A Multicentered Clinical Study of Serum as Adjuvant Therapy for Surgical Treatment of Macular Holes

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Objective: To evaluate and compare the risks and benefits of autologous serum as an adjuvant therapy in macular hole surgery for stage 3 or 4 macular holes.

Methods: Comparison of 2 consecutive (nonrandomized) cohorts using standardized methods for the determination of hole size and for surgical procedures, and using the same study surgeons. The serum cohort consisted of 106 eyes using autologous serum as an adjuvant, and the no serum cohort consisted of 58 eyes without adjuvants. The primary end point was the closure of the macular hole as determined by the 6-month fundus photographs. Secondary end points included the number and types of postoperative complications. Comparison in outcomes between the 2 cohorts used \( \chi^2 \) and logistic regression procedures, adjusting for preoperative differences between the study cohorts.

Results: At 6 months, the (unadjusted) rate of hole closure was significantly greater for the eyes treated with serum than for the eyes not treated with serum (90 [85%] of 106 vs 40 [69%] of 58, \( P = .04 \)). However, after adjusting for preoperative differences in hole diameter and the prevalence of epiretinal membranes, no overall difference in hole closure rates due to serum was found (\( P = .44 \)). In contrast, benefit due to serum for large holes (diameter >573 \( \mu \)m) was seen (12 [75%] of 16 vs 13 [57%] of 23, \( P = .04 \)). No differences in complication rates were found between the cohorts.

Conclusions: Any beneficial effect of serum used as an adjuvant to macular hole surgery is small, and, if present, the beneficial effect may be limited to larger holes. A randomized, prospective, controlled study in larger macular holes is needed.


The rate of anatomic success (hole closure) following vitrectomy surgery for stage 3 or 4 macular holes has been reported to be in the range of 58% to 91% without the use of any adjuvant therapy.\(^1\)\(^\text{-}\)\(^7\) The rate of anatomic success has been reported to be improved by the use of wound healing adjuvants that stimulate fibroblast proliferation and collagen synthesis.\(^3\)\(^-\)\(^7\) An early observational study\(^8\) using bovine transforming growth factor \( \beta \) as an adjuvant reported a hole closure rate of 91% compared with a hole closure rate of 53% without the use of an adjuvant. However, a subsequent randomized trial\(^8\) comparing recombinant transforming growth factor \( \beta \) with placebo showed no difference between treatments. A pilot study\(^8\) using serum as an adjuvant reported a 100% anatomic success rate in 11 eyes. In a subsequent report\(^10\) in a larger series of eyes, the anatomic success rate was reported to be 92%. Recently, an anatomic success rate of 88% was reported with the use of autologous platelet concentrate as an adjuvant.\(^11\)

The Vitrectomy for Macular Hole Study (VMHS) was a multicentered, randomized, clinical trial that compared vitrectomy surgery with observation alone in eyes with stage 3 or 4 macular holes. Compared with eyes randomized to observation, eyes randomized to vitrectomy surgery had a significantly greater hole closure rate (4% vs 69%, \( P<.001 \)) and showed significant improvements in several measures of vision.\(^12\) Complications due to vitrectomy surgery have also been documented.\(^13\) Subsequently, VMHS surgeons operated on a consecutive series of eyes with stage 3 or 4 macular holes using serum as an adjuvant. In this article, we compared the anatomic success rate in eyes treated by VMHS surgeons with vitrectomy surgery with and without the use of serum as an adjuvant. Because eyes were not randomized to the use of serum, appropriate statistical adjustments are made.
MATERIALS AND METHODS

COHORT OF EYES TREATED WITH SERUM

All patients in the cohort of eyes treated with serum had a macular hole classified as stage 3 or 4, with clear ocular media and a pupil of at least 5 mm in diameter. Patients were not included in the cohort if they had coexisting retinal disease that could affect the functional status of the macula, no symptoms of decreasing central vision, or a negative Watzke-Allen test result (a break seen through a slit beam projected onto the fovea). The self-reported date of onset of symptoms was recorded, and the duration of the macular hole was estimated. The risks and benefits of vitrectomy surgery with serum as an adjuvant were explained to all eligible patients, and informed consent was obtained. The resulting cohort consisted of 106 eyes from 106 subjects.

