could enable contamination of the medication, thereby increasing the risk of endophthalmitis.

Syringes prefilled with sterile medication eliminate the transfer process from storage vial to syringe, which reduces risk of contamination and may subsequently decrease the risk of infection. In June 2014, prefilled 0.5 mg ranibizumab syringes were commercially available in Japan. In October 2016, the United States Food and Drug Administration approved 0.5 mg prefilled ranibizumab syringes. The purpose of this study is to evaluate the rate and outcomes of endophthalmitis following prefilled vs conventional 0.5 mg ranibizumab intravitreal injection.

PATIENTS AND METHODS

• **OVERVIEW:** This multicenter retrospective comparative cohort study was prospectively approved by the Wills Eye Hospital Institutional Review Board as well as institutional review boards at each of the 10 participating centers. The study adhered to the tenets of the Declaration of Helsinki. Billing records were used to retrospectively identify all cases of endophthalmitis secondary to intravitreal injection of 0.5 mg ranibizumab (Genentech, South San Francisco, California, USA) in both conventional vials and prefilled syringes. Billing data were used to determine the total number of intravitreal injections, eyes, and patients; whether a conventional or prefilled injection was administered; sex; age; and indication for treatment. Charts of all patients who were treated for endophthalmitis were reviewed, and the diagnosis was confirmed. Recorded data included date of causative injection; date of tap and injection and/or vitrectomy; visual acuity before causative injection, at time of tap and inject and/or vitrectomy, at 3 and 6 months postprocedure, and at final follow-up; and microbial culture results.

• **INCLUSION AND EXCLUSION CRITERIA:** All eyes with presumed infectious endophthalmitis at each study site following intravitreal injection of ranibizumab were included. Suspected endophthalmitis was defined as any case in which clinical suspicion was high enough to warrant a tap and antimicrobial injection or pars plana vitrectomy. Culture-positive endophthalmitis was defined as any patient with bacterial growth on culture or a positive Gram stain from a vitreous or anterior chamber tap. Patients with presumed inflammatory endophthalmitis treated with topical steroids without tap and inject were excluded. Dates of inclusion were January 1, 2016 to December 31, 2017 for United States sites. As prefilled syringes were available earlier in Japan, dates of inclusion for Japanese sites were June 1, 2009 to December 31, 2017. We include dates prior to the availability of prefilled syringes within both countries during which only conventional preparation was available. Our study includes both conventional and prefilled syringes after the date on which prefilled syringes were approved in both countries.

• **INJECTION TECHNIQUE:** Across all 10 clinical sites, all injections were performed in office-based settings, either in a designated procedure room or in a clinical room where the examination was conducted. Eyes were prepped with a topical anesthetic and topical povidone-iodine per the routine of the injecting physician. Injection with a 30, 31, 32, or 33 gauge needle was performed 3.5 to 4.0 mm from the limbus. Physicians individually determined use of subconjunctival lidocaine, use of a bladed lid speculum, conjunctival displacement prior to injection, and superior vs inferior injection site. Injection techniques were not altered during the study period.

• **ENDOPHTHALMITIS TREATMENT PROTOCOL:** All eyes developing presumed infectious endophthalmitis immediately underwent a pars plana vitreous tap with aspiration and subsequent injection of intravitreal antibiotics and/or pars plana vitrectomy with vitreous culture and intravitreal antibiotics. If the physician was unable to obtain vitreous fluid, an aqueous tap was performed. Patients typically received intravitreal vancomycin (1 mg/0.1 mL) and ceftazidime (2 mg/0.1 mL). Intravitreal amikacin (400 μg/0.1 mL) was substituted for ceftazidime for patients with penicillin allergy at some sites. Patients were variably prescribed cycloplegic agents, topical antibiotics, and topical steroid drops.

