Adjuvant Methods in Macular Hole Surgery: Intraoperative Plasma-Thrombin Mixture and Postoperative Fluid-Gas Exchange

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■ **BACKGROUND AND OBJECTIVE:** The optimal method for surgical management of idiopathic macular holes remains unknown. Adjuvant methods including intraoperative cytokines and postoperative fluid-gas exchange with and without laser have been described. We report on the safety and final results of routine intraoperative autologous plasma-thrombin mixture and postoperative fluid-gas exchange when necessary as an adjunct to the surgical therapy of this disease.

■ **PATIENTS AND METHODS:** A consecutive series of 114 patients (mean age 66.9 years) with primary idiopathic full thickness Stage II, III, and IV macular holes were primarily treated by vitrectomy, fluid/perfluorocarbon gas exchange, and application of autologous plasma-thrombin mixture to the macular hole. Visible epiretinal membranes were peeled but the normal appearing internal limiting membrane was not routinely stripped. Outcome measures included final Snellen visual acuity, rate of macular hole closure, complications, and number of supplemental procedures performed.

■ **RESULTS:** Closed at one month, were 110 of 121 (91%) macular holes, including two that underwent repeat fluid/gas exchange and laser within the first two weeks after surgery. At the time of final follow-up (mean: 10.9 months), 110 of 121 (91%) macular holes were closed. This included 8 of 9 eyes that had reopening of the macular hole between one and 21 months successfully treated by repeat fluid-gas exchange and 2 eyes that underwent a second successful pars plana vitrectomy, membrane peeling, and repeat fluid-gas exchange. Overall, 98 of 121 eyes overall (81%) were successfully treated by a single surgery; 94 of 121 (78%) achieved two lines or greater of visual improvement; 83 of 121 (69%) achieved 20/70 or better vision; and 47 eyes (39%) achieved 20/40 or better vision. Complications in this series included infectious endophthalmitis (1 eye), intraoperative retinal break (2 eyes), late retinal detachment (5 eyes), transient mild intraocular pressure elevation (46 eyes), inflammatory response (six eyes), epiretinal membrane (6 eyes), intraretinal hemorrhages (1 eye), and cataract (33 of 99 phakic eyes underwent cataract extraction during the follow-up).

■ **CONCLUSION:** A combination of intravitreal perfluorocarbon gas and autologous plasma-thrombin mixture (tissue glue) was well tolerated in most patients and did not result in any specific long-term complications. The use of supplemental fluid-gas exchange when necessary improved the final success rate. Further well-controlled and randomized studies will be required to determine the efficacy of this as an adjunct or alternative to other methods of treatment for macular holes.

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INTRODUCTION

The optimal therapy for idiopathic macular hole in its various stages remains unknown.[1] The natural history of untreated stage III and IV macular holes is unfavorable with the majority of eyes falling to the level of 20/200 or less vision.[1-3] Early vitrectomy for stage I macular hole does not appear to appreciably improve the long-term prognosis.[4] However, vitrectomy, removal of the posterior cortical gel, fluid-gas exchange, and prone positioning have been shown to result in sealing of the edges of macular holes, resolution of the surrounding cuff of subretinal fluid, and improvement in Snellen acuity in selected cases.[4-7] Successful macular hole closure by these techniques has been further documented by histopathologic examination of several eyes.[8,9] Success rates for macular hole surgery appear to be improving as a result of several factors, including greater familiarity with the surgical techniques involved and possibly better case selection.[10] Despite these improvements, failure to initially close the macular hole following surgery and late reopening of macular holes have been reported to be continuing problems.[6,11]

Some investigators have reported improved success rates in macular hole surgery using adjunctive agents such as bovine TGF-beta[12-15] albeit it was recently shown that the use of recombinant human TGF-beta had no benefit in anatomic or visual outcome.[16] However, the conditions employed in the TGF-beta studies differ somewhat with respect to immediate postoperative positioning and choice of gas tamponade from the original series of patients treated, suggesting that other variables including choice of gas mixture,[17] immediate postoperative positioning, and case selection may also be important.[10] Investigators are evaluating other potential adjunctive agents including serum[18,19] that contain naturally occurring chemoattractants for the retinal pigment epithelium[20] as well as thrombin-plasma mixture, thrombin alone,[21] thrombin-autologous fibrinogen mixture,[22] and autologous platelet concentrate.[23] All of these are known to contain a variety of cytokines and mitogens that may play an important role in the vitreoretinal wound healing response.[24]

