**Intravitreal bevacizumab: safety of multiple doses from a single vial for consecutive patients**

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**Objectives**
To report the incidence of endophthalmitis after intravitreal injection of anti–vascular endothelial growth factor and the safety profile of multiple doses of bevacizumab from the same vial reused for multiple patients.

**Design**
Case series.

**Setting**
A private hospital in Hong Kong.

**Patients**
A systematic retrospective review of consecutive intravitreal anti–vascular endothelial growth factor injections between 5 June 2006 and 17 December 2010 at a single institute was conducted. Patients were identified from prospectively designed audit forms, and each patient’s medical record was reviewed for any documented complications. Bevacizumab 1.25 mg/0.05 mL to 2.50 mg/0.1 mL was aspirated from the designated vial, with a maximum of 10 consecutive injections being aspirated from the same vial. The opened vial was then discarded without overnight storage. Ranibizumab was aspirated from the commercially available 1 mg/0.1 mL single-use vial.

**Results**
A total of 1655 intravitreal anti–vascular endothelial growth factor injections into 392 eyes of 383 patients were evaluated during the study period. There were 1184 bevacizumab injections and 471 ranibizumab injections. There was one case of suspected endophthalmitis after ranibizumab injection, though culture of the vitreous tap was negative. The point prevalence of endophthalmitis was 0.06% (1/1655) for the total number of injections: 0.21% (1/471) after ranibizumab, and 0% after bevacizumab.

**Conclusion**
Although many centres aliquot multiple syringes from a single vial to be kept in a refrigerator for use, the current study shows that so long as proper sterile techniques are implemented, there were no cases of endophthalmitis from using the same vial, which was reused for a maximum of 10 consecutive injections. For intravitreal injection, bevacizumab costs approximately US$50 to US$100 per dose, as opposed to US$2000 per dose for ranibizumab. Sharing multiple doses of bevacizumab from a single vial can substantially reduce the cost of treatment.

**Key words**
Endophthalmitis; Intravitreal Injections; Ranibizumab; Receptors, Vascular endothelial growth factor; Bevacizumab

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**New knowledge added by this study**
- The frequency of endophthalmitis was 0.06% (1/1655) for the total number of injections, being 0.21% (1/471) after ranibizumab and 0% after bevacizumab.
- Using the same vial of bevacizumab for a maximum of 10 consecutive injections was safe when proper sterile techniques are implemented.

**Implications for clinical practice or policy**
- Bevacizumab is a safe alternative to ranibizumab.
- In a clinic setting, injections of bevacizumab can be safely used from the same vial for up to 10 consecutive injections.

**Introduction**
Anti–vascular endothelial growth factor (anti-VEGF) therapy plays an important role in many ocular diseases, particularly posterior segment pathologies characterised by choroidal neovascularisation (CNV) and macular oedema. While only ranibizumab (Lucentis; Genetech, San Francisco [CA], US) and pegaptanib (Macugen; Eyetech Pharmaceuticals,
New York, US) are labelled for intravitreal use, bevacizumab (Avastin; Genetech, San Francisco [CA], US) is currently also being used ‘off-label’ for the treatment of ocular diseases. Bevacizumab was approved by the US Food and Drug Administration (FDA) for treating patients with metastatic colorectal cancer in February 2004.3 Promising results were first reported using systemic bevacizumab in a case series of nine patients with age-related macular degeneration (AMD),2 followed by intravitreal injection of a smaller dose which also resulted in significant toxicity.3-5 Recently, the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT)—a large, prospective, multicentre, randomised controlled trial—concluded that monthly intravitreal injections of either anti-VEGF drug resulted in the same visual acuity outcomes at 1 year.4 Furthermore, the outcomes of the as-needed regimen with bevacizumab appeared similar to those of ranibizumab therapy. The data from the CATT study supported the use of bevacizumab, and the as-needed regimen appeared to be an acceptable alternative to the monthly regimen.

Commercially available bevacizumab comes in preservative-free 100 mg/4 mL vials, and is intended for use at relatively high concentrations on a single colon cancer patient. In this era of tremendous emphasis on health care cost containment in both developed and developing countries, it is a common practice among hospitals, clinics, and compounding pharmacies to divide the large volume of bevacizumab into smaller units that are suitable for single-use intravitreal doses for individual eyes.