• **OUTCOMES:** The primary outcome was the rate of endophthalmitis following intravitreal injection of ranibizumab. The secondary outcomes were visual acuity and microbial spectrum of culture-positive cases. Endophthalmitis

TABLE 1. Rates of Suspected and Culture-Positive Endophthalmitis for Prefilled Syringes vs Conventional Preparation of Ranibizumab

	Prefilled Syringes		Conventional Preparation		Odds Ratio	
	Injections	Cases (Incidence)	Injections	Cases (Incidence)	(95% Confidence Interval)	*P* Value
Suspected endophthalmitis	78 407	12 (0.015%) 1 in 6534 injections	165 347	43 (0.026%) 1 in 3845 injections	0.59 (0.31-1.12)	.10
Culture-positive endophthalmitis		2 (0.0026%) 1 in 39 204 injections		22 (0.013%) 1 in 7516 injections	0.19 (0.045-0.82)	.025

was considered culture-positive if there was a positive Gram stain and/or positive growth on culture plates as reported by the institutional microbiology laboratory. Snellen visual acuity was converted to logMAR equivalent. As established by prior studies,[17,18] vision levels of counting fingers, hand motion, light perception, and no light perception were assigned visual acuity values of 1.0/200, 0.5/200, 0.25/200, and 0.125/200 (logMAR equivalent 2.3, 2.6, 2.9, 3.2, respectively). Clinical variables were analyzed using Excel (Microsoft, Redmond, Washington, USA) and statistical analysis was performed using Stata Version 14 (StataCorp, College Station, Texas, USA).

RESULTS

• **RATE OF ENDOPHTHALMITIS:** During our study period, a total of 243 754 intravitreal injections of ranibizumab were administered to 43132 unique patients across all 10 institutions. Average patient age was 80.3 years and 61.5% of patients were female. Treatment indication was neovascular AMD for 82.1% of injections, branch retinal vein occlusion (BRVO) 7.9%, central retinal vein occlusion (CRVO) 5.3%, DME 0.5%, and other pathologies 4.2%, including myopic choroidal neovascularization. Overall, a total of 55 patients were treated for suspected endophthalmitis (0.023%, 1 in 4432 injections) and 24 cases were culture-positive (0.0098%, 1 in 10 156 injections).

In the conventional ranibizumab group, a total of 165 347 injections were administered. A total of 43 cases of suspected endophthalmitis occurred (0.026%; 1 in 3845 injections), of which 22 cases were culture-positive endophthalmitis (0.013%; 1 in 7516 injections) (Table 1). Thirty-three eyes received ranibizumab injection for neovascular AMD, 5 received injection for BRVO, 4 for CRVO, and 1 for DME. Causative organisms included 11 cases of coagulase-negative *Staphylococcus*, 3 cases of *Streptococcus viridans*, 3 cases of *Enterococcus faecalis*, 1 case of *Staphylococcus aureus*, 1 case of *Stenotrophomonas maltophilia* (a gram-negative rod), and 3 cases of gram-positive cocci on Gram stain with no growth on cultures (Table 2).

In the prefilled ranibizumab group, 78 407 injections were administered and 12 cases of suspected endophthalmitis occurred (0.015%; 1 in 6534 injections), of which 2 cases were culture-positive (0.0026%; 1 in 39 204 injections) (Table 1). Eight eyes received ranibizumab injection for neovascular AMD, 3 received injection for BRVO, and 1 for CRVO. Causative organisms included 1 case of coagulase-negative *Staphylococcus* and 1 case of *Staphylococcus aureus* (Table 3).

Compared to the conventional vial, use of prefilled syringes was associated with a trend toward decreased risk of suspected endophthalmitis (odds ratio 0.59; 95% confidence interval 0.31-1.12; $P = .10$) and a statistically significant decreased risk of culture-positive endophthalmitis (odds ratio 0.19; 95% confidence interval 0.045-0.82; $P = .025$) (Table 1). Oral-associated flora was found in 6 of the 22 (27.3%) cases of conventional ranibizumab culture-positive endophthalmitis (3 cases of *Streptococcus viridans*, 3 cases of *Enterococcus faecalis*) compared to 0 of the 2 culture-positive cases in the prefilled ranibizumab group ($P = 1.0$).