We elected to study the safety and possible efficacy of a mixture of autologous plasma and bovine thrombin, commonly referred to as "tissue glue," as an adjunctive agent in the surgical therapy of macular holes. Plasma, like serum, contains naturally occurring growth factors. Unlike serum, plasma is relatively deficient in platelet derived growth factor, but relatively rich in soluble fibrinogen, the substrate for thrombin resulting in the generation of insoluble fibrin.[24] Plasma thrombin mixture has been applied in other surgical disciplines, and functions as a hemostatic agent, as well as biodegradable tissue glue.[22,25,26] In addition to its physicochemical properties as a sealant, fibrin has been shown to affect the morphologic characteristics of retinal pigment epithelial cells involved in the proliferative response,[27] and has been used in ophthalmology both as a sealant for retinal defects,[28] and to close blepharoplasty incisions.[29] Bovine thrombin, at a concentration of 100 units per mL, has been shown to be an effective and relatively well tolerated thrombostatic agent in vitreoretinal surgery when administered as a component of the infusion solution.[30,31] In addition to its stimulation of fibrin plug formation, which may contribute to closure of macular holes, thrombin also has effects on retinal pigment epithelial proliferation and contraction in vitro that may further enhance macular hole closure in a method similar to that seen with TGF-beta.[24] In this study, we describe the results of a prospective consecutive series of 114 patients undergoing initial vitrectomy surgery for full-thickness stage II, III, and IV macular holes, augmented by the application of autologous plasma and bovine thrombin mixture.

PATIENTS AND METHODS

A total of 114 patients (121 eyes) were enrolled in the study between April 1992 and September 1996 at four university medical centers (Stanford Medical Center, Cornell Medical College, UC Davis Medical Center, and Rush Medical Center). Surgery was performed by one of four surgeons on all patients in a standardized fashion (MB, SC, LM, SDB) described below. Patients with Gass Stage II, III, or IV macular holes were eligible for enrollment.[1] Patients with greater than 6.0 diopters of myopia, a well-documented history of blunt trauma, previous macular hole surgery, or rhegmatogenous retinal detachment were specifically excluded. Each patient underwent complete ophthalmologic evaluation including determination of best-corrected Snellen visual acuity, measurement of intraocular pressure, evaluation of lens density, determination of the position of the posterior hyaloid, staging and measurement of the macular hole in microns, evaluation of the retinal periphery, and

color fundus photography. In selected instances, fluorescein angiography was performed.

The patients were evaluated one day, one week, one month, and six months following surgery at a minimum, with additional visits scheduled according to the judgment of the operating surgeon. Outcome variables measured included best corrected Snellen visual acuity, sealing of the edges of the macular hole, resolution of the cuff of subretinal fluid, additional procedures required, and intraoperative, early postoperative (≤ week) and late postoperative (> one week) complications. Differences between means were compared by Student's *t*-test, differences in frequencies between groups by the two-tailed chi square test. Additionally, multiple logistic regression analysis of preoperative variables (age, gender, preoperative visual acuity, lens status, as well as size, stage, and duration of the macular hole) was performed to analyze the visual and anatomic outcomes.