Endophthalmitis, although a rare complication of intravitreal injection with anti-VEGF agents, is a serious concern due to its devastating visual consequence and the increasing frequency of intravitreal injections being given worldwide. Scientific data and guidelines have been published on the necessary risk management procedures pre-injection, peri-injection, and post-injection.6,7 Nevertheless, there have been concerns about potential contamination associated with dividing the large volumes contained in vials of bevacizumab into smaller units. Though these misgivings have not been substantiated, a number of large series reporting complications after intravitreal bevacizumab did not provide information regarding how intravitreal doses of bevacizumab were prepared.8-13 Currently, there is no consensus within the ophthalmic community on whether compounding the large bevacizumab vial into several aliquots or reusing the same original vial for consecutive injections could minimise the risk of endophthalmitis.14-21 We report the safety profile of withdrawing multiple doses of bevacizumab from the original 4 mL vial and its reuse for multiple consecutive patients in an office setting.

Methods
This study was performed in accordance with the standards of the Declaration of Helsinki and approved by the Institutional Review Board of the Hong Kong Sanatorium and Hospital. Patients were informed about the off-label conditions of intravitreal bevacizumab. Women of childbearing age were also informed about the possible risks to the fetus and avoiding conception was advised for 3 months after injection. At each post-injection visit, patients were monitored for ocular side-effects (best-corrected visual acuity, intra-ocular pressure [IOP], indirect ophthalmoscopy, slit-lamp biomicroscopy) and systemic effects/adverse effects (medication changes, high blood pressure, clinical features of a cerebrovascular accident, myocardial infarction, or ischaemia).

Since 5 June 2006, a prospectively designed audit was carried out on every patient receiving an anti-VEGF injection (ranibizumab and bevacizumab) in the injection room of the out-patient clinic at the Hong Kong Sanatorium and Hospital. Each patient's
TABLE 1. Demographic characteristics of patients receiving intravitreal anti-vascular endothelial growth factor injections

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>No. of patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ranibizumab</td>
<td>Bevacizumab</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>68 ± 16</td>
<td>61 ± 17</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>158</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>163</td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>29</td>
<td>122</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7</td>
<td>79</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Taking aspirin</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Smokers</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>8</td>
<td>55</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation is shown

Results

A total of 1655 intravitreal anti-VEGF injections in 392 eyes of 383 patients were evaluated during the study period. There were 1184 intravitreal injections of 1.25 mg to 2.50 mg of bevacizumab, and 471 intravitreal injections of 0.5 mg ranibizumab. The average number of injections per eye was 4 (range, 1-31) per patient. Relevant demographic characteristics are summarised in Table 1. The most common indications for intravitreal anti-VEGF were CNV from AMD, followed by CNV from pathological myopia (Table 2).

Analysis of all injections documented only one instance of a clinically suspected endophthalmitis after an injection with ranibizumab and presented within 4 days of the procedure; subsequent vitreous tap and culture were negative. The ocular complications in this series are listed in Table 3. Thus, the frequency of suspected endophthalmitis was 0.06% (1/1655) for the total number of injections; 0.21% (1/471) after ranibizumab, and 0% after bevacizumab. There was also one case of rhegmatogenous retinal detachment, which presented 1 month after ranibizumab injection for CNV due to pathological myopia.

Case report

The patient was an 80-year-old man who had suspected left ocular ischaemic syndrome with a history of recurrent corneal erosions, chronic eye pain, and recurrent vitreous haemorrhage. He had a history of combined phacoemulsification, intraocular lens implantation, pars plana vitrectomy and had also undergone endolaser treatment 6 months earlier. On examination, his left eye visual acuity was hand movement and the IOP was 29 mm Hg. Neovascularisation over the iris and an open angle was noted. After thorough discussion of various options, he decided to receive a 0.5 mg ranibizumab intravitreal injection. Four days later he presented with mild left eye redness. Upon examination, his visual acuity remained at hand movement and the
IOP was 18 mm Hg. Slit lamp examination showed a 1 mm hypopyon, anterior chamber cells 3+, but less neovascularisation over the iris. Dilated fundoscopy revealed a mild vitreous haze with a flat retina. Infective endophthalmitis was suspected. Aqueous and vitreous taps for microscopy and culture were performed. The specimens were directly inoculated onto various culture media and slide plates, and immediately sent to the microbiological laboratory.

Intravitreal amikacin (0.4 mg in 0.1 mL) and vancomycin (1 mg in 0.1 mL) was given. Over 1 week the hypopyon gradually disappeared and there was clinical resolution of the endophthalmitis. The patient had persistent neovascular glaucoma with an IOP of 40 mm Hg. His left eye vision was reduced to light perception and he declined further interventions.

Aqueous and vitreous taps for microscopy and culture were all negative.

