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CLINICAL INVESTIGATION

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Autologous platelet concentrate for the treatment of full-thickness macular holes

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Abstract ● Background: To improve the anatomic success rate in the surgery of full-thickness macular holes, we tested, in a prospective pilot study, the effects of autologous platelet concentrate deposited on the macula at the end of surgery. ● Methods: Two consecutive groups of patients were compared. Twenty eyes (group 1, mean symptom duration 11 months) were operated on with injection of an autologous platelet concentrate on the macula after fluid-gas exchange. Another 20 eyes (*group 2*, mean symptom duration 11 months) were subsequently operated on without autologous platelet concentrate. For all stage 3 holes, posterior hyaloid was detached en bloc at the level of the optic disc. The patient was left supine for 24 h after surgery, and then remained face down for 10 days. ● Results: In *group 1*, 19 cases were an anatomic success, i.e. there was flattening of the retina surrounding the hole and reattachment of the edge of the hole to the retinal pigment epithelium; in 9 cases the hole was even undetectable. Final visual acuity was 0.5 or more in 9 eyes, and 0.4 or more in 14. Visual acuity improved by two lines or more in 17 of the 19 successfully operated eyes. In *group 2*, only 13 cases were an anatomic success. The functional results for the successfully operated eyes were identical to those of group 1. ● Conclusion: These results strongly suggested that autologous platelet concentrate could significantly improve the success rate in macular hole surgery and led us to begin a comparative, prospective, randomized trial.

Introduction

Since the publication by Kelly and Wendel in 1991 of the first successful surgery of full-thickness macular holes [11], many attempts have been made to reproduce and improve these results. In 1994 Patel and Wendel [18] reported a success rate of 86% in a large series of patients. This high success rate has not been reproduced by others without the use of a healing stimulator. Glaser and coworkers, using tranforming growth factor β2 (TGF-β2) in addition to vitreous surgery, obtained slightly better results [7, 21]. Other investigations currently under way involve autologous serum [5, 15], fibrin [23] or autologous plasma-thrombin mixture [2].

In the attempt to improve our success rate in the surgery of full-thickness macular holes, we decided to test the effects of autologous platelet concentrate, because platelet extracts are known to stimulate tissue healing [9, 12, 22]. Accordingly we conducted a prospective pilot study in which we compared the results for two groups of patients operated on consecutively for full-thickness macular holes with and without the addition of autologous platelet concentrate.

Patients and methods

Two groups of patients, operated on consecutively, were compared (Table 1). The study was performed in accordance with the 1964 declaration of Helsinki and after obtaining the patients' informed consent.

Group 1, operated on with an autologous platelet concentrate between July 1993 and January 1994, consisted of 20 eyes from 20 consecutive patients aged 34–77 years (mean 65 years). Mean symptom duration before surgery was 11.1 months (Table 1).

Group 2, operated on subsequently without autologous platelet concentrate between February and June 1994, consisted of 20 eyes from 20 consecutive patients aged 51–78 years (mean 67 years). Mean symptom duration before surgery was 11.2 months (Table 1).

Pre-operative examination

The eyes were examined pre- and post-operatively according to the same protocol. Visual acuity was measured with the best possible optical correction, determined after an objective refraction.

The fundus was examined biomicroscopically with a contact lens. Each macular hole was graded as stage 2, 3 or 4 according to the criteria described by Gass [6, 10].

Color and monochromatic green and blue filter photographs were taken under a 40° angle, and fluorescein angiography was performed in every case.

A scanning laser opthalmoscope (SLO) examination was also done, including assessment of the locus of fixation, screening for an absolute scotoma and the line test, which is an improved version of the Watzke test [1, 8, 13, 14, 20].

Surgical procedure

Surgery was performed by two operators (A.G., P.M.) according to the same procedure, beginning with three-port pars plana vitrectomy. In stage 4 macular holes, vitrectomy confirmed that the posterior hyaloid containing the Weiss ring was detached from the retina. For stage 2 and 3 macular holes, care was taken to leave the layer of cortical vitreous on the retinal surface during the vitrectomy [7, 17]. Then, for stage 3 holes, a special aspiration forceps was used to grasp the vitreous fibers over the optic disc. The forceps was connected to the aspiration system of the vitrectomy machine, preset to 400 mm Hg, and was positioned above the disc edge. Aspiration was then applied, resulting in a small traction of the vitreous on the optic nerve fibers. The forceps was then closed and traction was exerted to detach the vitreous from the disc edge. The maneuver was repeated until the Weiss ring was detached, dragging away the posterior hyaloid en bloc with the prefoveolar operculum. The hyaloid detachment was then extended out to the equator and the vitrectomy was completed, removing the vitreous as far out towards the periphery as possible, using a scleral depressor. For stage 2 macular holes, the posterior hyaloid detachment began around the macula by passive aspiration with a short silicone-tipped extrusion needle, to preclude sudden avulsion of the roof of the hole and widening of the hole [19]. In fact, no avulsion occurred, and in subsequent cases after the end of this study the detachment of the posterior hyaloid at the level of the optic disc did not result in the widening of stage 2 macular holes.

