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Prospective Study of Silicone Oil Microdroplets in Eyes Receiving Intravitreal Anti-Vascular Endothelial Growth Factor Therapy in 3 Different Syringes

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Purpose: To compare the prevalence of intravitreal silicone oil microdroplets detected by slit-lamp biomicroscopy in eyes with 6 or more injections of the same anti vascular endothelial growth factor (VEGF) drug.

Design: Prospective, cross-sectional case series.

Participants: A total of 260 consecutive eyes receiving 1 of 3 intravitreal anti-VEGF drugs for choroidal neovascularization, diabetic macular edema, or venous occlusive disease. The control group included 147 fellow eyes with no prior intravitreal injections.

Methods: The anterior and mid-vitreous were carefully examined using 12× to 16× magnification through dilated pupils with ocular saccades before an injection. Silicone oil microdroplets were graded on a scale from 0 to 4+ based on the number and size of droplets.

Main Outcome Measures: Presence and severity of silicone oil microdroplets in the vitreous.

Results: Silicone oil microdroplets were observed in 78.3% of eyes receiving bevacizumab in Becton Dickinson (BD, Franklin Lakes, NJ) 0.3-mL polypropylene syringes, 14.4% of eyes receiving ranibizumab in 1.0-mL BD polypropylene syringes or more recently glass prefilled syringes, 48.5% of eyes receiving aflibercept in 1.0-mL BD polycarbonate syringes, and 0% of eyes in controls. The differences among the 4 groups were statistically significant at $P < 0.001$. The severity of silicone oil microdroplets was significantly greater in eyes using BD 0.3-mL polypropylene syringes than BD 1.0-mL polypropylene syringes, BD 1.0-mL polycarbonate syringes, or controls ($P < 0.001$). The severity of silicone oil microdroplets in eyes using BD 1.0-mL polycarbonate syringes was significantly greater than BD 1.0-mL polypropylene syringes ($P = 0.012$) and controls ($P < 0.001$). There was no significant difference between silicone oil microdroplet severity between BD 1.0-mL polypropylene syringes and controls ($P = 1.0$).

Conclusions: The BD 0.3-mL polypropylene syringes with repackaged bevacizumab and the BD 1.0-mL polycarbonate syringes with aflibercept cause a higher likelihood of silicone oil microdroplets. Intravitreal injections in eyes receiving multiple regular anti-VEGF injections should be supplied in silicone-free syringes. *Ophthalmology Retina 2021;5:234-240 © 2020 by the American Academy of Ophthalmology*

The presence of intravitreal silicone oil microdroplets has been recognized most frequently in eyes receiving multiple intravitreal injections of anti vascular endothelial growth factor (VEGF). These anti-VEGF injections have become common in the treatment of choroidal neovascularization associated with age-related macular degeneration and other causes, such as myopia or histoplasmosis. Other common indications for treatment with anti-VEGF drugs include macular edema associated with venous occlusive disease and diabetic retinopathy. Many patients require these intravitreal injections every 4 to 12 weeks, and some patients have been receiving these injections since 2005.

The 3 anti-VEGF drugs commonly used are bevacizumab (Avastin, produced by Genentech/Roche, South San Francisco, CA), ranibizumab (Lucentis, produced by Genentech/Roche), and aflibercept (Eylea, produced by Regeneron Pharmaceuticals, Tarrytown, NY). Each of the 3 drugs was typically used in the United States in a different syringe type. Minute amounts of silicone oil are typically placed in the barrel of most syringes to lubricate the plunger.[1] The presence of silicone oil microdroplets was recognized more than 30 years ago with insulin syringes.[1] The silicone is important to prevent the plunger from sticking during manufacturing and shipping. It also helps to facilitate a smoother injection by minimizing the break-loose force to start the injection and glide force to complete the injections.[2] The syringe type and needle used are important because they can have an impact on the amount of silicone oil that is injected into the eye with an intravitreal injection. Bevacizumab is repackaged from vials into syringes by a compounding pharmacy or outsourcing facility. The prefilled syringe has typically