Surgery was performed according to methods previously described.[5-7,10] In brief, a three-port pars plana vitrectomy was performed using endoillumination. In Stage IV holes, the vitreous gel was excised with a vitreous cutter. In patients without posterior vitreous separation (Stage II or III holes), following removal of the central core of vitreous gel, the posterior hyaloid and posterior cortex were identified by their engagement with a silicone tipped catheter under moderate suction (200-300 mm Hg). The gel was then initially separated overlying the optic nerve and further advanced toward the retinal periphery in a gentle fashion, taking care to avoid traction or further enlargement of the macular hole. The remainder of the posterior cortical gel was then removed by the vitrector. Following this, the retinal periphery was carefully inspected by indirect ophthalmoscopy and any breaks identified and treated if present. Air-fluid exchange was then performed using either a high minus biconcave lens or 68 degree panoramic lens with associated image invertor (AVI) and continuous air insufflation at a setting of approximately 30 mm Hg. A tapered 30 gauge cannula was then used to evacuate the cuff of subretinal fluid through the center of the macular hole in most instances. Epiretinal membranes that produced significant distortion of the parafoveal tissues were routinely dissected. The normal internal limiting membrane of the retina was not routinely removed. Scleral plugs were then inserted and the eye allowed to equilibrate for an additional ten minutes under continuous air insufflation during preparation of the thrombin-plasma mixture. The remaining meniscus of subretinal fluid was then evacuated by the tapered cannula under suction, and the autologous plasma and bovine thrombin sequentially applied to the surface of the macular hole in the air-filled eye. One to two drops of sterile autologous plasma were delivered to the hole through the tip of a tapered 30 gauge cannula (approximate volume 10-20 µL) followed by a drop of bovine thrombin in a separate 30-36 gauge cannula, each expressed by gentle pressure on a tuberculin syringe by the surgeon or assistant. This resulted in the creation of a small translucent coagulum directly overlying the macular hole, measuring approximately 1.0 to 1.5 disc diameter size. Following this, the air in the eye was exchanged for a mixture of 10% to 16% gas, either C_2F_6 or C_3F_8, and the sclerotomies and conjunctiva closed. The patient was then placed in the prone position as soon as possible but in no case later than two hours after the surgical procedure.

Postoperatively, patients were instructed to remain in prone positioning at least 90% of the time for the first two weeks and at least 50% of the time for an additional two weeks. The importance of compliance was emphasized by the surgeon before and after surgery and family members were encouraged to assist patients in maintaining prone positioning after surgery. Topical beta blockers, topical alpha adrenergic agents, and systemic carbonic anhydrase inhibitors were given as clinically indicated for elevated intraocular pressure although not all patients were routinely placed on postoperative intraocular pressure lowering agents.

Upon entering the operating suite, approximately 10 mL of the patient's blood were withdrawn from the antecubital fossa after prepping with topical Betadine and transferred to two sterile (Vacutainer) tubes containing the anticoagulant Sodium Citrate. The tubes were then centrifuged at approximately 1000 rpms for 10 minutes and the supernatant withdrawn from the top of each tube, after removing the cap, through a filtered needle. The supernatant was then transferred to a labeled tuberculin syringe and connected to the 30-gauge cannula by a short length of silicone tubing.

Thrombin (Thrombinar/Armour Pharmaceutical Co., Kankakee, IL) was prepared as follows: 10 mL of sterile saline were added to 1000 units of lyophilized bovine thrombin and the solution mixed to yield a final concentration of 100 units per mL. One cc of this mixture was then withdrawn through a sterile fil-

Table 1. Baseline Demographics of the Patients Undergoing Macular Hole Surgery

Characteristics	n = 121 Eyes, 114 Patients
Age	66.9 (40 - 84) yr
Duration of symptoms	6.8 mon (1 w - 48 mon)
	(median - 3 mon)
Follow-up	10.9 (1 - 30) mo
Size	388 (100 - 1000) μm
Stage	
II	44 (36.4%)
III	53 (43.8%)
IV	24 (19.8%)
Pre-existing conditions	
Aphakia/pseudophakia	22 (18.2%)
Glaucoma	19 (15.7%)
Retinal breaks	2 (1.7%)
Prior herpetic keratouveitis	1 (0.8%)
Strabismus	1 (0.8%)

ter needle into a tuberculin syringe and attached to a 30-36-gauge blunt cannula through a length of short silicone tubing. Bioactivity of the thrombin-plasma mixture was then assayed by placing four drops of autologous plasma on a sterile glass slide. One drop of thrombin was added to each of two of the four drops of plasma on the glass slide and the resulting mixture allowed to stand at room temperature for two minutes. The resultant combined droplets were then lightly tapped with a 20-gauge needle and their coagulability compared with the plasma not treated by thrombin, serving as a control. In each case, the bioactivity of the plasma-thrombin mixture was ascertained prior to injection into the eye with the previously described procedure being performed during the 10-minute period of equilibration of air in the eye.

