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Silicone microbubbles after anti-vascular endothelial growth factor injections in patients with wet age-related macular degeneration: incidence, quantification and secondary optical coherence tomography artfacts

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ABSTRACT.

Purpose: **To report the incidence and quantity of silicone oil microbubbles and the relationship with the number of intravitreal anti-vascular endothelial growth factor (VEGF) injections and evaluate if microbubbles induce artefacts on optical coherence tomography (OCT) images.**

Methods: **Observational, descriptive, cross-sectional study. Patients with wet age-related macular degeneration were included who had been treated for 1 year minimally with anti-VEGF injections repackaged in the hospital pharmacy. Detection and quantification of silicone microbubbles by mydriatic biomicroscopic examination were conducted 1 month after the last injection. The numbers of microbubbles were quantified on a scale of 0–3: 0, none; 1 scarce (1–10 microbubbles); 2 moderate (10–30); or 3 numerous (>30). Shadowing on OCT images was classified as 0–3: 0, none; 1 obscuring some retinal layers; 2 obscuring all retinal layers; or 3 obscuring the retinal thickness.**

Results: **The study included 142 eyes of 98 patients (mean age, 82.4 years + 7.3; range, 65–97) treated with 2377 injections. Microbubbles were detected in 127 (89.4%) eyes, 62 (43.6%) with numerous microbubbles and 36 (25.4%) and 29 (20.4%), respectively, with scarce and moderate numbers. A positive correlation was found between the numbers of injections and intravitreal silicone (rho, 0.7). Optical coherence tomography (OCT) artefacts were detected in 11 eyes; the artefacts obscured all retinal layers in three eyes. No significant relationship could be established between the appearance of floaters and the microbubbles.**

Conclusion: **The presence and number of silicone microbubbles were correlated with the number of intravitreal injections. Microbubbles can produce OCT artefacts, which can hinder the treatment decision.**

Key words: age-related macular degeneration – intravitreal injections – macular degenerations – OCT artfacts – silicone microbubbles – wet

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Introduction

Age-related macular degeneration (AMD), which accounts for 8.7% of blindness worldwide, is the leading cause of blindness in people over age 50 in the Western world; the prevalence increases with age reaching 7.1% of people older than 75 years (Velez-Montoya et al. 2014). The neovascular or wet form of the disease (wAMD) progresses rapidly and aggressively, but intravitreal anti-vascular endothelial growth factor (VEGF) treatments can halt visual loss in most patients (Wong et al. 2007). The treatment must be repeated periodically, and the average number of intravitreal injections (IVI) varies widely among studies (4.2–7.5 the first year), although better results have been obtained in studies with higher numbers of injections (Chong 2016).

Anti-VEGF drugs are supplied in glass bottles with a drug volume that is several times greater than needed for a dose (0.05 ml). For economic reasons, hospital pharmacies prepare the doses/injection for each patient in disposable plastic syringes (Olea et al. 2020). During the manufacturing process of these syringes, silicone oil derivatives are used to coat the surfaces of the syringes and needles to improve the

ability of the plunger to slide, thus facilitating the injection (Krayukhina et al. 2015). Small microbubbles of this coating can penetrate the vitreous chamber when the drug is injected. The presence of silicone microbubbles in the vitreous has been observed with different injected agents, confirming that the origin of these microbubbles is the syringe or the needle and not the medication itself (Bakri & Ekdawi 2008; Sampat & Garg 2010). With the increasing numbers of patients undergoing treatment with IVIs and the increasing numbers of injections in the same patient, the occurrence of this complication can increase. The prevalence rates vary between 0.03%, 44% and 67.6% among published studies (Scott et al. 2009; Khurana et al. 2017; Melo et al. 2019). The usual method to evaluate the presence of silicone microbubbles is direct examination at the slit lamp. Optical coherence tomography (OCT) also has been performed to objectively and qualitatively assess vitreous opacities (Kennelly et al. 2015; Fernández-Avellaneda et al. 2019). It is also known that vitreous opacities can induce artefacts in OCT images, but the relevance of this fact in the case of silicone microbubbles has not been studied extensively. No systematic study has been performed to date to elucidate if the presence of silicone microbubbles causes other complications such as significant floaters (Schargus & Frings 2020).