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been shipped in the past to the ophthalmologist in a 0.3-mL insulin (Fig 1A) or 1.0-mL polypropylene tuberculin syringe (Fig 1B). It is important to recall that the bevacizumab is stored in the syringe for up to 3 months before use, based on the expiration date supplied by the repackager. These 2 types of syringes are typically produced by Becton Dickinson (BD, Franklin Lakes, NJ), a large supplier of syringes to the medical field. Ranibizumab was supplied in glass vials with a rubber stopper, and no BD syringe was in the kit supplied with the drug until early 2017 when a prefilled glass syringe became available for the 0.5-mg dose. The 0.3-mg ranibizumab glass prefilled syringes became available in 2018. Most ophthalmologists used the BD 1-mL tuberculin syringe (Fig 1B) to draw up and deliver ranibizumab before the introduction of the ranibizumab prefilled syringes. Aflibercept is supplied in a glass vial with a different BD 1.0-mL polycarbonate syringe supplied in the kit with the aflibercept (Fig 1C) before the introduction of glass prefilled aflibercept syringes in 2019. The 0.3-mL BD insulin syringe comes with a 31-gauge needle attached ("staked syringe"). Most ophthalmologists have attached the 30-gauge BD needle or 32-gauge needle (TSK Laboratory, Tochigi-Ken, Japan) because BD does not sell a disposable 32-gauge needle.

The presence of microdroplets of silicone oil in the vitreous of eyes receiving these injections was first reported in 2006 and was noted to be an infrequent complication after intravitreal injection of pegaptanib, an anti-VEGF drug in a prefilled syringe but rarely used today, and after triamcinolone acetonide injections.[3] A report 2 years later found silicone oil in 15 eyes of 1529 injections, but the number of patients is not reported, so a per patient prevalence cannot be calculated.[4] Subsequent publications have noted silicone oil droplets primarily in eyes treated with repackaged bevacizumab.[5-13] Silicone oil microdroplets have been reported less frequently after injection of ranibizumab[5,9] and aflibercept.[12] The purpose of the current study was to evaluate the prevalence and severity of silicone oil microdroplets in the vitreous of eyes that have received numerous intravitreal anti-VEGF injections of the same drug using the same syringe type for that particular drug.

Methods

The presence and severity of silicone oil microdroplets were evaluated prospectively in a consecutive series of all eyes in the author's practice on the day of an intravitreal anti VEGF injection before the injection for choroidal neovascularization or macular edema due to venous occlusive disease or diabetic retinopathy. The data were collected on eyes examined between November 5, 2018, and January 31, 2019. The evaluation was performed only when the eye was dilated because the eye is not routinely dilated on every visit. Eyes had to have a minimum of 6 injections of the same intravitreal drug (bevacizumab, ranibizumab, or aflibercept) in the same syringe type at the time of grading. Any eye that had a different anti VEGF drug/syringe injected at some point during their prior treatment was excluded. A total of 227 eyes were excluded primarily because they had more than 1 different anti VEGF drug injected or had fewer than 6 injections. This would allow comparison of the prevalence of silicone oil microdroplets among the 3 drugs that were each delivered using 3 different BD syringes, respectively, in addition to the prefilled syringe introduced for ranibizumab. Bevacizumab was supplied by Avella (Phoenix, AZ) in the BD 0.3 mL insulin syringes. Ranibizumab was delivered in the BD 1.0 mL tuberculin syringe until the prefilled syringes became available for the 0.5 mg and 0.3 mg dose in 2017 and 2018, respectively. The ranibizumab prefilled syringes were used once they became available. Aflibercept was delivered in