RESULTS

A total of 114 patients were enrolled in the study, ranging in age from 40 to 84 years, with a mean age of 66.9 years. There were 76 females (66.7%) and 38 males (33.3%). Stage II macular holes were manifested in 44 of 121 eyes (36.4%); 53 of 121 were stage III (43.8%); and 24 of 121 were Stage IV holes (19.8 %). The macular holes ranged in size from 100 to 1000 microns, and averaged 400 microns. The mean duration of symptoms was 6.7 months and ranged from 1 to 192 weeks; the median was 12 weeks. Mean follow-up was 10.85 months with all successful patients having minimum follow-up of 6 months or more. Twenty-two eyes of 121 (18.1%) were either aphakic or pseudophakic (Table 1).

Table 2. Anatomic Results Following Surgical Intervention With and Without Supplemental Treatment

	One Month	More Recent Follow -Up
Including supplemental treatment n = 121	91%† (110/121)	91%* (110/121)
Without supplemental treatment n = 108	89.3% (108/121)x	81% (98/121)

*Includes 2 patients open at week 2 who were closed by postoperative fluid-gas exchange with 20% C_3F_8 and light macular photocoagulation. †Includes 10 patients with closure at one month who reopened between 2 and 21 months after the primary surgery; 8/10 underwent successful supplemental fluid-gas exchange; 2/10 underwent a second pars plana vitrectomy, membrane peeling, and repeat fluid-gas exchange.

Of 121 eyes, 110 or 91% demonstrated macular hole closure at one month and a comparable number at six months. A total of 13 patients (10.7%) underwent supplemental therapy. Two eyes (1.7%) demonstrated macular hole closure after a second surgical procedure (repeat vitrectomy, membrane peeling and fluid-gas exchange). Eleven eyes (9.1 %) underwent postoperative fluid/gas exchange as an office procedure of which ten were successful. This included two patients who were noted to be open between two and four weeks after surgery, and eight of nine patients who demonstrated late opening of a macular hole between one and 21 months after surgery (Table 2).

Initial and final visual acuities are summarized in Table 3. No patients with visual acuity of 20/40 or greater were operated upon and only 19 of 121 eyes (15.7%) had presenting acuity of 20/70 or greater. In contrast, at the time of final exam, 47 eyes (38.84%) demonstrated visual acuity of 20/40 or greater and 83 of 121 eyes (68.6%) achieved 20/70 or greater postoperatively. Ninety-four eyes (77.1%) achieved two lines or greater improvement and only 5 eyes (4.1%) had one line or greater of visual decline (Table 3).

Table 3. Visual Results Reported for the Entire Group of Patients, Both Prior to and Following Surgery, Including Those Patients Who Underwent Supplemental Therapy. (Numbers of Patients Listed to the Right of the Percentages.)

Visual Acuity	Preoperative	Final
20/20-20/40	0% (0/121)	38.9% (47/121)
20/50-20/70	15.7% (19/121)	29.8% (36/121)
20/80-20/100	33.1% (40/121)	15.7% (19/121)
20/200-20/400	41.3% (50/121)	11.6% (14/121)
2/200-8/200	9.9% (12/121)	4.1% (5/121)
HM	0% (0/121)	0% (0/121)
LP	0% (0/121)	0% (0/121)
≥ 2 line improvement		77.7% (94/121)
≥ 20/70		68.6% (83/121)
≥ 20/40		38.9% (47/121)
≤ 20/200		15.7% (19/121)
≥ 2 line worsening		4.1% (5/121)

Univariate Analysis

Stratifying anatomic and visual results by preoperative variables, patients with macular hole symptoms of 6 months or less achieved closure overall at a rate of 93.3% (84 of 90 eyes), and a final average vision of 20/40 or greater in 39 of 90 eyes (43.3%) (Table 4). Patients with initial acuity of 20/80 or better were more likely ($P < 0.03$) than patients with initial visual acuity of 20/100 or worse to achieve vision levels of 20/70 or greater and 20/40 or greater (Table 5).