The objectives of the current study were to report the incidence of vitreous silicone microbubbles in patients with wAMD treated with intravitreal anti-VEGF agents in our daily clinical practice, quantify them and determine the possible relationship to the numbers of injections administered. We also evaluated to what extent these microbubbles induced artefacts that could preclude an OCT evaluation, the findings of which are important for treatment decisions. The appearance of permanent floaters after IVIs also was studied.

Methods

Study design

This was an observational, descriptive, cross-sectional study of patients with wAMD undergoing intravitreal anti-VEGF treatment.

Study subjects

Patients were included who were diagnosed with wAMD, evaluated in the Ophthalmology Department of Palencia U. Hospital Complex between October 2018 and June 2019, and treated with intravitreal anti-VEGF injections for at least 1 year.

Patients were excluded who had any ocular media opacities that could have affected direct visualization of the silicone microbubbles. Other exclusion criteria were cognitive impairment or intellectual deficiency, previous intravitreal corticosteroid treatments or patient refusal to participate in the study. All patients included in the study always received treatment with anti-VEGF drugs repackaged in our hospital pharmacy.

Drug repackaging

The hospital pharmacy in the Palencia U. Hospital Complex is in a separate building located 4 km from the Ophthalmology Department. Drug repackaging began to be performed in the hospital pharmacy in the Palencia U. Hospital Complex in 2008. Pharmacists collect approximately 1 ml of the drug, that is several vials in the case of ranibizumab (Lucentis, Genentech Inc., South San Francisco, CA, USA) and aflibercept (Eylea, Regeneron Pharmaceuticals, Tarrytown, NY, USA), in a sterile disposable syringe luer slip (Caress CCAR 00101000) with a BD Blunt Fill Needle Filter (Becton Dickinson and Company, Franklin Lakes, NJ, USA). The syringe is placed face up, and 0.06-ml aliquots were extracted using several BD Microfine^{TM}+ (packaged as BD Ultrafine in other countries) with a staked-in needle syringes (Becton Dickinson and Company) resulting in about nine doses of the drug.

Study variables

The following variables were recorded from the patient files: age, gender, number of injections since the start of treatment, duration of treatment, and unilateral or bilateral anti-VEGF treatment.

Detection and quantification of silicone microbubbles were conducted 1 month after the last injection by biomicroscopic examination in mydriasis, without a lens used to view the anterior vitreous and with a 78-dioptre lens to visualize the posterior vitreous. The numbers of microbubbles were quantified as 0 indicating absent, 1 scarce (1–10 microbubbles), 2 moderate (10–30) or 3 numerous (>30) Video S1.

During the same visit, the patients were asked about the appearance of permanent and significant floaters after the beginning of the intravitreal treatment. Significant floaters were defined as floaters that were appreciated not only in a bright environment but also in other darker light environments and caused moderate or severe discomfort in daily life.

All patients were evaluated based on the findings on 'fast macular' OCT scans obtained using the Spectralis® (Heidelberg Engineering, Heidelberg, Germany) with the follow-up protocol centred on the fovea. The recorded OCT scans obtained at the last visit were considered for the study. The following scale was used to classify the OCT artefacts: 0 indicated no artefacts, 1 artefacts that obstructed observation of some retinal layers, 2 artefacts that obstructed observation of all retinal layers and 3 artefacts that obstructed observation of the retinal thickness.