**Discussion**

The frequency of endophthalmitis after intravitreal anti-VEGF reported in the scientific literature varies from 0.01% to 1.6%. In a meta-analysis of 105,531 injections from all major US-based studies from 2005 to 2010, McCannel reported an endophthalmitis frequency of 0.049% (approximately 1 of 1949 injections). There is a definite, albeit small, risk of developing endophthalmitis following an intravitreal injection. During the study period lasting almost 4.5 years, the frequency of endophthalmitis we encountered after intravitreal anti-VEGF injections was 0.06%, which is within previously published ranges.

Our single case of suspected endophthalmitis after intravitreal ranibizumab was negative for bacteria and fungi when assessed by microscopy and cultures. Sterile endophthalmitis has been reported after intravitreal anti-VEGF; endotoxin introduced through the injection site has been proposed as a cause of severe intra-ocular inflammation. However, such acute intra-ocular inflammation was found to present early (within 1 day) post-injection. By contrast our patient presented 4 days after the injection, which is more typical of bacterial endophthalmitis. Thus, we could not exclude the possibility of very small microorganism load that was not detected in the vitreous tap sent to our laboratory.

We did not encounter endophthalmitis after intravitreal bevacizumab. Hence, based on our series, reusing the same bevacizumab vial for multiple injections seems not to increase its frequency in comparison to single-use ranibizumab vials. To date, other large series found no difference in the endophthalmitis risk in patients receiving

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**TABLE 2. Indications for intravitreal anti–vascular endothelial growth factor injections**

<table>
<thead>
<tr>
<th>Indications*</th>
<th>No. of eyes</th>
<th>No. of injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ranibizumab</td>
<td>Bevacizumab</td>
</tr>
<tr>
<td></td>
<td>Ranibizumab</td>
<td>Bevacizumab</td>
</tr>
<tr>
<td>CNV (AMD)</td>
<td>29</td>
<td>89</td>
</tr>
<tr>
<td>CNV (pathological myopia)</td>
<td>11</td>
<td>80</td>
</tr>
<tr>
<td>DME</td>
<td>5</td>
<td>49</td>
</tr>
<tr>
<td>PCV</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>CRVO</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>BRVO</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Pseudophakic CME</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Idiopathic CNV</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>NVG</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Vitreous haemorrhage (DMR)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Other secondary CNV</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Chronic CSC</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Uveitis CME</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>327</td>
</tr>
</tbody>
</table>

* CNV denotes choroidal neovascularisation, AMD age-related macular degeneration, DME diabetic macular oedema, PCV polypoidal choroidal vasculopathy, CRVO central retinal vein occlusion, BRVO branch retinal vein occlusion, CME cystoid macular oedema, NVG neovascular glaucoma, DMR diabetic retinopathy, and CSC central serous chorioretinopathy

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**TABLE 3. Ocular complications after intravitreal anti–vascular endothelial growth factor injections**

<table>
<thead>
<tr>
<th>Type of complications</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subconjunctival haemorrhage</td>
<td>61</td>
</tr>
<tr>
<td>Corneal injury</td>
<td>1</td>
</tr>
<tr>
<td>Rhegmatogenous retinal detachment</td>
<td>1</td>
</tr>
<tr>
<td>Suspected endophthalmitis</td>
<td>1</td>
</tr>
</tbody>
</table>
bevacizumab as opposed to ranibizumab.\textsuperscript{6,13-15,19,22} The recent CATT study revealed no statistically significant difference between the endophthalmitis rates after bevacizumab and ranibizumab; the respective rates being 2/5449 (0.04\%) injections in 599 patients, and 4/5508 (0.07\%) injections in 586 patients.\textsuperscript{6} Nonetheless, the CATT study had limited statistical power to detect important adverse events and could not definitively conclude that both drugs had similar rates of post-injection endophthalmitis.