In all cases a short silicone-tipped extrusion canula with passive suction was gently swept over the posterior pole surface, avoiding any contact with the edges of the hole. When an epiretinal membrane or a softer friable substance was engaged, it was cautiously lifted around the hole with a blunt retinal pick and removed, or resected when it adhered to the hole margin.

A fluid-air exchange was performed, followed by a 10-min pause before the residual intraocular fluid was aspirated.

Then, in the patients of group 1, 0.1 ml of an autologous platelet concentrate solution was injected with a canula introduced into the middle of the air-filled vitreous cavity. After closure of the two superior sclerotomies, a gas mixture (17%, C_2F_6 , 83% air) was exchanged for the air in the vitreous cavity. The patients of group 1 were maintained in a supine position for the first 24 h after surgery and then remained face down as much as possible for 10 days, whereas the patients of group 2 were turned face down immediately after surgery.

Platelet concentrate preparation

The platelet concentrate was prepared at the beginning of the operation from the patient's blood as follows:

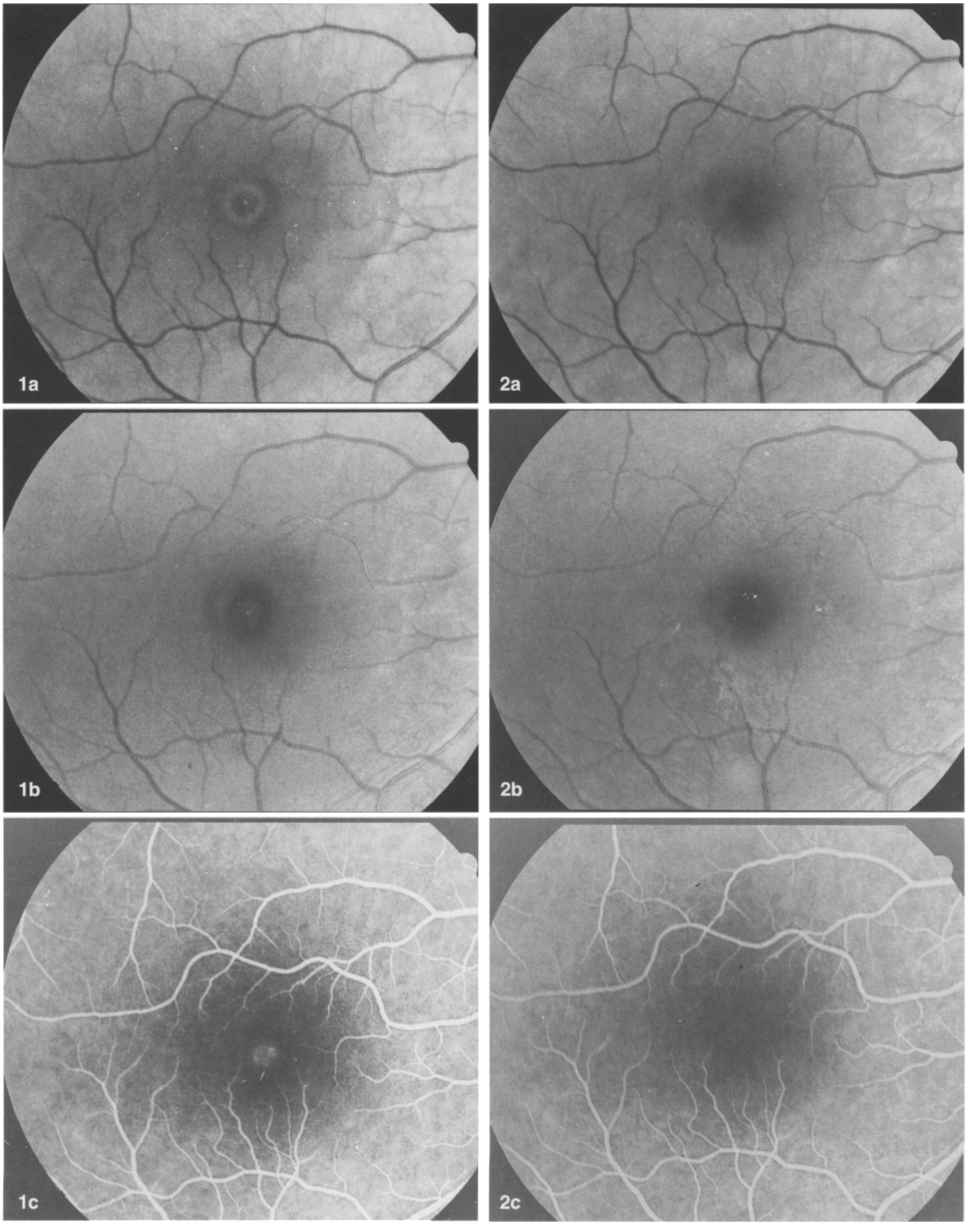
Just before the operation, 40 ml of venous blood was collected into a syringe containing 6 ml of acid citrate dextrose, formula A (ACDA), and the two were gently mixed. The syringe containing the blood sample was then brought to the blood bank and treated under sterile conditions. The blood was transferred into a tube and immediately centrifuged at 280 g for 15 min at room temperature. The platelet-rich plasma devoid of red blood cells was decanted and mixed with 1/8 volume ACDA before a second centrifugation at 1000 *g* for 10 min at room temperature. The supernatant platelet-poor plasma was drawn off, and the packed platelets were gently mixed with 0.6 ml of isotonic sodium chloride to obtain a platelet suspension devoid of agglutinates. The average concentration of this suspension was 10^9 platelets/ml. The