Overall, patients with presumed endophthalmitis presented an average of 6.0 days after injection (range 0-61 days). No patients had any intraocular surgeries or procedures between administration of the intravitreal injection and presentation with presumed endophthalmitis. The vast majority of cases presented within 7 days of intravitreal injection (85.5%). Eight patients (6 in the conventional group, 2 in the prefilled group) presented more than 7 days after injection. Of these 8 patients with delayed presentation, 6 patients presented within 3 weeks of injection. One patient in the prefilled group presented 24 days after injection; however, this patient reported severe pain and vision loss beginning approximately 1 week after injection. One patient in the conventional group presented 61 days after injection; this patient reported pain and decreased vision for several weeks prior to returning to the clinic for further evaluation. Patients receiving conventional ranibizumab injection presented an average of 5.9 days after injection compared to an average of 6.8 days for patients receiving prefilled syringes ($P = .70$). Regardless of method of drug preparation, culture-positive cases presented an average of 7.3 days after injection (range 1-61 days) compared to 5.1 days (range 0-24 days) for culture-negative cases ($P = .42$). Fewer of the prefilled ranibizumab cases were culture-positive (16.7%; 2/12) compared to the conventional ranibizumab group (51.1%; 22/43) ($P = .049$).

TABLE 2. Visual Acuity Outcomes Following Endophthalmitis From Conventional Preparation of Ranibizumab

Patient	VA at Injection	VA at 3 Months Post Endophthalmitis	VA at 6 Months Post Endophthalmitis	VA at Final Follow-up	Culture Results
1	20/25	CF	CF	CF	*Streptococcus viridans*
2	20/400	CF	CF	CF	*Streptococcus viridans*
3	20/400	CF	CF	20/400	*Streptococcus viridans*
4	20/50	CF	HM	HM	*Enterococcus faecalis*
5	20/200	20/400	20/200	20/200	*Enterococcus faecalis*
6	20/30	HM	LP	LP	*Enterococcus faecalis*
7	20/25	20/30	20/20	20/20	*Staphylococcus epidermidis*
8	20/40	20/60	20/40	20/60	*Staphylococcus epidermidis*
9	20/70	20/200	20/80	20/60	*Staphylococcus epidermidis*
10	20/20	20/30	20/25	20/100	*Staphylococcus epidermidis*
11	20/40	20/70	20/50	20/50	*Staphylococcus capitis*
12	20/50	LP	LP	LP	*Staphylococcus aureus*
13	20/30	20/200	20/60	20/40	Coag- *Staphylococcus*
14	20/60	20/80	20/60	20/80	Coag- *Staphylococcus*
15	20/50	20/50	20/30	20/40	Coag- *Staphylococcus*
16	20/50	CF	20/400	20/200	Coag- *Staphylococcus*
17	20/30	CF	20/200	20/200	Coag- *Staphylococcus*
18	20/80	20/100	20/60	20/60	Coag- *Staphylococcus*
19	CF	n/a	n/a	NLP	*Stentrophomonas maltophilia*
20	20/30	20/50	20/40	20/25	Gram-positive cocci (Stain)
21	20/60	CF	CF	CF	Gram-positive cocci (Stain)
22	20/70	n/a	n/a	CF	Gram-positive cocci (Stain)
23	20/200	20/200	20/200	20/200	Negative
24	20/70	20/400	20/400	20/400	Negative
25	20/40	20/30	20/30	20/30	Negative
26	4/200	n/a	n/a	CF	Negative
27	20/40	20/30	20/30	20/30	Negative
28	20/80	20/50	20/40	20/40	Negative
29	20/40	20/100	20/60	20/200	Negative
30	20/80	20/60	20/200	20/200	Negative
31	1/200	5/200	3/200	3/200	Negative
32	20/60	20/200	n/a	20/200	Negative
33	20/30	20/50	20/60	20/40	Negative
34	20/200	20/60	20/60	20/40	Negative
35	CF	CF	CF	CF	Negative
36	20/40	20/200	20/200	20/60	Negative
37	20/25	20/25	20/25	20/25	Negative
38	20/25	NLP	NLP	NLP	Negative
39	20/25	20/80	20/30	20/30	Negative
40	20/50	20/25	20/30	20/25	Negative
41	20/30	20/30	20/50	20/40	Negative
42	HM	HM	HM	CF	Negative
43	20/30	20/40	20/30	20/25	Negative