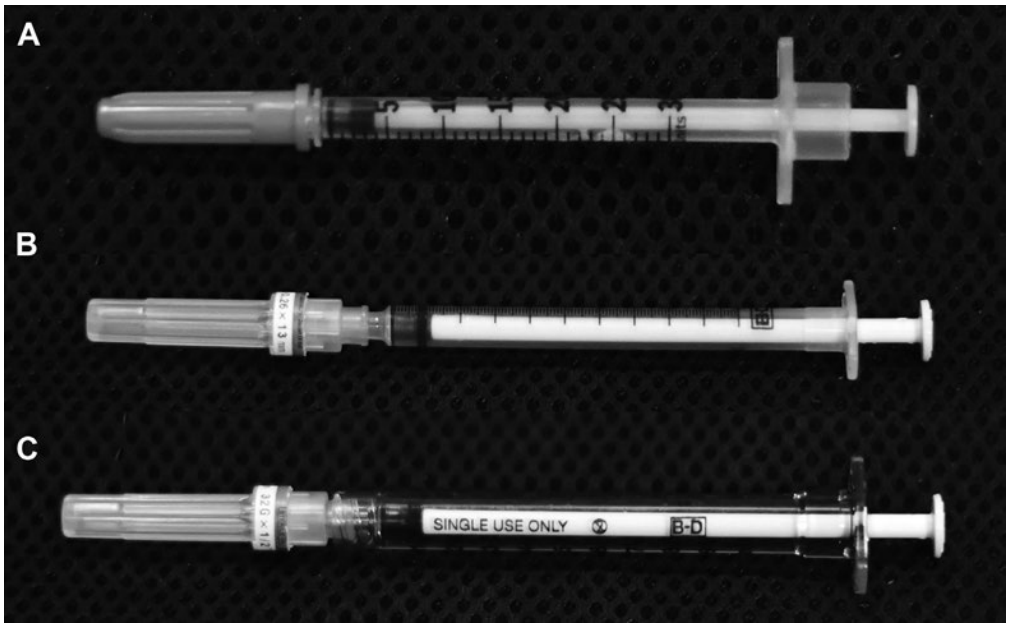

Figure 1. A, Becton Dickinson (BD, Franklin Lakes, NJ) 0.3-mL polypropylene insulin syringe. **B,** BD 1.0-mL polypropylene syringe. **C,** BD 1.0-mL polycarbonate syringe.

Table 1. Grading Scale for Silicone Oil Microdroplets in the Vitreous

Silicone Oil Severity Grade	
0	No droplets
0.5	1–2 microdroplets < 0.1 mm diameter
1.0	3–5 microdroplets < 0.1 mm diameter
2.0	5–7 microdroplets < 0.1 mm diameter or 1 large droplet > 0.1 mm diameter
3.0	8–10 microdroplets < 0.1 mm diameter or 2 larger droplets > 0.1 mm
4.0	>10 microdroplets < 0.1 mm or ≥3 larger droplets > 0.1 mm diameter

the BD 1.0 mL polycarbonate syringe supplied in the aflibercept kit, and none of the eyes in this study had aflibercept from the prefilled syringe that was not available at completion of data collection. The BD 30 gauge needle was used on all ranibizumab and aflibercept doses until switched to the TSK Laboratory 32 gauge needles in mid 2018. Other exclusion criteria were prior vitrectomy because the oil would float to the top of the eye and not be visible by slit lamp biomicroscopy, poor pupillary dilation, active uveitis with vitreous cells, and asteroid hyalosis because the author found it difficult to accurately identify silicone oil microdroplets under these circumstances. Eyes were excluded if they had not received anti VEGF injections within the past year. All eyes with prior injection of other drugs such as triamcinolone or dexamethasone implant were excluded. Institutional Review Board approval was obtained by The Greater Baltimore Medical Center Institutional Review Board. The study adhered to the tenets of the Declaration of Helsinki. The data was collected during routine care of the patients, and the IRB did not require consent.

The presence or absence of silicone oil microdroplets was assessed by an unmasked observer using an oblique slit beam at 12× to 16× magnification focused into the mid vitreous. The silicone oil microdroplets could be distinguished from other floaters or cells in the vitreous because they reflected light, causing them to appear like shiny bubbles. Patients were asked to saccade up and down, side to side several times because this improved detection of the silicone oil microdroplets. This examination of the vitreous typically took approximately 30 seconds, depending on patient cooperation with the saccades, and all grading was performed by the author. It was not possible to mask the author to the intravitreal anti VEGF drug received by the patient, but the goal was to find silicone oil microdroplets in all eyes, including controls. Silicone oil severity was graded using the following approximate scale because it was somewhat difficult to precisely count the number of silicone oil microdroplets (Table 1). The control group included fellow eyes of patients who were receiving intravitreal anti VEGF injections, but not necessarily those included in the study. They could be any fellow eye from a patient receiving intravitreal anti VEGF injection that was examined during the same time interval.

Statistical Analyses

The prevalence of any silicone oil was determined for each group and compared with one another using the chi square test with correction for multiple comparisons. The severity of silicone oil was compared among the 4 groups, including the controls using univariate analysis of variance with Bonferroni correction for multiple comparisons. Linear regression analysis was used to compare the severity of silicone oil and the number of injections for each of the 3 anti VEGF drugs. All analyses were performed using Statistical Package for the Social Sciences Subscription (IBM, Chicago, IL).