Due to the lack of evidence on any increased risk of endophthalmitis associated with preparing bevacizumab in small doses for intravitreal use, there is no current consensus on the optimal protocol to minimise the risk of contamination during the handling of this drug. Artunay et al\textsuperscript{25} reported three eyes (of 3022 injections, 0.066\%) with endophthalmitis after intravitreal bevacizumab, in which multiple doses had been withdrawn from a single vial in an out-patient setting. Other studies have reported endophthalmitis, possibly related to contamination during the compounding procedures of bevacizumab. Yamashiro et al\textsuperscript{18} reported 14 out of 19 consecutive cases of culture-negative endophthalmitis after intravitreal injection from a single batch of bevacizumab. Lee et al\textsuperscript{16} reported two patients who received intravitreal bevacizumab on the same day that developed \textit{Serratia marcescens} endophthalmitis. Subsequent molecular typing confirmed that the strains were identical, suggesting contamination during compounding. However, they did not find any case of endophthalmitis in another group of patients that received intravitreal bevacizumab aspirated from the same vial that was reused for multiple consecutive injections just before each procedure and discarded on the same day. Similarly, the Pan-American Collaborative Retina Study Group (PACORES) reported more frequent endophthalmitis (6/1833 injections, 0.33\%) in eyes injected using previously compounded aliquots than in eyes given injections from the same multidose vial (1/2470 injections, 0.04\%) that was reused appropriately.\textsuperscript{17} Contrary to the perceived higher risk of contamination from re-utilisation of a single vial, a higher frequency of endophthalmitis was reported in studies utilising compounded aliquots.

In 2001, there was an outbreak of 11 \textit{S marcescens} infections, including meningitis, epidural abscesses, and septic arthritis following epidural or joint injections of betamethasone that was compounded at a single pharmacy.\textsuperscript{26} An investigation of the pharmacy revealed cross-contamination of the clean room environment and stock solutions, as well as inadequate autoclaving temperature, lack of terminal sterilisation, insufficient training of pharmacy staff, and absence of end-product sterility tests, all of which could have contributed to the outbreak.\textsuperscript{27} Whilst the frequency of endophthalmitis after intravitreal bevacizumab is fairly low, the two recently reported cases of \textit{S marcescens} endophthalmitis had devastating visual consequence and destruction of the eye despite surgical treatment.\textsuperscript{16} To minimise the risk of contamination, a recommendation was made to compound drugs in an accredited pharmacy adhering to professional standards such as those espoused by the FDA,\textsuperscript{28} American Society of Health System Pharmacists,\textsuperscript{29} and US Pharmacopeia (USP chapter 797\textsuperscript{29}).

According to the USP (chapter 797), the preparation of multiple doses of bevacizumab for intravitreal injection can be categorised as a medium-risk compounded sterile preparation, and the quality assurance procedures recommended for compounding included adequate personnel garb for sterile preparation within a laminar-airflow workbench, routine disinfection, air quality testing to maintain an International Organization for Standardization (ISO) Class 5 (3520 particles/m\textsuperscript{3}) environment, and annual media-fill tests of aseptic manipulations of every pharmacy staff member involved in compounding.\textsuperscript{30}

Drawing multiple doses from the same vial immediately before injection has the potential for contamination. Increasing the number of punctures also increases the risk of contamination. This could be due to the rubber septum increasingly wiped by fingers or a dirty gauge, rubber stopper leakage, poor aseptic technique (eg entering the vial without alcohol swabbing), injection of air into the vial before removal of the solution, a contaminated needle or syringe used to draw medication, and inappropriate storage durations and temperatures.\textsuperscript{32-34} Similar to the protocol of intravitreal injection of bevacizumab devised by Artunay et al,\textsuperscript{25} our protocol had a limit of 10 punctures for each opened vial, as studies have found that contamination was rare (<1 colony forming unit/1000 perforations) when multidose vials were punctured up to 10 times in hospital use.\textsuperscript{25,34,35}

Clinicians from other specialties had longer experience in administering multidose injections retrieved from a single vial. Examples in the literature include subcutaneous injections for the immunotherapy for allergy,\textsuperscript{36} intravenous injections of contrast media in radiology and anaesthetic medications for non-emergency procedures.\textsuperscript{37} The safety of these injections were also ensured by hand washing, proper personnel garb, and using sterile equipment during drug transfer, disinfection of the rubber diaphragm with ≥70% alcohol or 10% povidine-iodine, using sterile needles and syringes each time a vial is entered, and avoiding touching the needle and rubber diaphragm. Furthermore, wearing surgical masks during withdrawal of medications from the vial and peri-injection procedures in the injection room may have prevented respiration-associated bacterial contamination by species such
as *Streptococcus*. Meta-analysis of endophthalmitis after intravitreal anti-VEGF injections reported the risk of *Streptococcus*-associated cases was 3 times more frequent than after intra-ocular surgery. While the most common microbial sources in postsurgical endophthalmitis were believed to originate from the patient's conjunctiva, *Streptococcus* species were not commonly identified in cultured isolates from conjunctival flora in patients undergoing intravitreal injections. *Haemophilus influenzae* endophthalmitis was reported by Artunay et al following intravitreal bevacizumab injections from a shared single vial. Although insufficient evidence supports the protective effect of wearing masks to avoid endophthalmitis, this practice may have prevented contamination by aerosolised bacteria.