Macular hole size at time of surgery appeared to be another predictor of anatomic and visual success (Table 6). Of those patients with macular holes estimated to be 450 microns in size or less, 96.2% of all patients were closed either with one procedure or supplemental fluid-gas exchange, contrasted with 81.4% of those with 450 microns or greater sized holes. Strikingly, 39 of 78 patients with macular hole size 450 microns or less achieved vision of 20/40 or better (50.0%) contrasted with only 8 of 43 (18.6%) of those with macular holes of 500 microns or greater (significant difference, $P = 0.001$) (Table 6).

Multivariate Analysis

Multivariate logistic regression analysis of the preoperative variables revealed that none correlated significantly with anatomic closure although the duration of the macular hole approached significance ($P = 0.051$). Both duration of the macular hole ($P = 0.034$) and preoperative visual acuity ($P = 0.004$) were significant predictors of visual acuity better than 20/70 while only the latter correlated significantly ($P = 0.034$) with visual acuity better than 20/40. Epiretinal membrane peeling was not a significant predictor of

Table 4. Anatomic and Visual Results Stratified by Duration of Symptoms. Results are Expressed in Columns, Both as Absolute Number of Patients as well as Corresponding Percentages

	Anatomic and Visual Results Stratified by Duration of Symptoms			
Duration of Symptoms	Closure With One Procedure	Closure With Supplement	VA ≥ 20/70	VA ≥ 20/40
≤ 6 months (n = 90)	83.3% (75/90)	93.3% (84/90)	74.4% (67/90)	43.3% (39/90)
> 6 months (n = 31)	74.2% (23/31)	83.9% (26/31)	51.6% (16/31)	25.8% (8/31)
(n = 121)	81% (98/121)	91% (110/121)	68.6% (83/121)	38.9% (47/121)

Table 5. Anatomic and Visual Results Stratified by Initial Visual Acuity

Initial Visual Acuity	Closure With One Procedure	Closure With Supplement	VA ≥ 20/70	VA ≥ 20/40
VA ≥ 20/80 (n = 37)	89.2% (33/37)	91.2% (34/37)	86.5% (32/37)	54.0% (20/37)
VA < 20/80 (n = 84)	77.4% (65/84)	90.5% (76/84)	60.7% (51/84)	32.1% (27/84)
(n = 121)	81% (98/121)	91% (110/121)	68.6% (83/121)	38.9% (47/121)

either final visual acuity (20/40 or better, $P = 0.512$; 20/70 or better; $P = 0.304$) or anatomic closure. Eighty-eight of 121 (72.7%) patients underwent attempted epiretinal membrane peeling.

Complications

Complications were relatively infrequent (Table 7). Five of 121 eyes (4.1%) developed a retinal detachment and two of 121 eyes (1.7%) developed an intraoperative retinal break and subclinical detachment. One eye (0.8%) demonstrated retinal hemorrhages. Six eyes (5.0%) developed a microhypopyon and mild cellular response in the anterior chamber that cleared with topical steroids within 48 hours. Forty-five of 121 eyes (37.2%) developed transient intraocular pressure elevations ranging from 21 to 30 mm Hg that was easily controlled with topical and/or oral medications. Twenty-eight (23.1%) of these demonstrated transient pressure elevations above 40 mm Hg, one of whom required paracentesis of the gas bubble, without further complications. All other patients were successfully managed with topic intraocular pressure lowering agents. Some of these patients gave a positive history of prior glaucoma. Thirty-three eyes of 99 phakic eyes (33.3%) have undergone subsequent cataract extraction and lens implantation to date following surgery. Twenty-two of 121 eyes (18.2%) were aphakic or pseudophakic prior to macular hole surgery. Thus, 66 of 121 eyes (54.5%) remain phakic and may further benefit visually from cataract extraction in the future. Six eyes (5.0%) developed an epiretinal membrane. One eye (0.8%) developed a choroidal detachment. The most serious complication in this series involved one eye (0.8%) that developed a hypopyon associated with *Staphylococcus aureus* endophthalmitis on the fifth postoperative day. This was successfully treated by repeat vitrectomy and intravitreal antibiotic injection, although the patient ultimately developed a retinal detachment that required repeat vitrectomy, scleral buckling, and silicone oil injection. The final visual acuity in this patient four months following silicone oil injection and six months following macular hole surgery was 20/100, comparable to the preoperative acuity.