CF = count fingers; Coag- = coagulase negative; HM = hand motion; LP = light perception; n/a = not available; NLP = no light perception; VA = visual acuity.

Overall, affected eyes received an average of 19 injections (range 1-106 injections) prior to developing suspected endophthalmitis. Patients treated with conventional ranibizumab injection received an average of 18 injections prior to developing suspected endophthalmitis vs an average of 24 injections for patients receiving prefilled syringes ($P = .50$). Regardless of method of drug preparation, culture-positive cases received an average of 19 injections compared to 20 injections for culture-negative cases ($P = .98$).

• **VISUAL OUTCOMES:** Mean follow-up for all suspected endophthalmitis cases was 11.3 months (range 1 day to 43.4 months). Average follow-up for patients with endophthalmitis receiving conventional preparation was

TABLE 3. Visual Acuity Outcomes Following Endophthalmitis From Prefilled Syringes of Ranibizumab

Patient	VA at Injection	VA at 3 Months Post Endophthalmitis	VA at 6 Months Post Endophthalmitis	VA at Final Follow-up	Culture Results
1	20/25	20/30	n/a	20/30	*Staphylococcus epidermidis*
2	20/50	n/a	n/a	20/50	*Staphylococcus aureus*
3	20/70	n/a	n/a	20/50	Negative
4	20/200	20/400	20/400	20/400	Negative
5	20/20	20/20	20/20	20/20	Negative
6	20/100	20/200	20/200	20/200	Negative
7	20/30	20/40	20/40	20/40	Negative
8	20/30	20/40	20/30	20/30	Negative
9	20/25	20/25	20/30	20/30	Negative
10	20/200	20/40	20/40	20/40	Negative
11	20/25	20/30	20/30	20/30	Negative
12	20/40	20/40	20/60	20/60	Negative

n/a = not available; VA = visual acuity.

TABLE 4. Visual Acuity Outcomes at Final Follow-up for Endophthalmitis Following Prefilled Syringes vs Conventional Preparation of Ranibizumab

	Prefilled Syringes (N = 12)	Conventional Preparation (N = 43)	*P* Value
Average lines of Snellen visual acuity lost from baseline	0.38	4.45	.0062
Average visual acuity logMAR (approximate Snellen equivalent)	0.42 (20/50)	1.13 (20/250)	.00039
Visual acuity of count fingers or worse	0%	27.9%	.050

12.8 months and 6.1 months for prefilled syringes ($P = .0017$). Overall average baseline visual acuity was logMAR 0.58 (approximately 20/80) with no statistically significant difference between conventional syringes (logMAR 0.63; approximately 20/80) and prefilled syringes (logMAR 0.39; approximately 20/50) ($P = .091$). By 6 months post endophthalmitis a minority of eyes (35.4%) lost 3 or more lines of visual acuity compared to baseline vision.