Results

There were 69 eyes receiving bevacizumab in BD 0.3 mL polypropylene syringes, 125 eyes receiving ranibizumab in BD 1.0 mL polypropylene syringes, and 66 eyes receiving aflibercept in BD 1.0 mL polycarbonate syringes. The control group consisted of 147 fellow eyes of patients receiving any anti VEGF injection with no prior intravitreal injections in that eye. The eyes receiving bevacizumab had a mean of 29 injections, eyes receiving ranibizumab had a mean of 33 injections, and eyes receiving aflibercept had 26 injections. There was no significant difference in the number of injections among the 3 syringe types by analysis of variance using Bonferroni correction for multiple comparisons (Table 2). Figure 2 shows the percentage of eyes with any visible silicone oil droplets in the vitreous. Silicone oil microplets were visible in 78% of bevacizumab delivered with the BD 0.3 mL BD polypropylene insulin syringe and 14% of ranibizumab delivered with the 1.0 mL BD polypropylene syringe or prefilled syringe. Silicone oil microdroplets were not seen in any of the 14 eyes receiving ranibizumab in the prefilled syringe only, but these eyes had many fewer injections because this syringe was more recently introduced. Silicone oil microdroplets were detected in 49% of aflibercept eyes delivered with the BD 1.0 mL polycarbonate syringe and none of the control eyes. The prevalence of silicone microdroplets was significantly greater in BD 0.3 mL polypropylene (bevacizumab) eyes than all other groups as well as BD 1.0 mL polycarbonate (aflibercept) compared with ranibizumab and control ($P < 0.001$ for all comparisons by Pearson chi square). The prevalence of any silicone oil microdroplets was significantly greater in BD 1.0 mL polypropylene (ranibizumab) eyes compared with control eyes ($P < 0.001$ by Pearson chi square). Figure 3 shows a graph of the severity of silicone oil for each of the 4 groups.

The silicone microdroplet severity score was compared using univariate analysis of variance with Bonferroni correction for multiple comparisons (Table 3). The severity grade of silicone oil microdroplets with bevacizumab in 0.3 mL BD insulin syringes was significantly greater ($P < 0.001$) than aflibercept in BD polycarbonate 1.0 mL, ranibizumab in BD 1.0 mL polypropylene, and controls. The severity of silicone oil microdroplets with aflibercept in 1.0 mL BD polycarbonate syringes was significantly greater than ranibizumab in BD 1.0 mL polypropylene ($P = 0.012$) and control eyes ($P < 0.001$). The severity of silicone oil microdroplets with ranibizumab in BD 1.0 mL polypropylene or prefilled syringes was not significantly different from that of controls ($P = 1.0$). Linear regression analysis was performed to see if the severity of silicone oil microdroplets correlated with the number of injections. The number of injections was associated with the

Table 2. Number of Eyes and Intravitreal Injections

Anti-VEGF Drug	No. of Eyes	Mean No. of Injections (Range)	Median	P Value (with Bonferroni Correction)	
Bevacizumab	69	29 (6–121)	19	Bevacizumab vs. Ranibizumab	0.0593
				Bevacizumab vs. Aflibercept	1.00
Ranibizumab*	125	33 (6–90)	29	Ranibizumab vs. Aflibercept	0.055
Aflibercept	66	26 (6–69)	26		
Control (fellow eye with no prior intravitreal injections)	147	0			

VEGF vascular endothelial growth factor.
*A total of 14 eyes had ranibizumab delivered only in prefilled syringes. Most eyes treated with ranibizumab had a combination of injections with BD polypropylene and later with prefilled syringes.

severity of silicone oil for bevacizumab in BD 0.3 mL polypropylene ($P = 0.011$) and ranibizumab in BD 1.0 mL polypropylene ($P = 0.005$) but was not significant for aflibercept in BD 1.0 mL polycarbonate syringes ($P = 0.085$). The correlation was weak for all 3 anti VEGF drugs with R^2=0.093 for bevacizumab, R^2=0.064 for ranibizumab, and R^2=0.046 for aflibercept. The relatively low value for R^2 means that less than 10% of the silicone oil severity is explained by the mean number of injections. This suggests that the amount of silicone oil is not constant in each syringe but varies from lot to lot. Otherwise, eyes with a greater number of injections would be expected to have more silicone oil microdroplets than eyes with fewer injections.