Fundus photography and fluorescein angiography were performed at baseline and 6 months postoperatively. Hole size (total diameter in micrometers) was determined at baseline and 6 months postoperatively from the fundus photographs that were read by one of us (A.S.B.), and independently reviewed by the principal investigator (W.R.F.) until consensus was reached. The opinion of the surgeon was used in the case of photographs that could not be evaluated. This method was also applied to the no serum cohort. Visual acuity was measured at baseline and 6 months postoperatively using nonstandardized Snellen visual acuity charts; preoperative refraction was not performed as part of the protocol.

For the preparation of autologous serum, 5 mL of venous blood was placed into a sterile red-topped blood tube, and the clotted blood was centrifuged at 350g for 10 to 15 minutes. The serum was collected in a sterile syringe using a sterile technique for intraocular use.

The surgical technique has been previously described.12,13 In brief, the procedure included a standard 3-port pars plana vitrectomy with removal of all visible vitreous (scleral depression was not required). A soft tip extraction needle was used to sweep over the retina to induce a complete posterior vitreous detachment, if not already present. The vitreous gel and epiretinal membranes (ERMs) (if present) were then gently removed from the macular area with a bent microvitreoretinal blade. Examination of the peripheral retina was performed to detect any peripheral retinal breaks, which, if found, were lasered. Perfluoropropane gas (16%) was used in all cases as an intraocular tamponade, and rigorous facedown positioning for 2 weeks postoperatively was mandated. Air-fluid exchange was then performed to flatten the macular hole, and attempts were made to drain the hole dry, keeping the soft-tipped suction cannula close to the hole. After complete air fill, the surgeon waited 10 to 15 minutes to reextrude all fluid. Using a bent 30-gauge cannula, 60 µL (~3 drops) of autologous serum was then placed over the hole.

COHORT OF EYES NOT TREATED WITH SERUM

For comparison, we used the cohort from 58 subjects’ (of 64) enrolled eyes with stage 3 or 4 macular holes randomized to vitrectomy surgery without the use of serum and with anatomic status determined at the 6-month follow-up visit. (Six eyes did not have complete 6-month follow-up data.) Anatomic status was determined by photographs for 52 eyes and by surgeon opinion for 6 eyes (because of poor photograph quality). Anatomic, visual acuity, and complication outcomes have been previously reported by the VMHS group.12,13 For this cohort, all eligibility and exclusion criteria; methods for the determination of hole size preoperatively and at 6 months, and surgical procedures were the same as those used for the cohort of eyes treated with serum. In contrast to eyes in the serum cohort, the best-corrected visual acuity was measured in a standardized manner at baseline and 6 months postoperatively using the Early Treatment for Diabetic Retinopathy Study charts. Table 1 shows that both groups were similar for age and sex. The same surgical technique was used in both cohorts.

STATISTICAL ANALYSES

The primary end point was the closure of the macular hole as determined by the 6-month fundus photographs and the frequency and types of postoperative complications. The assessment of hole closure was made by photographic readers masked to the treatment status. Comparison in anatomic outcomes between the 2 cohorts used χ² and logistic regression procedures, adjusting for preoperative differences between the study cohorts. Because visual acuity testing in patients treated with serum was performed without refraction or Early Treatment for Diabetic Retinopathy Study charts, as contrasted to visual acuity measurements in the nonserum group (which were performed using standardized refraction with Early Treatment for Diabetic Retinopathy charts), visual acuity was not considered an outcome measure.

Table 1 summarizes the baseline characteristics for the 106 eyes in the serum cohort and the 58 eyes in the no serum cohort. No differences were found in the distributions of age, sex, and self-reported hole duration between the study cohorts. In contrast, the hole size was significantly larger for the eyes in the no serum cohort compared with those in the serum cohort. In addition, the prevalence of ERMs was significantly greater for the eyes in the no serum cohort. For this reason, all analyses of hole closure rates were adjusted for hole size and the prevalence of ERM. Differences were also found in baseline visual acuity.