Visual acuity outcomes of endophthalmitis cases were generally better for patients receiving prefilled ranibizumab compared to patients receiving conventional ranibizumab. Average vision loss at final follow-up was significantly worse for eyes that developed endophthalmitis from the conventional preparation compared to the prefilled syringe group (4.45 lines lost from baseline acuity vs 0.38 lines lost; $P = .0062$) (Table 4). While average baseline vision was not significantly different between the groups, at 6 months post endophthalmitis treatment average visual acuity for the prefilled group returned nearly to baseline, with logMAR 0.44 (approximately 20/50) compared to logMAR 1.07 (approximately 20/250) for the conventional ranibizumab group ($P = .0060$). At final follow-up, average visual acuity for the prefilled group was logMAR 0.42 (approximately 20/50) compared to logMAR 1.13 (approximately 20/250) for the conventional ranibizumab group ($P = .00039$). Overall, 12 patients (21.8%) who developed endophthalmitis had visual acuity of count fingers or worse at final follow-up—all of whom received an injection of conventional ranibizumab. Patients who developed endophthalmitis after receiving the conventional preparation of ranibizumab were more likely to have vision of count fingers or worse at final follow-up (27.9%) compared to patients receiving prefilled ranibizumab (0%) ($P = .050$).

Visual outcomes were generally worse for culture-positive endophthalmitis cases compared to culture-negative cases regardless of ranibizumab preparation. Average vision loss at final follow-up was significantly worse for eyes with culture-positive endophthalmitis compared to culture-negative endophthalmitis (6.47 lines lost from baseline acuity vs 1.32 lines lost; $P = .015$) (Table 5). Average visual acuity 6 months post endophthalmitis was 1.22 logMAR (approximate Snellen equivalent 20/320) for culture-positive cases compared to 0.77 logMAR (approximate Snellen equivalent 20/125) for culture-negative cases ($P = .12$). At final follow-up, average visual acuity for culture-positive cases was logMAR 1.23 (approximately 20/320) vs logMAR 0.78 (approximately 20/125) for culture-negative cases

TABLE 5. Visual Acuity Outcomes at Final Follow-up for Culture-Positive and Oral-Associated Culture-Positive vs Culture-Negative Endophthalmitis Cases Regardless of Ranibizumab Preparation

	Culture-Positive Endophthalmitis (N = 24)	Culture-Negative Endophthalmitis (N = 31)	*P* Value
Average lines of Snellen visual acuity lost from baseline	6.47	1.32	.015
Average visual acuity logMAR (approximate Snellen equivalent)	1.23 (20/320)	0.78 (20/125)	.089
Visual acuity of count fingers or worse	33.3%	12.9%	.10
	Oral-Associated Culture-Positive Endophthalmitis (N = 6)	**Culture-Negative Endophthalmitis (N = 31)**	***P* Value**
Average lines of Snellen visual acuity lost from baseline	11.73	1.32	.068
Average visual acuity logMAR (approximate Snellen equivalent)	2.06 (<20/2000)	0.78 (20/125)	.0060
Visual acuity of count fingers or worse	66.7%	12.9%	.013

($P = .089$). For culture-positive cases, 33.3% of cases had visual acuity of count fingers or worse at final follow-up compared to 12.9% of culture-negative patients ($P = .10$). While the number of culture-positive cases in the prefilled group was too small for statistical comparison, we compared results of culture-negative cases between prefilled and conventional preparation. For culture-negative cases, average vision loss at final follow-up compared to baseline acuity was no different between conventional injections and prefilled syringes (1.8 lines lost vs 0.4 lines lost; $P = .26$).