Discussion

Intravitreal anti-VEGF injections have become common because of their potent effect on decreasing leakage from neovascular and damaged blood vessels in the retina and choroid. Frequent injections are required to maintain the beneficial response in most eyes, leading to numerous intravitreal injections over a period of many years. The BD syringes often used for these injections were not designed for delivering intravitreal drugs and contain silicone oil droplets to lubricate the barrel of the syringe to make the injection easier. The needles may also contain small amounts of silicone oil, but this appears to be a less important source of silicone oil microdroplets based on the results of studies of the BD syringes and syringes from other manufacturers. The same needles were used for the ranibizumab and aflibercept, so this would not explain the difference in frequency of silicone oil between the different syringe types. One limitation of the current study is that the anti-VEGF drug and syringe type in each patient was not masked to the grader. It was the goal of the grader to find silicone oil microdroplets in all eyes, even the control eyes. There was also no precise quantitative way to measure the number and size of the silicone oil microdroplets, but all eyes were examined by 1 grader using the same criteria.

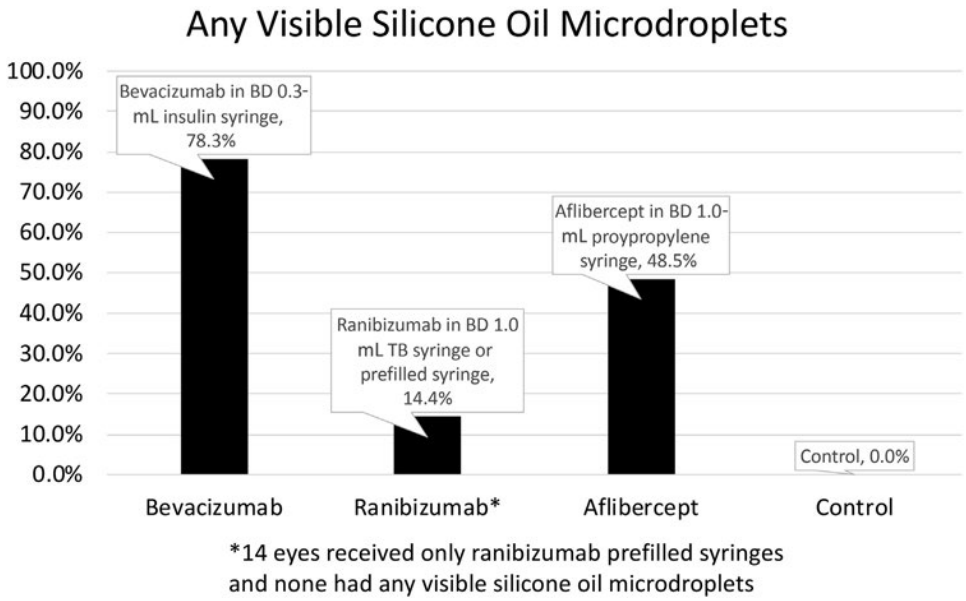

Figure 2. Percentage of eyes with any silicone oil microdroplets visible in the vitreous by slit-lamp biomicroscopy for bevacizumab in BD 0.3-mL polypropylene syringes, ranibizumab in BD 1.0-mL polypropylene syringes, aflibercept in BD 1.0-mL polycarbonate syringes, and controls. BD Becton Dickinson.