The locations of the artefacts were classified into foveal [Early Treatment Diabetic Retinopathy Study (ETDRS) 1 mm] or extrafoveal (EDTRS 3 mm). Artefacts on OCT images were attributed to silicone oil microbubbles when sections of the scans were missing as a result of the vitreous opacities that corresponded to the microbubbles. In cases in which shadow artefacts were attributed to silicone microbubbles, the first OCT scans obtained before treatment were reexamined to rule out pretreatment vitreous opacities.

Statistical analysis

Statistical analyses were performed using SPSS 20 (IBM Corp. Released 2011.IBM SPSS Statistics for Windows, version 20.0 Armonk, NY: IBM Corp. USA). $p = 0.05$ was considered statistically significant. The Lilliefors (Kolmogorov–Smirnov) test was used as a normal test or the Shapiro–Wilk test was used for small samples. Qualitative variables were expressed as percentages. The distributions of continuous quantitative variables were

expressed as the averages, standard deviations, minimums and maximums.

The Spearman coefficient was used to correlate the IVIs and the frequency of the microbubbles. Pearson's chi-square test was used to assess the relationship between two qualitative variables. If the expected frequencies were small, Fisher's exact test was used. The Student *t*-test or, when the assumption of normality was not valid, the non-parametric Mann–Whitney *U*-test alternative was used to compare the differences between the means of two independent groups.

All patients provided written informed consent to participate in the study. The Medical Research Ethics Committee of the Palencia U. Hospital Complex approved the study, which was conducted in accordance with the recommendations of the Declaration of Helsinki.

Results

The final sample included 142 treated eyes of 98 patients (mean age, 82.4 years + 7.3; range 65–97) treated with 2377 injections. The patients had been treated for an average of 44.7 ± 26.8 months and had received an average of 16.74 + 10.4) IVIs before the evaluation. Information about sex of patients, treated eye and drug injected is shown in Table 1.

A total of 127 (89.4%) injected eyes presented with silicone microbubbles in the vitreous chamber. Ninety-two patients (93.9%) presented with microbubbles in one or both eyes. Sixty-two eyes (43.6%) had numerous microbubbles, 36 (25.4%), 29 (20.4%) had moderate amounts, and 36 (25.4%) had few microbubbles (Fig. 1). In many cases with numerous microbubbles, the microbubbles appeared in clusters (Fig. 2).

Table 1. Demographic and study data.

	Frequency (%)
Gender	
Men	40 (40.8)
Women	58 (59.2)
Total	98 (100)
Treated eyes	
Right eye	29 (29.6)
Left eye	25 (25.5)
Both eyes	44 (44.9)
Total	142 (100)
Drug injected	
Lucentis®	1.281 (53.9)
Eylea®	933 (39.3)
Avastin®	163 (6.9)
Total	2.377 (100)

The eyes with no silicone microbubbles had received an average of 8.6 IVIs, while the eyes with microbubbles had received an average of 17.7 IVIs ($p < 0.001$). A positive correlation was seen between the number of injections and the frequency of the appearance of silicone microbubbles (rho = 0.7; $p < 0.001$).

Thirty-six (36.7%) patients reported having permanent and significant floaters that began after the intravitreal treatment. In 34 patients, the microbubbles were detected in one or both eyes; the other two patients did not have microbubbles in either eye. A significant relationship could not be established between the appearance of floaters after intravitreal treatment and the presence of microbubbles (Fisher p value ~1). There was not a statistically difference in number of microbubbles (the 0–3 scale) between patients reporting significant floaters and patients not reporting them ($p = 0.561$).

OCT artefacts were detected in 11 (7.7%) treated eyes of 9 patients (9.2%; Fig. 3). In eight eyes, artefacts prevented observation of some retinal layers; in three eyes, artefacts prevented observation of all retinal layers. There were no cases where artefacts prevented assessing the retinal thickness. In two cases, they were located in the foveal region and in nine were extrafoveal.

Discussion

We found both a high incidence of silicone microbubbles in the vitreous chamber of patients with wAMD who were treated with anti-VEGF and a positive correlation between the number of IVIs and the number of microbubbles. The presence of microbubbles precluded an OCT evaluation in 7.7% of eyes.