DISCUSSION

The issue of whether adjuvant agents are required for the optimal therapy of macular holes continues to be actively debated. While it is now well established that macular holes can be closed in a high percentage of cases, a distinction exists between closure rates and preferred or optimal practice patterns for this disease. This difference is based on several important variables including final vision, complication rates, ease of the procedure, and cost. To date, while surgery has been shown to be effective in closing macular holes when compared to observation,[14] there have been no trials

Table 6. Anatomic and Visual Results Stratified by Macular Hole Size

Hole Size	Closure With One Procedure	Closure With Supplement	VA ≥ 20/70	VA ≥ 20/40
≤ 450 µm n = 78	88.5% (69/78)	96.2% (75/78)	82.0% (64/78)	50.0% (39/78)
> 450 µm n = 43	67.4% (29/43)	81.4% (35/43)	44.2% (19/43)	18.6% (8/43)
n = 121	81% (98/121)	91% (110/121)	68.6% (83/121)	38.9% (47/121)

Table 7. Complications of Surgical Repair of Macular Holes (n = 121 eyes)

Intraoperative	
Retinal break with subclinical detachment	1.7% (2/121)
Early Postoperative	
Transient elevation IOP (21-30)	37.2% (45/121)
Retinal hemorrhage	0.8% (1/121)
Sterile hypopyon*	5.0% (6/121)
Endophthalmitis (*Staph. aureus*)	0.8% (1/121)
Late Postoperative Complications	
Retinal detachment	4.1% (5/121)
Cataract requiring extraction	27.2% (33/121)
Chronic uveitis	0.8% (1/121)
Macular pucker	5.0% (6/121)

*One patient in whom 1000 µ/mL thrombin was inadvertently used.

comparing one adjuvant with another, or both versus placebo. As a result, pilot data indicating the safety, methodology employed for different adjuvants, as well as noncomparative closure and visual results are relevant to the search for the preferred therapy that can be subjected to a comparative trial.

This is the first reported study of autologous plasma and bovine thrombin as an adjuvant in macular hole surgery. Our results suggest that vitrectomy, vitreous cortex removal, gas tamponade, and application of autologous plasma-thrombin tissue glue are a relatively safe and effective method for macular hole closure. These results compare favorably with the natural history of the disease,[1-3] and are at least comparable to several recently published large scale studies.[10,16,18] Wendel, et al originally reported anatomic success in 73% of 170 eyes, two lines of vision improvement in 75%, and visual acuity of 20/40 or greater in 29%.[10] Paques et al reported anatomic success rates as high as 98% in 53 patients treated with autologous platelet concentrate as a surgical adjuvant vs. 82% in 57 control eyes.[23] Banker et al reported anatomic success rates in 78% of 166 total eyes, and 85% of 106 eyes treated with serum as adjuvant therapy.[19] Visual acuity was not considered an outcome measure in this study. Thompson et al reported anatomic success in 70% of 120 eyes, and 77.8% of 63 eyes treated with recombinant TGF-beta as adjuvant therapy.[16] In that series, final visual acuity of 20/40 or better was obtained in 17.5% of eyes and visual acuity improved two or more lines in 44.2%. In contrast, in our group of 121 eyes, anatomic success with one procedure was 81% (91% with supplemental treatment), visual improvement of two lines occurred in 77.6%, and 38.9% achieved 20/40 or better vision (Table 5).

