Correspondence

To the Editor:

We read with great interest the article by Nagpal et al.[1] In the article, the authors have prospectively compared the effects of 20-millisecond Pattern Scanning Laser (Pascal) with 200-millisecond single-spot, 532-nm, solid-state green laser (GLX) in nonproliferative and proliferative diabetic retinopathy. They claimed that Pascal panretinal photocoagulation (PRP) showed lesser collateral damage and was less painful for the patient compared with GLX. We would like to address several points in this study that may provide a more open interpretation of their findings to the journal readership.

Currently, the standard laser pulse durations for PRP range from 20 milliseconds to 100 milliseconds. The use of 200-millisecond pulse laser appears rather excessive and not in keeping with current laser practice. Inevitably, the laser–tissue interactions and pain responses using 200-millisecond pulse laser will be considerably greater when compared with 20-millisecond PRP.

"The Pascal and GLX systems showed average fluences of 191 and 40.33 J/cm^2, respectively." These data are clearly erroneous because shorter pulse duration will deliver a lower fluence compared with higher pulse durations.[2] These data may be a typographic error as the abstract results conflict with the data presented in the article's results section and Table 1. The authors report significant differences in fluence between Pascal and GLX; however, these data would appear inconsistent to readers and require clarification by the authors.

A Mainster 165 PRP lens (magnification, $\times$1.96) was used in the study, but there is no mention of adjusted laser spot size and fluence calculations by the authors. The current Pascal system has an integrated laser lens correction mode that will automatically adjust the fluence according to the laser lens used by the operator. This lens magnification factor requires clarification by the authors in order that these laser parameter data may clearly be interpreted. The fluence data presented by the authors would suggest that a 100-μm spot size was used for PRP, and the final 200-μm spot would be as a result of laser spot magnification. Otherwise, the reported fluence values should be corrected by $\times$1.96 magnification, and this will produce lower fluence levels. GLX fluence range would be 33.1 J/cm^2 to 66.3 J/cm^2 (average, approximately 48 J/cm^2), and Pascal fluence range would be 6.6 J/cm^2 to 16.6 J/cm^2 (average, approximately 11 J/cm^2). The corrected fluence for 20-millisecond Pascal PRP would significantly be less than the authors' report, and further clarification is required by Nagpal et al.

To achieve threshold Grade 3 burns, the Pascal system required an average power of 630 mW (range 400–1000 mW), whereas the GLX needed 288 mW. The power used at 20-millisecond appears excessively high for threshold 20-millisecond PRP. The Pascal photocoagulator system was introduced to Manchester in 2006. We undertook a large retrospective evaluation of Pascal laser at Manchester Royal Eye Hospital and have recently published our experience using 20-millisecond to 30-millisecond Pascal PRP in proliferative diabetic retinopathy.[3,4] On average, laser powers between 350 mW and 400 mW were required for 20-millisecond to 30-millisecond PRP, and levels of PRP power greater than 800 mW have not been encountered in our practice. There may be a higher risk of choroidal rupture and subretinal neovascularization using very high power at short pulse duration. The Manchester Pascal Study is a randomized clinical trial that evaluated the clinical effects of Pascal 20-millisecond versus 100-millisecond PRP (Archives of Ophthalmology 2010, in press). The laser powers used in our study were significantly lower than the Nagpal study, and we were able to demonstrate effective PRP responses using these lower laser power parameters. Furthermore, we performed 100-millisecond laser using much lower power settings than the Nagpal study, and so we would welcome the authors' views on laser burn titration for PRP in their study.

The authors report the changes in laser burn size over time. The laser burns in both groups increased up to 3 months, with doubling of the 200-millisecond burns and 1.5 times increase in average sizes of 20-millisecond burns. The expansion of 20-millisecond burns in this study is in contrast to the observations of laser–tissue interactions in animal studies.[5] In the last few years, short-pulse laser burns have been found to progressively reduce in size, and this has been reported in vivo and in histopathologic work.[5,6] We recently completed a randomized controlled trial of laser–tissue interactions, comparing 20-millisecond and 100-millisecond PRP burns.[7] We observed a reduction in burn size using 20-millisecond laser lesions in the short-term. We presume that the enlargement of 20-millisecond burns in the study by Nagpal is related to the higher powers

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used to perform PRP, and so their findings should be interpreted closely with the laser parameters used and may not be universally applied to in vivo laser–tissue interaction.

