2. Lipham WJ, Tawfik HA, Dutton JJ. A histologic analysis and three-dimensional reconstruction of the muscle of Riolan. *Ophthal Plast Reconstr Surg*. 2002;18(2):93-98.

3. Shah CT, Blount AL, Nguyen EV, Hassan AS. Cranial nerve seven palsy and its influence on meibomian gland function. *Ophthal Plast Reconstr Surg*. 2012;28 (3):166-168.

4. Call CB, Wise RJ, Hansen MR, Carter KD, Allen RC. In vivo examination of meibomian gland morphology in patients with facial nerve palsy using infrared meibography. *Ophthal Plast Reconstr Surg*. 2012;28(6):396-400.

Trypan Blue Vital Dye Staining vs TUNEL Technique to Detect Corneal Endothelium Toxic Effects

To the Editor We read the article titled "Efficacy and Safety of Antifungal Additives in Optisol-GS Corneal Storage Medium" by Layer et al[1] with interest. In their article, the authors investigated the efficacy and safety of voriconazole and amphotericin B in reducing *Candida* species contamination of Optisol-GS under normal storage conditions. Layer and colleagues used 0.4% trypan blue as a vital stain to evaluate corneal endothelium toxic effects.

Trypan blue is primarily an indicator of membrane integrity and does not identify corneal endothelium undergoing apoptosis.[2] Apoptosis is the principal form of cell death in the corneal endothelium under organ culture conditions.[3] It was found that the mean percentage of cell death at the end of storage assessed by the terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) technique is about 8-fold higher than when assessed by the trypan blue technique.[2] We think the authors should also have shown apoptosis of corneal endothelium in their study.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

1. Layer N, Cevallos V, Maxwell AJ, Hoover C, Keenan JD, Jeng BH. Efficacy and safety of antifungal additives in Optisol-GS corneal storage medium. *JAMA Ophthalmol*. 2014;132(7):832-837.

2. Gain P, Thuret G, Chiquet C, et al. Value of two mortality assessment techniques for organ cultured corneal endothelium: trypan blue versus TUNEL technique. *Br J Ophthalmol*. 2002;86(3):306-310.

3. Albon J, Tullo AB, Aktar S, Boulton ME. Apoptosis in the endothelium of human corneas for transplantation. *Invest Ophthalmol Vis Sci*. 2000;41(10): 2887-2893.

In Reply There is no gold standard or widely accepted staining protocol for assessing endothelial cell death in human corneas after storage. We followed the vital dye staining protocol proposed by Park et al,[1] who validated the ability of trypan blue to detect endothelial damage by comparing corneas exposed to hydrogen peroxide with those exposed to balanced salt solution and found that the optimal dye concentration was 0.4%. The only other study, to our knowledge, of an antifungal additive in Optisol-GS also used the trypan blue technique to evaluate corneal endothelial toxic effects.[2]

Gain et al[3] demonstrated that the TUNEL technique revealed a higher mean percentage of endothelial cell death compared with trypan blue staining, but they also found a significant correlation between the 2 methods. This implies that implementing the TUNEL technique would not change the relative values of our findings. However, we appreciate the potential of the TUNEL technique to reveal nonviable endothelial cells undetected by trypan blue staining, and we agree that it would be worthwhile to include this more sensitive method in further assessments of antifungal toxic effects.

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1. Park S, Fong AG, Cho H, et al. Protocol for vital dye staining of corneal endothelial cells. *Cornea*. 2012;31(12):1476-1479.

2. Ritterband DC, Shah MK, Meskin SW, et al. Efficacy and safety of voriconazole as an additive in Optisol GS: a preservation medium for corneal donor tissue. *Cornea*. 2007;26(3):343-347.

3. Gain P, Thuret G, Chiquet C, et al. Value of two mortality assessment techniques for organ cultured corneal endothelium: trypan blue versus TUNEL technique. *Br J Ophthalmol*. 2002;86(3):306-310.

Comments on Infant Aphakia Treatment Study 4.5-Year Results

To the Editor We read with interest the article on 4.5-year visual acuity outcomes for the Infant Aphakia Treatment Study (IATS).[1] This is clearly a well-thought-out and meticulously devised study.