Visual acuity outcomes for culture-positive endophthalmitis cases associated with oral flora were poor. Overall, 6 cases of culture-positive endophthalmitis were caused by oral flora (3 cases of *Streptococcus viridans*, 3 cases of *Enterococcus faecalis*), all of which occurred in the conventional ranibizumab group. Average vision loss at final follow-up was 11.73 lines from baseline for oral flora–associated endophthalmitis compared to 1.89 lines for non–oral flora–associated culture-positive endophthalmitis ($P = .19$). Average visual acuity 6 months post infection for the oral flora–associated endophthalmitis cases was 2.23 logMAR (Snellen equivalent <20/2000) compared to 0.78 logMAR for non–oral flora–associated culture-positive cases (approximately 20/125; $P = .0012$). At final follow-up, average visual acuity for the oral flora–associated endophthalmitis cases was 2.06 logMAR (Snellen equivalent <20/2000) compared to 0.96 logMAR for non–oral flora–associated culture-positive cases (approximately 20/160; $P = .014$) (Table 5). For oral flora–associated endophthalmitis cases, visual acuity of count fingers or worse was present in 5 of 6 eyes at 6 months and 4 of 6 eyes at final follow-up.

Initial treatment was intravitreal tap and injection of antibiotics in 50 cases and primary pars plana vitrectomy with injection of antibiotics in 5 cases (4 in the conventional group, 1 in the prefilled group). Pars plana vitrectomy was performed as a secondary procedure in 6 cases (5 in the conventional group, 1 in the prefilled group). No difference in final average visual outcomes was found between endophthalmitis cases receiving initial procedure of tap and injection (logMAR 0.93; approximate Snellen 20/160) vs primary pars plana vitrectomy (logMAR 1.44; approximate Snellen 20/500; $P = .41$).

DISCUSSION

ENDOPHTHALMITIS FOLLOWING INTRAVITREAL INJECTION remains an uncommon event. Any prophylaxis measure that might lower the risk of infection requires assessment of a large number of injections in order to achieve adequate power to detect a significantly decreased risk. In our study of 10 institutions across the United States and Japan, we assessed endophthalmitis rates after nearly 250 000 intravitreal ranibizumab injections with detailed confirmation of the diagnosis and clinical course. Although the difference in suspected endophthalmitis rates was not statistically significant, there was a trend toward a lower rate in the prefilled syringe group. Prefilled syringes of ranibizumab were associated with lower rates of culture-positive endophthalmitis and with improved visual outcomes, driven in part by fewer cases of endophthalmitis caused by oral flora, which had worse outcomes.

Currently, few studies have investigated the impact of prefilled syringes on endophthalmitis risk. A recent nationwide study in France of acute endophthalmitis with intravitreal injections of corticosteroids or anti-VEGF agents reported a lower risk of endophthalmitis with prefilled injections.[19] In this study, prefilled syringes of ranibizumab decreased the rate of endophthalmitis by 40% compared to room preparation of nonprefilled ranibizumab and by 46% for aflibercept, only available as a nonprefilled medication.

One prior study evaluated the effect of prefilled syringes on endophthalmitis risk with intravitreal injection of bevacizumab. Endophthalmitis rates were reduced from 1 in 425 injections with bevacizumab drawn multiple times from the same vial to 1 in 2000 injections with prefilled syringes of bevacizumab made by a compounding pharmacy ($P < .003$).[20] Important differences exist between this study and our own. A single manufacturer makes ranibizumab, whereas bevacizumab must be compounded by a pharmacy. Prefilled syringes of bevacizumab indicate that a compounding pharmacy—not the manufacturer—has filled the syringes with the medication prior to arrival in the clinic. Additionally, in our study, we compare prefilled ranibizumab syringes to ranibizumab drawn in a clinic from an individually assigned vial injected into a single patient. In the aforementioned study, prefilled syringes of bevacizumab were compared to bevacizumab repeatedly drawn in a clinic from a large vial that was injected into multiple patients.