The incidence of microbubbles in the current study is higher than in other published studies (Scott et al. 2009; Melo et al. 2019), for which several explanations are possible. The systematic search for microbubbles could have increased the reporting of this complication. In addition, variations in the injection technique, repackaging and transport processes, or possible mishandling of the repackaged doses (sudden variations in temperature, exposure of the syringes to light, agitation of the syringes) also could have affected this data (Dias Júnior et al. 2020; Melo et al. 2020a; Melo et al. 2020b; Schargus & Frings 2020). Type of syringe used could also have had an important role as other authors have found BD syringes to release a significant amount of silicone oil, even ranking highest among others (Emerson 2017; Melo et al. 2020b). Melo et al. (2019) also reported a high incidence of this complication and a positive correlation between the number of IVIs and the presence of silicone microbubbles.

To the best of our knowledge, no other published studies have quantified the number of microbubbles, so we have no comparative references. It is noteworthy that many of our patients had clusters of numerous microbubbles. We believe that such grouped bubbles could came from the same IVIs.

To what extent the presence of silicone microbubbles leads to meaningful clinical complications such as intraocular pressure elevation resulting from blockage of the trabecular meshwork, inflammation, or significant floaters have been discussed extensively (Schargus & Frings 2020). Large bubbles seem to cause floaters; in some cases, the intensity was such that vitrectomy was considered (Avery et al. 2019). While floaters appear frequently immediately after IVIs, their persistence is less usual. In the current study, permanent floaters were not uncommon, but they also developed in patients who had no microbubbles. Therefore, we cannot conclude that the presence of microbubbles increases the risk of significant and permanent floaters. Posterior vitreous detachment is more frequent in patients treated with IVIs and it also may trigger floaters (Geck et al. 2013).

Although the presence of microbubbles rarely prevented correct evaluation of the OCT scans, it was especially problematic in 7.7% of the eyes studied in which treatment decisions were more difficult, and this is not negligible.

It would be desirable to reduce the incidence of this problem by using syringes and needles without silicone or with the least amount of silicone possible, such as syringes with

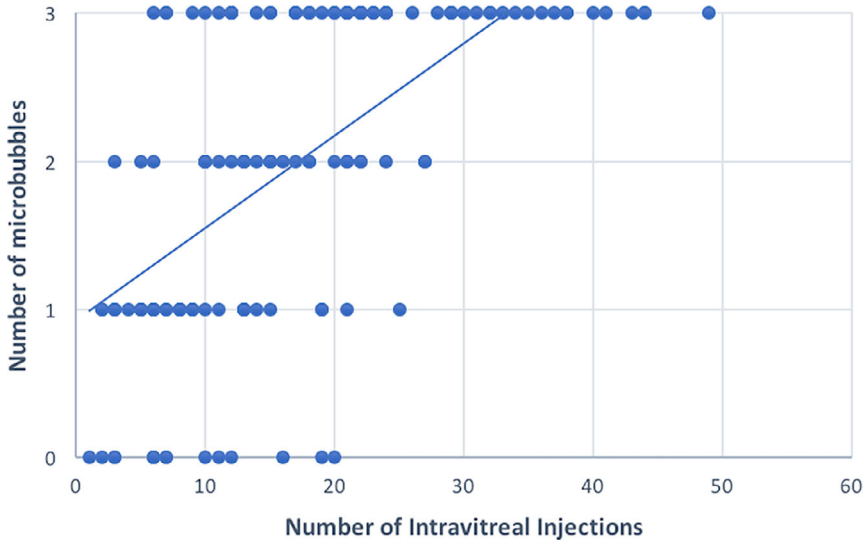

Fig. 1. The graph shows the direct relationship between the number of intravitreal injections (IVIs) per eye and the number of microbubbles in the vitreous. The number of intravitreal injections that an eye has received is on the *x*-axis, and the quantity of microbubbles (0: no microbubbles; 1: scarce, 1–10 microbubbles; 2: moderate, 10–30; and 3: numerous >30) is on the *y*-axis.