Previous investigators have correlated duration of macular hole with macular hole size in natural history studies[1,3] and have also associated duration of macular hole with a more unfavorable anatomic and visual outcome.[5,10] In our series, by univariate analysis, initial size of the macular hole appeared to have the strongest predictive value regarding final anatomic and visual outcome in conjunction with presenting visual acuity. Eyes with macular holes of 450 µm or less in size and visual acuity of 20/80 or greater prior to surgery had a more favorable outcome than eyes with larger holes or lesser presenting acuity. Thirty-nine of 78 (50%) eyes with macular holes 450 µm or less in size prior to surgery achieved 20/40 or better vision postoperatively, contrasted with only 8 of 43, or 18.6% of eyes with macular holes greater than 450 microns in size. Similarly, 20 of 37 (54%) eyes with visual acuity of 20/80 or greater at time of presentation achieved 20/40 or better vision after macular hole surgery contrasted with only 27 of 84 (32.1%) eyes with 20/100 or worse vision preoperatively. This difference is statistically significant ($P = 0.03$).

Multivariate logistic regression analysis of the preoperative variables revealed that only the duration of the macular hole approached significance ($P = 0.051$) with anatomic outcome and that only preoperative visual acuity correlated significantly ($P = 0.034$) with a visual outcome of 20/40 or better. Thompson et al, also using a multivariate model in their series of 120 eyes, reported these same preoperative variables as significant predictors of anatomic outcome and final visual acuity, respectively.[16]

The question remains open as to whether adjuvant therapy is required for the successful treatment of most macular holes and, if so, which type. The potential advantages of an adjuvant such as either TGF-beta, plasma-thrombin mixture or serum must be weighed against the potential disadvantages that include lack of availability, cost, or complications specific to the use of an adjuvant. Prior randomized studies confirmed the apparent superiority of either 660 ng or 1330 ng of bovine TGF-beta 2 compared with placebo in the treatment of a macular hole, when the patient was placed in the supine position for the day of surgery,

and 16% C_3F_8 gas is employed as the tamponade.[15] More recent studies have suggested a modest improvement in closure rates, not adjusted for initial hole severity, with serum or autologous platelet concentrate although visual results were less notable.[19,23] Success rates are concomitantly lower when either a lower gas concentration, lower concentrations of TGF-beta, or a hyaluronic acid vehicle is employed.[12,17] In this study, we employed a surgical and positioning protocol comparable to that of Wendel et al, with the exception that the plasma-thrombin mixture was placed on the macular hole prior to prone positioning.[5,10]

The theoretical basis for the choice of thrombin in this pilot study was several fold. Like TGF-beta, thrombin is known to be a potent cytokine, producing both mitogenic and cytoskeletal changes in pigment epithelial cells in culture, which in turn are thought to participate in the wound healing response associated with macular holes. Twenty units of bovine thrombin maximally stimulate human RPE cells to proliferate to 162% of control levels in culture.[24] Concentrations of 10 units of thrombin will stimulate a collagen gel to contract to 23% of its original volume within 48 hours in vitro, and 100 units to 7% of its original volume.[24] Both TGF-beta as well as fibrin have been shown to produce similar morphologic changes in RPE cells[26,27] and TGF-beta will also stimulate contraction of collagen gels[32] and facilitate experimental retinal wound healing in the rabbit.[28] Comparable studies on rat and human skin confirm the stimulatory effects of plasma-thrombin tissue glue on wound healing as well.[25,26]