Table 2 summarizes the anatomic outcomes for the 2 study cohorts. At 6 months, the (unadjusted) rate of hole closure was significantly greater for the eyes treated with serum than it was for eyes not treated with serum. However, after adjusting for preoperative differences in hole diameter (coded as quartiles), and the prevalence of ERM, no overall difference in hole closure rates due

in the analyses to account for preoperative differences between the 2 cohorts of eyes.
to serum was found. Of interest, however, was the observed benefit due to serum for the largest holes (diameter > 573 µm). The prevalence of ERMs for the largest holes was not significantly different between the 2 cohorts: 11 (69%) of 16 eyes for the serum cohort compared with 20 (87%) of 23 eyes for the nonserum cohort (P = .16). This is in contrast to the holes with a diameter of less than 573 µm or smaller, in which case the prevalence of ERM was significantly greater for the nonserum cohort: 27 (79%) of 34 eyes for the nonserum cohort compared with 20 (87%) of 23 eyes for the serum cohort (P < .001).

The most common postoperative complication was the formation of facettike retinal pigment epithelium changes in or around the macular hole (Table 2). No other complications were observed with the use of the serum.

The primary goal of macular hole surgery is to close the macular hole, reappose the detached rim of neurosensory retina to the retinal pigment epithelium, and thus improve visual function. Macular hole surgery may be performed with or without biochemical adjuvants. Adjuvant therapy is used in an attempt to increase the hole closure rate and, therefore, potentially improve visual results. The first adjuvant therapy used to treat macular holes was the application of transforming growth factor β. The initial studies of this substance appeared promising, with clinical trials suggesting an increased hole closure rate and better visual acuity when this adjuvant therapy was used. Subsequently, a randomized, prospective, clinical trial showed no benefit. Liggett and associates first reported the use of autologous serum as an adjuvant and in an unrandomized series reported a high (100%) hole closure rate. Other adjuvants have been used, including autologous platelet concentrate and bovine thrombin. All of the adjuvants that have been used contain numerous biologically active cytokines and growth factors that may have an effect on the retinal pigment epithelium and adjacent tissues to promote retinal reattachment.

We chose to study the use of autologous serum because it is readily available and because of the promising results reported by Christmas et al in the laboratory and by Liggett et al in a preliminary report in patients. One concern with the use of such autologous growth factors is sterility and the possibility that there may be an adverse effect on the hole closure rate or that the factor may improve hole closure but might in some way adversely affect vision. For these reasons, the VMHS group chose to compare the results of 2 consecutive series of patients operated on with and without serum adjuvant. The cohort of eyes operated on with serum was a consecutive series operated on in subsequent years to those in the initial VMHS cohort operated on without serum. Consequently, eyes operated on with serum had smaller holes and holes of shorter duration and had less associated ERM. The serum cohort might be expected to benefit from more experience of the surgeons and improvements in technique and patient management. For this reason, it was necessary to statistically correct for this reason, it was necessary to statistically correct for this imbalance to evaluate whether there was an improved hole closure rate using serum.

Our data show that after adjusting for preoperative differences in hole diameter and the prevalence of ERMs, no overall difference in hole closure rates due to serum

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**Table 1. Baseline Characteristics of the Study Cohorts**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum Group (n = 106)</th>
<th>No Serum Group (n = 58)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y§</td>
<td>67.2 ± 8.6 (37-88)</td>
<td>69.5 ± 6.9 (50-83)</td>
<td>.07</td>
</tr>
<tr>
<td>Hole, mean ± SD, µm§</td>
<td>447.5 ± 152.6 (177-917)</td>
<td>541.1 ± 159.2 (215-875)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ERM prevalence‡¶</td>
<td>38 (37)</td>
<td>48 (83)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Baseline visual acuity§</td>
<td>20/148 (20/50-20/1002)</td>
<td>20/120 (20/46-20/833)</td>
<td>NA</td>
</tr>
</tbody>
</table>

* ERM indicates epiretinal membrane; NA, statistical testing is not applicable because of different vision testing methods used in the 2 studies.
†n = number of eyes and patients (1 eye per patient).
‡Presence or absence of ERM was not available for 3 eyes in the serum group.
§The range is given in parentheses.
¶Hole diameter was not recorded for 1 eye in the no serum group.