The authors state that the study follows the intention-to-treat principle. We feel that as not all the study participants met the outcome criteria, this study does not fit the definition of an intention-to-treat study, which should include all the participants. For example, only the participants who had at least 3 adherence assessments were included in the analysis.

The authors conclude with this comment: "This study did not demonstrate any visual benefit from implanting an IOL [intraocular lens]...."[1] With all due respect, this conclusion may be an overstatement. The study only looked at visual acuity

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as a marker for vision. It is well known that there are other parameters of visual function that may be equally as important as visual acuity, such as contrast sensitivity, visual fields, and color vision.

The authors did not comment on the vision-threatening complications present only in the contact lens group (Table 2 in the article). This group of patients had 2 retinal detachments, 1 case of endophthalmitis, and 1 case of phthisis bulbi; none of these appeared in the IOL group. We appreciate that in this relatively small study these may have been present by chance alone, but they may be statistically significant and should be mentioned.

When analyzing the visual acuity data, it would have been useful to compare the difference in the visual acuity between the operated eye and the fellow eye in the contact lens and IOL groups. This method has been used in other similar studies[2] and would allow for any differences between patients on account of poor cooperation with visual acuity testing.

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1. Lambert SR, Lynn MJ, Hartmann EE, et al; Infant Aphakia Treatment Study Group. Comparison of contact lens and intraocular lens correction of monocular aphakia during infancy: a randomized clinical trial of HOTV optotype acuity at age 4.5 years and clinical findings at age 5 years. *JAMA Ophthalmol*. 2014;132(6):676-682.

2. Autrata R, Rehurek J, Vodicková K. Visual results after primary intraocular lens implantation or contact lens correction for aphakia in the first year of age. *Ophthalmologica*. 2005;219(2):72-79.

In Reply We appreciate the interest of Sueke and Chandna in the outcomes of the IATS.

The intention-to-treat strategy is used in randomized clinical trials to ensure that treatment groups remain similar.[1] This strategy requires that patients are analyzed with the groups to which they were originally randomized even if they received a different treatment. One patient in our study was randomized to the IOL group but did not undergo IOL implantation, and 3 patients in the contact lens group received secondary IOLs. All of these patients were analyzed with the group to which they were originally randomized. Missing data can also be an issue. We were fortunate that this was not a major problem for the clinical outcomes assessed at follow-up examinations since only 1 patient was lost to follow-up, and for the primary outcome of optotype visual acuity at age 4.5 years, only 1 additional patient did not have data as a result of developmental delay. In contrast, patching adherence (which was not a clinical outcome) was assessed using recall telephone interviews every 3 months and a patching calendar completed by the child's primary caregiver for 1 week each year. Because not all caregivers completed these calendars and some recall telephone interviews were missed, there were more missing data for patching adherence (missing data: year 1, 10%; years 2-5, <25%) than for the clinical outcomes.

The primary outcome of the IATS was optotype visual acuity at age 4.5 years using the Amblyopia Treatment Study HOTV test. There was no difference in the visual acuity outcome between the 2 groups (for both: median visual acuity, 0.90 logMAR [Snellen equivalent, 20/159]; $P = .54$).[2] While it would have been interesting to have collected data for other parameters of visual function, many of the children in the IATS would have had difficulty performing these tests when aged 4.5 years.

Two patients in the contact lens group had serious vision-threatening complications. We discussed these complications in detail in previous publications describing the 1-year outcomes.[3,4] The percentage of patients with serious vision-threatening complications was not significantly different between the 2 groups using the Fisher exact test (contact lens group, 2 of 57 patients [3.5%]; IOL group, 0 of 57 patients; $P = .50$).

The decision to use the visual acuity in the treated eye as the primary end point, rather than the difference in visual acuity between the treated and fellow eyes, was made prior to the start of the study and in consultation with the Data and Safety Monitoring Committee. The decision was based primarily on statistical considerations. The use of the interocular difference in the analysis is a form of adjusting for a postrandomization covariate in which the estimated effect on the visual acuity in the treated eye when using an IOL rather than a contact lens is adjusted for the visual acuity in the fellow eye. Given the disadvantages of adjusting for a postrandomization covariate,[5] particularly the possibility of introducing bias into the estimate, the decision was made to use the visual acuity in the treated eye as the primary end point.

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1. Hollis S, Campbell F. What is meant by intention to treat analysis? survey of published randomised controlled trials. *BMJ*. 1999;319(7211):670-674.