A final comment relates to the pain responses reported by the authors. The use of 200-millisecond pulse PRP will visibly produce a more traumatic laser burn, and this is clearly shown in the authors' infrared images at 3 months. At this high fluence level, pain responses will be clinically relevant and well reported. The visual analog scale reported for 20-millisecond Pascal is 0.33. This value appears quite low considering the high level of power and fluence used for the 20-millisecond Pascal technique. The method of pain assessment is not published by the authors, and it would be relevant for readers to know whether pain responses were assessed using a masked and independent strategy to reduce observer bias. The Manchester Pascal Study has reported mild pain responses using 20-millisecond Pascal PRP, and our results are higher than those published by Nagpal et al; however, as previously mentioned, we used lower fluence laser pulse PRP (accepted for publication, British Journal of Ophthalmology, 2010).

We congratulate the authors for the effort; however, we believe that the issues raised within this letter require clarification by them because short pulse Pascal may offer a minimally traumatic and more comfortable method of retinal laser therapy. However, the in vivo dynamics of short-pulse laser photocoagulation should be accurately interpreted in clinical studies, to allow translation of effective and safe Pascal laser photocoagulation into clinical practice.

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Reply

To the Editor:

We thank Stanga et al for their interest in our article[1] about comparison of single-spot standard argon laser (GLX) versus pattern scan laser (PASCAL) for panretinal photocoagulation (PRP).

In this correspondence, Stanga et al have emphasized that standard laser pulse duration for PRP ranges from 20 milliseconds to 100 milliseconds and 200-millisecond burns are excessive. We point out a recent publication of the Diabetic Retinopathy Clinical Research Network Study,[2] which allowed laser exposure durations ranging from 50 milliseconds to 200 milliseconds. Our purpose was to compare two different laser modalities about efficacy, collateral damage, and convenience. Parameters of each were calibrated to achieve a replicable Grade 3 burn. In this set of patients, 100-millisecond duration did not consistently result in Grade 3 burns in our hands. Moreover, due to shorter laser pulse, PASCAL laser needs higher power settings to achieve the same-quality burn compared with longer pulse lasers. In our experience, a consistent Grade 3 burn, adequate for direct comparison with GLX burns, was achieved at an average power of 630 MW. While we note that Sanghvi et al[3] have used average laser powers between 350 MW and 400 MW, a recent study from Montoya et al[4] has reported a consecutive series of 1,301 cases treated by PASCAL in a Mexican population, with the average power settings varying between 520 MW and 590 MW for a 20-millisecond to 30-millisecond duration. These differences may represent the inherent biologic responses to the PASCAL laser system in the different populations being treated. We must also add that in none of the cases of our study did we encounter a choroidal rupture or any sign of a subretinal neovascularization.

Thus, while we note that Stanga et al achieved effective PRP responses using lower laser power parameters than ours, the aim of our study was not to derive the lowest possible power and duration settings to achieve adequate PRP responses. It only was aimed

at a comparison of two different modalities of laser and to study the efficacy of PASCAL. To determine the lowest efficacious power settings, a different study design will be applicable.

"The PASCAL and GLX systems showed average fluences of 191 J/cm^2 and 40.33 J/cm^2, respectively" is indeed a typographical error. The correct statement should be as follows: "The PASCAL and GLX systems showed average fluences of 40.33 J/cm^2 and 191 J/cm^2, respectively." We apologize to the journal readership for this typographical error. The fluence values mentioned in our article have been derived from the PASCAL screen. These values are calculated automatically by the laser machine based on the power, duration, and spot size of the laser procedure. Stanga et al are right when they say that this reading does not take into account the magnification factor of the lens. Fluence values would further reduce if the magnification factor is taken into consideration, although essentially the ratio of PASCAL versus GLX fluences would remain the same. As mentioned in the "discussion" section, the fluence used by PASCAL is indeed much lesser than that used by GLX.