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**Table 2. Anatomic Outcomes and Complication Rates at 6 Months**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum Group (n = 106)</th>
<th>No Serum Group (n = 58)</th>
<th>OR (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis of Effect of Treatment (Unadjusted for ERM and Diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (unadjusted)</td>
<td>90 (85)</td>
<td>40 (69)</td>
<td>2.5 (1.2-5.5)</td>
</tr>
<tr>
<td>Analysis of Effect of Treatment (Adjusted for ERM and Diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERM‡</td>
<td>Not present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>56/56 (86)</td>
<td>9/10 (90)</td>
<td>1.0</td>
</tr>
<tr>
<td>Present</td>
<td>51/38 (82)</td>
<td>31/46 (65)</td>
<td>0.6 (0.2-1.6)</td>
</tr>
<tr>
<td>Diameter, µm§</td>
<td>≤354</td>
<td>33/36 (92)</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>≥354</td>
<td>355-480</td>
<td>23/27 (85)</td>
<td>11/14 (79)</td>
</tr>
<tr>
<td>≥481</td>
<td>481-573</td>
<td>22/27 (81)</td>
<td>11/14 (79)</td>
</tr>
<tr>
<td>≥573</td>
<td>573-875</td>
<td>12/16 (75)</td>
<td>13/23 (57)</td>
</tr>
<tr>
<td>Treatment (adjusted)</td>
<td>. . .</td>
<td>. . .</td>
<td>1.4 (0.6-3.4)</td>
</tr>
</tbody>
</table>

*OR indicates odds ratio; CI, confidence interval; ERM, epiretinal membrane; RPE, retinal pigment epithelium; and ellipses, data are not applicable.
†n = number of eyes and patients (1 eye per patient). Values are given as number (percentage) of eyes and patients.
‡Presence or absence of ERM was not available for 3 eyes in the serum group.
§Diameter was not recorded for 1 eye in the no serum group.
was found (P = .44). However, there was a possible benefit of serum, but this appeared to be present only in holes of relatively large size (>573 µm) (P = .04). These analyses only adjust for ERMs and hole size, but there may be imbalance in other unobservable variables that could affect the results. For example, increased experience of surgeons in the serum (second) cohort could account for the higher closure rate in the more difficult to close larger holes. It is not possible to quantify this potential confounder. As previously reported, the 2 most common complications of macular hole surgery include pigmentary changes in the macula and retinal detachment. No differences in complication rates for pigmentary changes or retinal detachments were found between the cohorts. There were no cases of microbial endophthalmitis.

Visual acuity was not a primary outcome in this study. Unfortunately, it was not possible to evaluate the effect of adjuvant serum on visual acuity because of limitations in the study design. Because standardized visual acuity measurements were not performed in the serum cohort and because some eyes were not refracted preoperatively, preoperative visual acuity may have been underestimated. On the other hand, postoperative visual acuity was more likely to have been determined with refraction.1,2

In summary, this multicentered nonrandomized trial does suggest that if there is any benefit of serum as an adjuvant for macular hole surgery, it is limited to larger holes. We were not able to evaluate whether this potential benefit was associated with significant improvement in vision. A prospective, randomized, clinical trial would be necessary to determine whether this potential benefit is real. Using data from this study, we estimate that 118 eyes would be needed in each treatment arm (α = .05, β = .80, 2-sided test of hypothesis). For most macular holes, there does not appear to be an anatomic benefit to the use of serum.

Accepted for publication April 13, 1999.

This study was supported by a grant from Alcon Inc, Fort Worth, Tex; and core grant for vision research NEI- EY-03040 from the National Institutes of Health, Bethesda, Md (Dr Azen). Dr Freeman is the recipient of the Lew Wasserman award from Research to Prevent Blindness, Inc, New York, NY.

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REFERENCES