2. Lambert SR, Lynn MJ, Hartmann EE, et al; Infant Aphakia Treatment Study Group. Comparison of contact lens and intraocular lens correction of monocular aphakia during infancy: a randomized clinical trial of HOTV optotype acuity at age 4.5 years and clinical findings at age 5 years. *JAMA Ophthalmol*. 2014;132(6):676-682.

3. Lambert SR, Buckley EG, Drews-Botsch C, et al; Infant Aphakia Treatment Study Group. A randomized clinical trial comparing contact lens with intraocular lens correction of monocular aphakia during infancy: grating acuity and adverse events at age 1 year. *Arch Ophthalmol*. 2010;128(7):810-818.

4. Plager DA, Lynn MJ, Buckley EG, Wilson ME, Lambert SR; Infant Aphakia Treatment Study Group. Complications, adverse events, and additional intraocular surgery 1 year after cataract surgery in the Infant Aphakia Treatment Study. *Ophthalmology*. 2011;118(12):2330-2334.

5. Rochon J. Issues in adjusting for covariates arising postrandomization in clinical trials. *Drug Inf J*. 1999;33(4):1219-1228. doi:10.1177/009286159903300425.

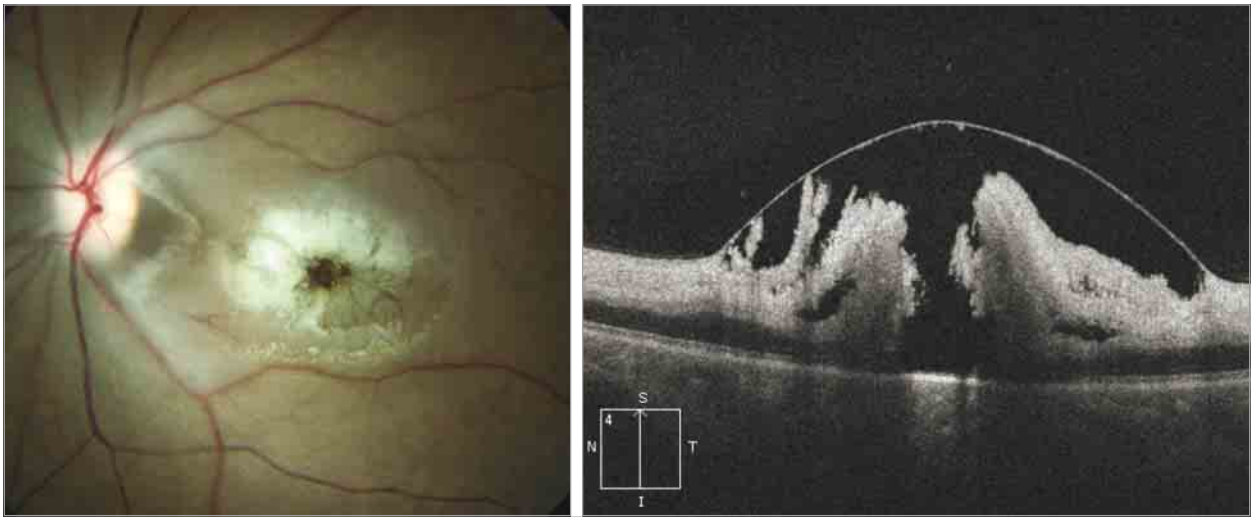
CORRECTION

Incorrect Information in Table: In the Original Investigation entitled "The Risk of Toxic Retinopathy in Patients on Long-term Hydroxychloroquine Therapy" published online October 2, 2014, in *JAMA Ophthalmolgy* (doi:10.1001/jamaophthalmol.2014.3459), incorrect information appeared. In Table 2, the confidence intervals for Duration of use in 5-y increments should have read as 2.03 (1.72-2.40). This article was corrected online.

OPHTHALMIC IMAGES

Macular Hole From a Central Retinal Artery Occlusion

Ashleigh L. Levison, MD; Andrew P. Schachat, MD



A 24-year-old woman with lupus and antiphospholipid syndrome presented with sudden vision loss. By clinical examination and fluorescein angiography, she was diagnosed with a central retinal artery occlusion in the left eye. The retinal edema was associated with a macular hole documented on an optical coherence tomography image.

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