We noted a spot size increase on the infrared images taken on the Heidelberg retinal angiogram in both our subgroups in the study. Muqit et al[5] mentioned that the spot sizes of 20-millisecond PASCAL burn decrease in size on follow-up. However, it should be noted that the maximum follow-up in their study has been 4 weeks while we have looked at 3 months. Hence, a direct comparison may not apply. Moreover, it has been documented that laser spots increase over time. The spots increase much more in the first 2 years after application and can increase further up to 4 years.[6] Thus, a 4-week follow-up might not be adequate to justify the end point of laser spot change.

We have clearly mentioned in our paper (p. 454, para 2)[1] the strategy used for assessing the pain response. As mentioned, we used the standardized Visual Analogue Scale to elicit these responses and it was done using a masked strategy by an independent observer. Panretinal photocoagulation was done in two sessions per eye and we reversed the order of PASCAL versus standard laser treatment in the first and second sessions to avoid any bias.

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To the Editor:

Dr Mallinowski[1] recently highlighted the importance of obtaining undiluted vitreous in a controlled way. The technique describes the essential elements of what we detailed in our letter in *Retina* in 2003.[2] More recently, we reported in 2009 at the European Vitreo-Retinal Society meeting an update on our experience based on 50 cases, which supported the usefulness and safety of this technique,[3] I believe it would have been appropriate for the author to acknowledge the original descriptors.

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Reply

To the Editor:

I value Dr Polkinghorne's response to my article, "The vitreous trap: a simple, surgeon-controlled

technique for obtaining undiluted vitreous and subretinal specimens during pars plana vitrectomy.''[1] Even if this is a similar technique, Dr Polkinghorne's letter to the editor from 2003[2] and his 2009 poster abstract[3] did not show up in any search of the peer-reviewed literature. No disrespect was intended and I commend Dr Polkinghorne on his fine technique.

Susan M. Malinowski, MD, FACS

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To the Editor:

We read with interest the recent article entitled ''Infectious endophthalmitis after intravitreal injection of antiangiogenic agents'' by Diago et al.[1] The authors investigated the risk factors for infectious endophthalmitis after intravitreal injection of anti–vascular endothelial growth factor agents and retrospectively identified 3 cases of infectious endophthalmitis among 3,875 injections. The authors concluded that failure to use a lid speculum and injection by a non–retina specialist may be risk factors for infectious endophthalmitis. The study did not examine the relationship between infectious endophthalmitis and structural characteristics of the vitreous cavity, such as posterior vitreous detachment (PVD). We propose that PVD may be an important risk factor for postinjection infectious endophthalmitis.

A recent study by de Caro et al[2] demonstrated a 13% rate of positive bacterial cultures of the bulbar conjunctiva after topical povidone–iodine and antibiotics and a 2% rate of bacterial contamination of needles used for injection. A similar study by Nentwich et al[3] identified bacterial contamination on 0.36% of 550 needles used for intravitreal injection. Both studies identified rates of needle contamination significantly higher than the postinjection endophthalmitis rate of approximately 0.02% to 0.05% seen in large prospective studies.[4,5] These findings suggest that ocular surface bacteria seeds the vitreous cavity at the injection site with a much higher frequency than the rate of infectious endophthalmitis. We posit that progression to endophthalmitis may be related to a combination of the seeding bacterial cell count, bacterial characteristics, intraocular immune mechanisms, and fluidic or anatomical characteristics of the vitreous cavity. The last of these factors is most easily studied, and we conducted an experiment to examine the possible role of PVD in development and progression of postinjection bacterial endophthalmitis. Our a priori hypothesis was that eyes with PVD would be less likely to develop endophthalmitis because an intact vitreous body could serve as a scaffold for the bacteria to travel to the retina in eyes without PVD but not in eyes with PVD.

Eighteen New Zealand albino rabbits were divided into two groups. In Group 1 (n = 9), the left eyes were injected with 0.25 mg of microplasmin (Thrombogenics, Dublin, Ireland) in 0.1 mL to enzymatically induce PVD. Seven days later, the left eyes of both groups received intravitreal injections of 0.05 mL of *Staphylococcus epidermidis* (approximately 550,000 colonies). On Postinjection Days 1, 3, and 7, 3 eyes from each group underwent enucleation for histologic examination. All surviving rabbits at each time point underwent clinical examination and B-scan ultrasonography. The right eyes of each rabbit served as control eyes.