Coleman et al were the first to suggest the use of biologic plasma-thrombin tissue adhesive as a method for closure of macular hole associated with retinal detachment.[28] It has also been successfully used for closure of blepharoplasty incisions.[29] Continuous intravitreal infusion of thrombin has been described and found to be nontoxic in the rabbit in concentrations of 100 units per mL.[30] In one human study of intravitreal thrombin to control hemorrhage during vitrectomy, a hypopyon developed in up to one-third of the patients, limiting its usefulness.[31] In our study, we encountered a sterile hypopyon in one patient in whom a concentration of 1000 units per ml of thrombin was inadvertently employed, rather than the 100 units in the other 120 eyes. Based upon an approximate droplet size of 10 μL with a 30 gauge needle, we calculated the total dose of thrombin used in these patients to be in the range of 1 unit. This is in contrast to the intravitreal thrombin study where total doses as high as 10 000 units of thrombin were administered (in over 100 mL of irrigation fluid containing 100 units per mL). Because of its hemostatic properties, there is a theoretical risk of thrombosis associated with the use of thrombin and hence intravascular administration is not recommended. In our series, one patient developed intraretinal hemorrhages possibly related to vigorous vitreous separation. These changes resolved spontaneously and were not associated with any definite visual loss or ocular morbidity. Higher dosages of thrombin (200 units per mL) have been associated in several patients with small hypopyon and intraretinal hemorrhages (personal communication Len Joffee, MD). One final potential complication of intraocular thrombin use is that of delayed sensitization to thrombin and factor V.[33] This has been described experimentally in rabbits, and recently in two children exposed to large quantities of fibrin glue during the course of cardiac surgery.[34] In this series, we elected to use non-concentrated autologous plasma rather than donated single-source or pooled plasma to avoid the potential risks of transmissible viruses, and the expense and difficulty associated with fractionation of plasma to yield higher concentrations of fibrinogen. This can be achieved either by cryoprecipitation or other chemical precipitation methods, including ethanol.[22] We found that bioassay of the autologous plasma and bovine thrombin in the operating room confirmed the enzymatic conversion of soluble fibrinogen to insoluble fibrin.

The use of plasma-thrombin mixture was associated with the undesirable effect of elevated intraocular pressure rise after surgery. Yet, this phenomenon is probably not specific to this adjuvant in particular as recombinant TGF-beta 2 has also been associated with an increased risk of postoperative intraocular elevations.[16] Furthermore, it is difficult to attribute the elevation to the surgical adjuvant alone as multiple factors are probably involved in the intraocular pressure rise particularly but not exclusively the level and concentration of intraocular gas. Other pharmacologic adjuvant series did not specifically address intraocular pressure as a postoperative parameter so it is not clear whether this occurrence was observed in these series as well. In any case, the elevated intraocular pressure in our series did respond to treatment with topical and systemic agents without the development of secondary glaucoma.

We were not able to identify any other complica-

tions felt to be specific for the use of plasma-thrombin mixture. One patient developed mild central pigment epithelial changes that may have been related either to gentle intraoperative trauma to the pigment epithelium during evacuation of submacular fluid, or supplemental photocoagulation treatment.[35] One patient developed small petechial perimacular hemorrhages that may have been related either to dissection, vitreous separation, or the use of thrombin.

The optimal method for the preparation of the plasma suitable for use in macular hole repair remains unknown. We elected to use sterile sodium citrate test tubes, although both EDTA and heparin containing tubes are also available for this purpose. EDTA was not chosen because of potential concerns regarding retinal toxicity, and heparin was not chosen because of its known ability to inhibit the vitreoretinal wound healing response and modulate the surface binding characteristics of thrombin.[36,37]

One other adjuvant technique employed in this series and not previously reported as a contributor to the final success rate in other studies was the use of supplemental fluid-gas exchange with laser photocoagulation.[38] The final success rate in this large series of patients was increased from 81% to 91% as a result of 11 patients who underwent fluid-gas exchange with laser photocoagulation following surgery for either persistent opening or reopening and two additional patients who underwent another vitrectomy. This may reflect an accurate estimate of the likelihood for success rather than the results looking only at a single surgery.

In summary, we have demonstrated the relative safety of autologous plasma-bovine thrombin mixture as a surgical adjuvant in the primary repair of macular holes and the incremental benefits of postoperative fluid-gas exchange when necessary. Because of differences in methodology between this and other studies employing either no adjuvant or serum and TGF-beta as adjuvants, no definitive conclusions can be drawn regarding the relative merits of these therapies compared with one another. Future randomized studies employing similar surgical methodologies and postoperative positioning paradigms should shed further light on the safety, efficacy, and cost-effectiveness of these regimens.

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