Several studies have reported that oral-associated flora are more common with endophthalmitis occurring after intravitreal injection than after other vitreoretinal procedures.[2,21–24] For the 22 culture-positive cases of endophthalmitis in the conventional group, 6 cases grew *Streptococcus* or *Enterococcus* species (27.3%), which could be secondary to oral droplet transmission. One difference between intravitreal injections and other ocular procedures is that injections are often performed in an office-based setting, often with variable to no masking of the physician, patient, or technician. In contrast, all persons in the operating room are masked and, in the case of the patient, draped. Some studies have suggested rates of endophthalmitis may be lower when performed in an operating room setting.[24] One suggested strategy to reduce droplet transmission is cessation of talking during the injection, which has shown modest evidence of lowering endophthalmitis risk.[13]

Several possibilities could contribute to a decreased rate of endophthalmitis with prefilled syringes. By eliminating several steps required to transfer medication from vial to syringe with conventional preparation of anti-VEGF medications, there may be a lower risk of introducing bacteria during this process. Given that the drug transfer with conventional preparation typically is performed in a nonsterile environment, the possibility of contaminating the medication exists. If an individual touches the top of the vial or the transfer needle, skin flora may be introduced. Additionally, oropharyngeal droplets may be inadvertently introduced onto the uncapped vial or transfer needle. While our results trended toward lower rates of suspected endophthalmitis for prefilled syringes, we did find a statistically significant decreased risk of culture-positive endophthalmitis with prefilled syringes. Interestingly, in our study approximately one quarter of culture-positive endophthalmitis cases were caused by oral-associated flora in the conventional preparation group compared to no cases within the prefilled group, which lends support to the hypothesis that fewer steps to prepare the drug for injection limits exposure to aerosolized droplets containing oral bacteria.

Visual acuity outcomes following endophthalmitis were better in eyes receiving prefilled ranibizumab syringes compared to conventional preparation. Average loss of vision from baseline acuity was less than half of one line for the prefilled group and 4.5 lines for the conventional preparation. While baseline visual acuities were not significantly different between the groups, average postendophthalmitis visual acuity at final follow-up was substantially better for prefilled injections compared to the conventional group, with approximate Snellen acuities of 20/50 vs 20/250. The difference in visual outcomes between the groups appears to be primarily driven by culture-positive and oral flora–associated endophthalmitis cases, both of which were more frequent in the conventional preparation group. Visual outcomes of culture-negative cases were no different between conventional preparation and prefilled syringes.

Regardless of medication preparation, visual outcomes were worse for culture-positive cases, with an average loss of 6.5 lines of vision from baseline acuity compared to a loss of 1.3 lines for culture-negative cases. At final follow-up, average acuity was approximately 20/320 for culture-positive cases compared to 20/125 for culture-negative cases. Oral flora–associated endophthalmitis cases lost an average of almost 12 lines of vision and average Snellen acuity at final follow-up was less than 20/2000, while non–oral flora–associated culture-positive cases lost 2 lines of vision and averaged a Snellen acuity of 20/160 at final follow-up. Prior studies have also found that endophthalmitis from oral flora—primarily *Streptococcus* and *Enterococcus* species—tend to have a poor visual prognosis.[23,25] While our data show that culture-positive and oral flora–associated endophthalmitis have worse visual outcomes, it remains unclear precisely why conventional drug preparation might have a higher rate of culture positivity.

While most baseline characteristics of patients who developed endophthalmitis were not significantly different between the conventional and prefilled ranibizumab groups—baseline visual acuity, number of injections prior to endophthalmitis, days to presentation—there was a significantly longer average clinical follow-up for endophthalmitis cases within the conventional group. This difference is owing to the shorter time period during which prefilled syringes have been available—particularly in the United States. The difference in follow-up length is unlikely to affect our study's conclusions, as rates of endophthalmitis should not be affected by this follow-up difference. If a bias were potentially introduced from the difference in average follow-up, we would expect the data to bias toward worse visual outcomes for the prefilled group, given the shorter time for treatment. However, with our finding of improved visual outcomes for the prefilled group, we do not believe this difference between the groups substantially affects our conclusions.