One day after bacterial inoculation, 8 of 9 rabbits in Group 1 (enzymatic PVD) showed mild to moderate signs of infection on clinical examination, while only 1 of 9 rabbits in Group 2 (no PVD) showed any signs of infection. Examination with B-scan ultrasonography in Group 1 demonstrated at least moderate vitreous opacification, including 5 eyes that demonstrated a ''triangle sign,'' with discrete vitreous opacification extending from the anterior periphery to the posterior pole (Figure 1). In Group 2, all 9 eyes demonstrated mild to moderate vitreous opacification and no eyes demonstrated the triangle sign. Histologic examination of 3 eyes in Group 1 demonstrated discrete areas of preretinal fibrinous adhesions to focal areas of retinal destruction and inflammation. Histologic examination of 3 eyes in Group 2 demonstrated diffuse vitreoretinal adhesion with neutrophilic infiltration of the vitreous over corresponding areas of retinal destruction and inflammation.

Seven days after bacterial inoculation, all three surviving rabbits in each group demonstrated moderate to severe signs of infection. All three rabbits in each group demonstrated moderate to severe diffuse vitreous opacification on B-scan ultrasonography. Histologic examination of the remaining three eyes in each group demonstrated diffuse retinal destruction in all eyes.

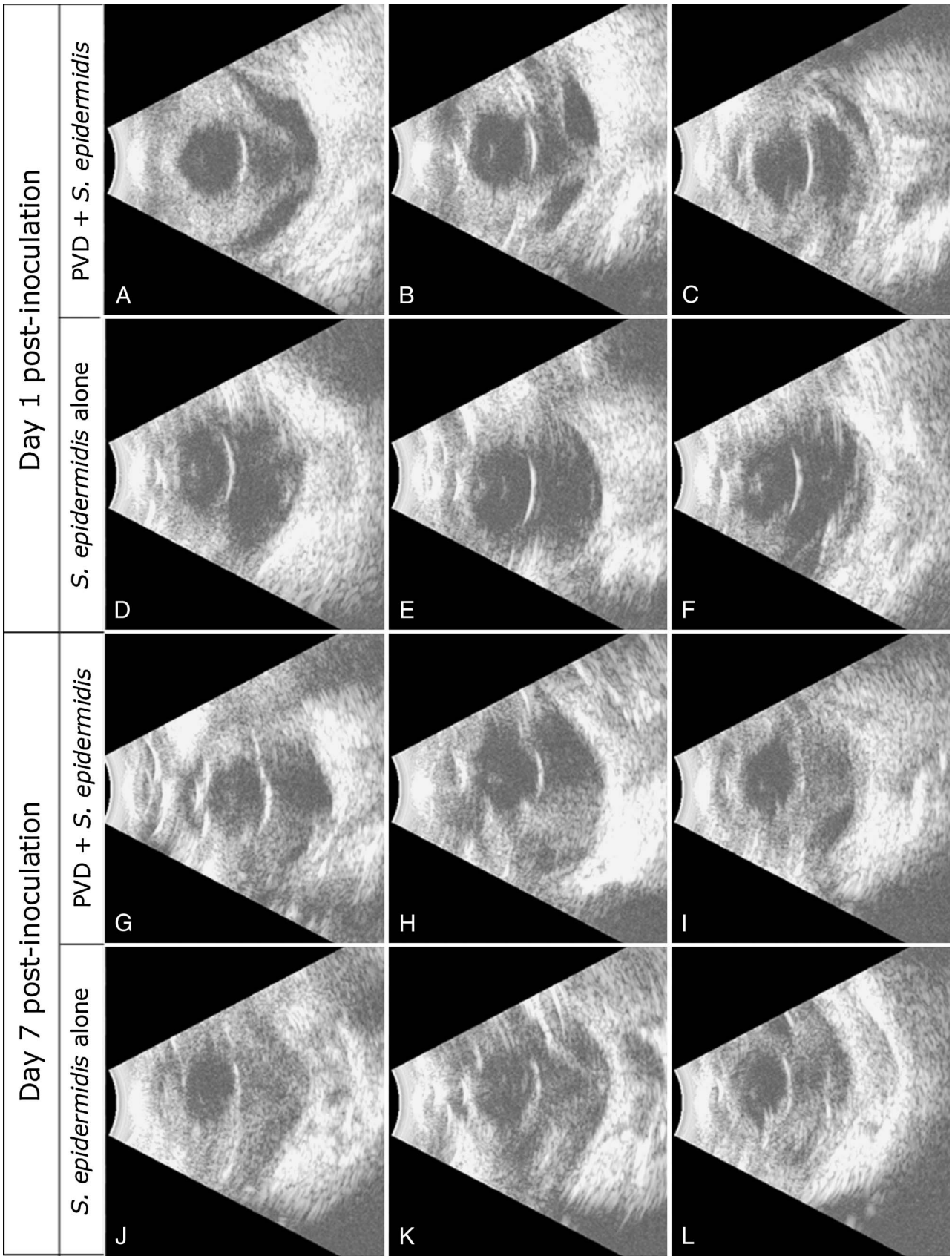

Fig. 1. At Postinoculation Day 1, eyes in Group 1 (PVD) demonstrated more signs of inflammation, including a triangular opacity on ultrasonography (**A**–**C**), compared with those (no PVD, **D**–**F**) in Group 2, whereas by Day 7, both groups appeared similarly affected (Group 1, **G**–**I** compared with Group 2, **J**–**L**).

At Postinoculation Day 1, eyes in Group 1 (PVD) demonstrated more signs of infection and inflammation on both clinical examination and ultrasonography, whereas by Day 7, both groups appeared equally affected. The triangle sign seen in some eyes in Group 1 is likely to represent a fibrin bridge between detached vitreous and the neurosensory retina. Given the consistent findings of PVD in eyes with lower doses of microplasmin in other studies, this finding is unlikely to represent incomplete PVD.[6,7] Histologic examination demonstrated that focal areas of retinal destruction and inflammation in Group 1 corresponded to areas of likely fibrin bridge formation in the retina. Findings on Postinoculation Day 3 were intermediate between those on Days 1 and 7 in both Groups 1 and 2.