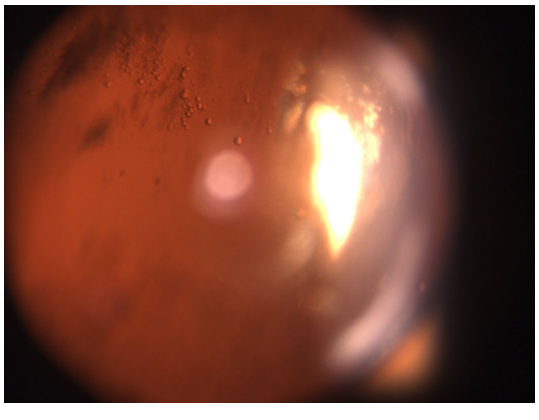

Fig. 2. Numerous microbubbles in the vitreous disposed as clusters in the same location.

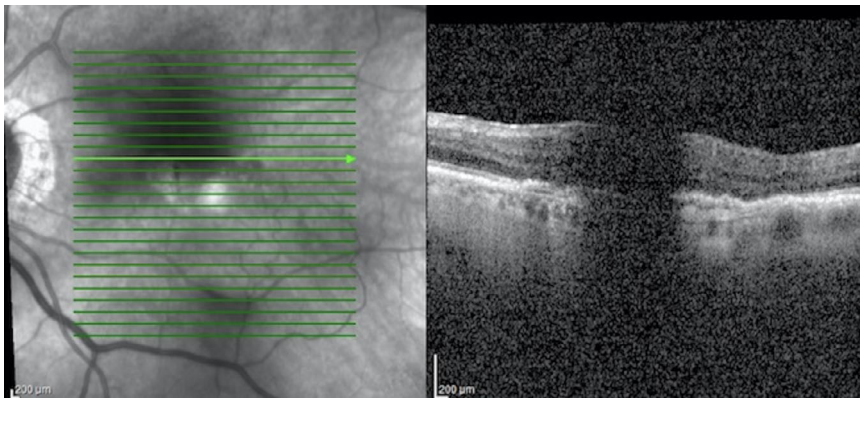

Fig. 3. Example of optical coherence tomography (OCT) shadowing artefacts due to silicone microbubbles that precluded a precise OCT evaluation and interfered with treatment decisions.

optimized siliconization and baked-on siliconization, or to use prefilled syringes (Gerhardt et al. 2015; Melo et al. 2020a). Prefilled syringes are subjected to an optimized siliconization process that minimizes the risk of transfer of silicone to the drug solution (Sassalos & Paulus 2019). In addition, they are not manipulated, which could further reduce the release of silicone microbubbles into the drug and in turn into the vitreous (Liu et al. 2011).

Patients should be informed about the probability of accumulation of silicone microbubbles in the vitreous and the possible consequences (Schargus & Frings 2020). Further studies are needed to better understand the real clinical consequences of silicone microbubbles after anti-VEGF injections. Determining the correlation between the presence and abundance of silicone microbubbles and intraocular pressure elevation in our sample will be addressed in our next study.

Although the sample can be considered representative because the demographic data agreed with the demographics of wAMD described in a Spanish population and other reports (Spanish Eyes Epidemiological Study Group 2011; Mitchell et al. 2018), the current study was conducted in one hospital and there may be inherent issues in the pharmacy, type of syringe, packaging process, among others, that could be study limitations.

In summary, the presence and abundance of silicone microbubbles were correlated with the number of IVIs. Besides the possible clinical complications, microbubbles also can cause OCT artefacts that can impact treatment decisions.

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The data that support the findings of this study are available from the corresponding author upon reasonable request. The supplementary material supports the study findings.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Video-Image of numerous micro bubbles in motion in the vitreous.