While the vast majority of patients in our study were treated for AMD, BRVO, or CRVO, ranibizumab 0.5 mg is approved for treatment of diabetic indications in Japan. Some evidence suggests endophthalmitis rates may be higher for patients with diabetes.[7] We did not exclude patients with diabetes or patients receiving injections for diabetic indications, nor did we separately evaluate these patients, which could be a source of bias. However, only 0.5% of injections were given for diabetic indications and only 1 patient developed endophthalmitis following an injection for diabetic macular edema.

Strengths of this study include the large number of intravitreal injections performed across multiple institutions. Importantly, identification of endophthalmitis cases was not dependent on physician recall. All endophthalmitis cases were confirmed with detailed chart review, which is not possible with insurance claim databases. Limitations of this study are related to its retrospective, cohort nature. Visual acuity measurements in retrospective studies may be variable between and within centers. It is possible that a patient could have developed endophthalmitis and sought treatment at an outside institution, but this is unlikely and was not reported by any institution. Furthermore, this potential loss to follow-up would be unlikely to have affected one medication preparation group over another. Culture-positive cases were more frequent in the conventional group. However, a study examining organism identification between traditional culture, polymerase chain reaction, and bio representation is situ karyotyping (BRisK) did not find a significant difference in bacteria or bacterial DNA detection between the 3 testing modalities.[26]

Our study may be limited by an imbalance in the number of prefilled and conventional syringes. The dates of inclusion for this study were chosen to balance the amount of time before and after release of prefilled syringes within each country. For the United States, we chose a 2-year period and for Japan, we chose an 8.5-year period. While the time periods were roughly balanced, differing rates of prefilled syringe adoption led to an imbalance in the number of injections in each group. The number of injections with prefilled syringes was less than half the number of injections in the conventional preparation, which could lead to an ascertainment bias in the rate of endophthalmitis, representing an additional limitation of our study.

Further limitations of the study include the lack of a standardized protocol for intravitreal injections across the institutions. At some centers, physicians prepare the ranibizumab for conventional injection while other sites rely on nonphysician staff to transfer the medication from vial to syringe. We were unable to assess the number of injections prepared by nonphysician staff, and it is possible that experience of personnel preparing the medication could affect the endophthalmitis rate. Numerous confounding factors could contribute to our findings and the association should not be interpreted as causational. However, it is notable that despite the possibility of confounding, our multicenter study did reach significant findings. Additionally, a recent study in France also found that prefilled syringes were associated with lower rates of postinjection endophthalmitis.[19]

While all vitreous and aqueous taps were processed by certified microbiological laboratories, no standardized culture protocol was in place. Furthermore, the risk of endophthalmitis was evaluated over time—a 2-year period in the United States and a 9-year period in Japan. It may be possible that other factors could have contributed to the decreased risk of injection observed in our study. However, no other interventions were implemented during these time periods that have been shown to decrease the risk of infection. While these limitations are inherent in this type of multicenter retrospective study, our results reflect real-world experience across multiple locations and represent the environment in which many retina practices operate. Ideally, a randomized controlled study could evaluate the risk of endophthalmitis with prefilled syringes vs conventional preparation. However, the low incidence of endophthalmitis makes such a study prohibitive. Assuming that the risk of suspected endophthalmitis with conventional preparation is 1 in 3800 injections and that prefilled syringes may have a relative risk of 0.6 (similar to our findings), a study would need 596 596 injections to be sufficiently powered to detect a significant difference between the 2 groups with a confidence level of 0.95 and power of 0.8.

While the incidence of endophthalmitis following intravitreal injection remains low, treatment of retinal pathologies can require years of repeated injections. Our patients received an average of 19 injections prior to developing endophthalmitis, with a range reaching 106 injections. With increased injections comes an increased cumulative risk. Consequently, any intervention that may lower the risk of endophthalmitis following intravitreal injection should be evaluated. In our large, multicenter, retrospective study, our findings indicate that prefilled syringes of intravitreal medication are associated with a decreased rate of endophthalmitis and improved visual outcomes.

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