In our experiment, eyes were inoculated with more than 500,000 colonies of *S. epidermidis*, a much greater bacterial load than would be expected with inadvertent vitreous seeding after intravitreal injection. Eyes with PVD fared worse than those without PVD, with the most dramatic difference seen in the first day after injection. If these findings are extrapolated to a much lower bacterial load, we believe that our study supports the notion that postinjection bacterial seeding of the vitreous cavity is more likely to progress to endophthalmitis in eyes with PVD than in those without PVD. Our findings stand in contrast to our a priori hypothesis. It is possible that in eyes with PVD, fluid currents posterior to the hyaloid face serve as an efficient conduit for the bacteria to travel to the retina, with concurrent or subsequent formation of fibrin bridges across this fluid-filled space.

Clearly, further work is needed to elucidate the role of the vitreous body and vitreous detachment in the development and progression of bacterial endophthalmitis after intravitreal injection. We applaud the efforts by Diago et al[1] to identify risk factors for infectious endophthalmitis after intravitreal injection, a rare complication, and we hope that future studies will increasingly pursue the role of vitreous structure and dynamics in the development of this complication.

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Reply

To the Editor:

We value the interest showed by Ranchod et al to our article ''Infectious endophthalmitis after intravitreal injection of antiangiogenic agents.''[1] Their hypothesis of posterior vitreous detachment as a risk factor for postinjection endophthalmitis is interesting as well as their study conducted with rabbits. Ranchod et al obtained higher incidence of inflammation after the inoculation of bacterial colonies in the eyes of the rabbits with enzymatic posterior vitreous detachment. However, more studies are needed to elucidate the role of posterior vitreous detachment as a higher risk in the development of endophthalmitis after intravitreal injections. We encourage the authors to do more studies after their hypothesis and try to demonstrate it with clinical studies in humans.

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