No scientific data have revealed how best to divide the large bevacizumab volume in each vial into smaller units so as to minimise the risk of endophthalmitis. To date, the reported frequency of drug preparation–related endophthalmitis was higher when it was compounded into aliquots as opposed to drawing multiple doses out of the original vial immediately before consecutive injections (Table 4). A possible reason might be that accredited compounding facilities were not available in many localities and compliance with the standards of sterile compounding were poor. In our protocol moreover, the opened bevacizumab vials were not stored overnight, which was also practised for Lee et al's cohort in which no cases of infective endophthalmitis were encountered. The revised USP (chapter 797) incorporated microbial contamination into the determination of beyond-use dating, quite apart from the chemical stability of the products.

Although previous studies have demonstrated the stability and sterility of compounded bevacizumab stored at 4°C for 15 days and up to 6 months, avoidance of overnight storage may have greatly reduced the microbial burden risk within vials, especially as the manufactured bevacizumab was preservative-free.

We routinely prescribed moxifloxacin eye drops to patients after injection. A recent antibiotic susceptibility pattern study among conjunctival isolates from patients undergoing intravitreal injection found most organisms to be sensitive to gentamicin (≥85%). Nonetheless, the protective role of post-injection antibiotics is controversial. Prospective controlled trials found that the omission of peri-injection antibiotics did not increase the rate of endophthalmitis. Some concern exists about the possibility of microbial resistance with repeated short-term topical antibiotic use.

The one case of rhegmatogenous retinal detachment in this series may or may not be related to the intravitreal injection, as it can develop as part of the natural history of highly myopic eyes. No adverse systemic events occurred during the study period. Nonetheless, complications after intravitreal anti-VEGF injections in this study could have been under-recognised owing to retrospective collection of some of the data.

For intravitreal injections, ranibizumab costs approximately US$2000 per dose while bevacizumab costs approximately US$50 to US$100 per dose. Since treatment with both drugs entails multiple doses, the cost differential is substantial. In the CATT study, the average 1-year costs for regular monthly treatment with ranibizumab and bevacizumab were US$23 400 and US$95, respectively. Because

### TABLE 4. Summary of studies and reported frequencies of suspected endophthalmitis related to the preparation of bevacizumab in smaller units before intravitreal injection

<table>
<thead>
<tr>
<th>Study</th>
<th>Shared original vial</th>
<th>Compounded vials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of injections</td>
<td>No. of endophthalmitis (incidence)</td>
</tr>
<tr>
<td>Wu et al (PACORES)17</td>
<td>2470</td>
<td>1 (0.04%)</td>
</tr>
<tr>
<td>Lee et al16</td>
<td>1420</td>
<td>0</td>
</tr>
<tr>
<td>Artunay et al25</td>
<td>3022</td>
<td>3 (0.066%)</td>
</tr>
<tr>
<td>Pilli et al19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jonas et al20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Velpandian et al21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CATT Research Group6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Present study</td>
<td>1184</td>
<td>0</td>
</tr>
</tbody>
</table>

* N/A denotes not available
they are less costly, in the treatment of neovascular AMD, intravitreal injections of bevacizumab will increase in popularity with the availability of level I evidence on their efficacy and safety in comparison to ranibizumab.4 Many practices in both developed and developing countries have no alternative but to prepare small aliquots for intra-ocular injections in their own facilities before its use is licensed.21 Jonas et al20 reported that the risk of endophthalmitis could increase by 0.1% for every injection of bevacizumab. As endophthalmitis is a rare complication following intravitreal injection, it is difficult to arrive at any statistical conclusion regarding a comparison between bevacizumab and ranibizumab. However, this retrospective study spanning 4.5 years is already of reasonable duration. The theoretical increased risk of endophthalmitis associated with the additional procedures in fractionating the large bevacizumab vial for ‘off-label’ ophthalmic indications should not be neglected. Currently, there is no consensus regarding the preparation of bevacizumab into small doses for ophthalmic indications, and prospective study with a large sample size and long follow-up duration is needed to investigate the optimal protocol to minimise the risk of contamination. For this purpose, many centres aliquot multiple syringes from a single vial to be kept in a refrigerator for use. However, this study showed that there were no cases of endophthalmitis after the same vial was reused for a maximum of 10 consecutive injections, overnight storage of opened vials was avoided, and wearing surgical masks during intravitreal injections was mandated.

Disclaimer

None of the authors had relevant financial relationships to disclose regarding this study.

References