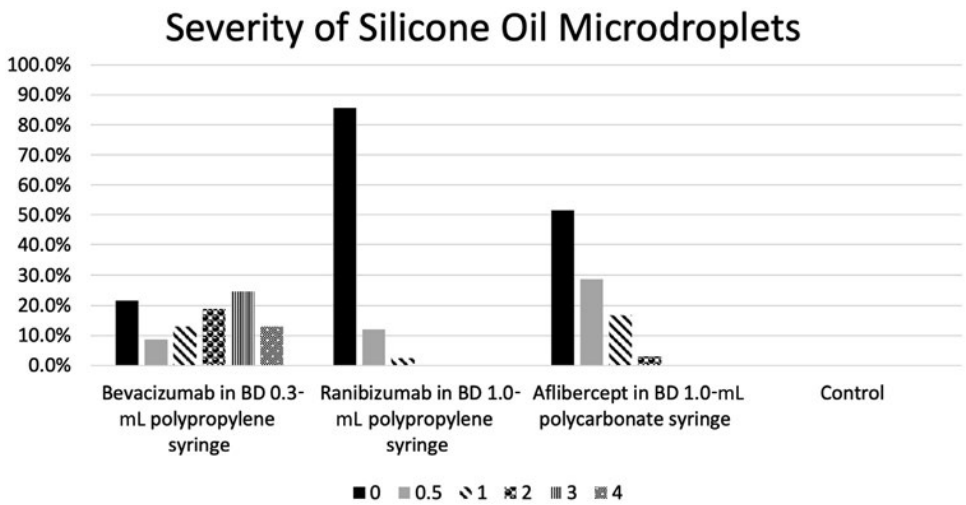

Figure 3. Percentage of eyes with silicone oil visible in the vitreous for bevacizumab in BD 0.3-mL polypropylene syringes, ranibizumab in BD 1.0-mL polypropylene syringes, aflibercept in BD 1.0-mL polycarbonate syringes, and controls using a severity scale from 0 to 4+. BD Becton Dickinson.

Early reports of silicone oil microdroplets after intravitreal injections suggested this was an infrequent finding and related to particular batches of syringes with greater amounts of silicone oil.[3,4] Several studies have noted increased detection of silicone oil microdroplets in the vitreous during one time period.[10] A larger series of 6632 bevacizumab injections found 60 of 6632 eyes (0.904%) with silicone oil droplets with a greater prevalence from injections during one time period in 2016 (1.7%) compared with a different time period in 2015 2016 (0.03%).[7] The variability in silicone oil detection during different time intervals was confirmed in a more recent study.[14] The storage of the prefilled bevacizumab syringes also influences the likelihood of injecting silicone oil microdroplets into the eye. For example, if the bevacizumab is frozen and then thawed because of temperature variability during refrigeration, the amount of siliconle oil increases by approximately 4-fold in 0.3-mL insulin syringes, but not 1.0-mL tuberculin syringes.[3] Several studies have recognized that syringes from different manufacturers have variable amounts of silicone oil. The BD 0.3-mL syringes were noted to have more silicone oil when fluid was expressed through them compared with BD tuberculin syringes and HSW silicone-free syringes (Henke Sass Wolf [HSW], Tuttlingen, Germany).[8] More oil is also expressed at the end of injection if the plunger is depressed all of the way to the end with the BD 0.3-mL insulin syringes compared with the beginning of the injection.[6,8] Agitation of the syringes by flicking them to remove bubbles also appears to increase delivery of silicone oil.[15] These 2 factors could also explain why the eyes treated with aflibercept had more silicone oil microdroplets. The volume of aflibercept in the glass vial supplied with the drug is 0.1-mL, so this means there is less drug compared with ranibizumab, which contained 0.23-mL in their glass vial. This means that it was more common to have to push the plunger of the syringe containing aflibercept all the way down to deliver the full 0.05-mL dose compared with ranibizumab, for which there was sufficient drug to start with the syringe at 0.1-mL and depress the plunger until it read 0.05-mL to deliver the same dose. Aflibercept is also more viscous than ranibizumab, so there is a tendency for the aflibercept to contain bubbles when it is drawn up from the glass vial into the syringe. It is common practice to flick the syringe and withdraw additional air into the syringe to expel the air bubbles during priming, before the aflibercept is injected into the eye because patients are bothered by the small air bubbles for 1 or 2 days after their intravitreal injection.

Table 3. Comparison of Silicone Oil Microdroplets Severity in Different Syringe Types by Univariate Analysis of Variance

Anti-VEGF Drug/Syringe	Compared to Anti-VEGF Drug/Syringe	*P* Value (with Bonferroni Correction)
Bevacizumab in 0.3-mL BD insulin syringe	Aflibercept in 1.0-mL BD polycarbonate syringe	*P* <0.001
	Ranibizumab in 1.0-mL BD polypropylene/prefilled syringe	*P* <0.001
	Control (no injections)	*P* <0.001
Aflibercept in 1.0-mL BD polycarbonate syringe	Ranibizumab in 1.0-ml BD polypropylene/prefilled syringe	*P* 0.012
	Control (no injections)	*P* <0.001
Ranibizumab in 1.0-mL BD polypropylene/prefilled syringe	Control	*P* 1.0

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