Table 1 Characteristics of the patients (n=20 eyes in each group)

Characteristic	Group 1 (autologous platelets)	Group 2 (without platelets)
Age (years)		
30–49	1	0
50–59	3	4
60–69	8	9
70–79	8	7
Mean	65.1 years	66.7 years
Symptom duration (months)		
0–6	12	10
7–12	4	4
13–18	3	3
>18	1	3
Mean	11.1 months	11.3 months
Stage of hole		
Stage 2	3	0
Stage 3	11	14
Stage 4	6	6

Fig. 1a–c A stage 3 macular hole before surgery: VA 0.2. **a** Green filter photograph: typical appearance of full-thickness macular hole, with elevated edges. **b** Blue filter photograph: a central defect in the foveolar xanthophyll pigment. **c** Fluorescein angiography: central hyperfluorescence in the hole

Fig. 2a–c Same eye as in Fig. 1, 3 months after surgery using autologous platelet concentrate: anatomic and functional success, VA 0.6. **a** Green filter photograph: complete disappearance of the hole. **b** Blue filter photograph: disappearance of the central defect. **c** Fluorescein angiography: disappearance of the central hyperfluorescence


1a
2a
1b
2b
1c
2c

suspension was brought back to the operating room about 1 h after blood sampling. There, the platelet concentrate was put into a small cup and aspirated in a 1-ml sterile syringe for injection as described above.

Post-operative evaluation

A complete post-operative examination was performed between 10 and 12 weeks after surgery, using the same procedure as pre-operatively. If the objective refraction had changed (for instance, on account of a myopic error due to early nuclear sclerosis changes), visual acuity was recorded with the best optical correction determined on the basis of the autorefractometer data. The Fischer exact test permitted comparison of anatomic success rate for groups 1 and 2.

All the patients were also examined about 6 months (from 5 to 7 months) after surgery.

Results

During surgery, an epiretinal membrane or a yellowish gelatinous premacular substance was found in only 5 of the 28 stage 2 or 3 holes, but in 11 of the 12 stage 4 holes.

No peroperative complications occurred except for one peripheral retinal break, which was treated immediately by laser photocoagulation.

The early postoperative course was similar in both groups. No inflammatory reaction was observed. In both groups we observed some deposits on the posterior face of the cornea, known as "good positioning spots" [24], but in the platelet group (group 1) these deposits were often a little more pronounced and occurred earlier. In one third of the cases a moderate increase in intraocular pressure occurred, which never exceeded 30 mm Hg, was controlled by medical treatment and usually resolved after 1 week. Retinal detachment occurred in one case, 10 days after surgery, due to an inferior peripheral break, and was successfully operated.

Three weeks after surgery, the gas bubble filled about 40% of the vitreous cavity, and it was already possible to assess the anatomic results.

Anatomic success was defined as the flattening of the cuff of fluid surrounding the macular hole, with the edge of the hole reattached to the retinal pigment epithelium. Although this edge was extremely thin when reattached, in some cases it remained faintly detectable on biomicroscopic examination through a contact lens with high-power magnification. In other cases, however, the hole seemed to have close in on itself and could not be detected at all.

In *group 1*, 19 of the 20 cases were an anatomic success, and in 9 cases the hole was undetectable (Figs. 1, 2). Mean visual acuity improved from 0.18 to 0.43. Final visual acuity was 0.5 or more in 9 eyes, and 0.4 or more in 14 eyes. Visual acuity improved by two lines or more in 17 of the 19 successfully operated eyes, and by three lines or more in 10 eyes (Table 2). We compared the pre- and post-operative results of SLO examination in the 19 cases of anatomic success. At presentation, the line was seen as broken in 15 cases, despite the presence of an absolute scotoma with the 5′ test in all the patients. On final examination the line was still seen as broken in only 7 cases, but an absolute central scotoma with the 5′ test, though of smaller size, was still detectable in 8 cases.

Table 2 Pre- and post-operative visual acuity. Visual acuity (no. of eyes)

Group	Pre-operative		Post-operative	
	≥0.4	≥0.5	≥0.4	≥0.5
Group 1 Success: 19/20 eyes	3	0	14	10
Group 2 Success: 13/20 eyes	0	0	10	5

In *group 2*, only 13 of the 20 cases were an anatomic success, and in 9 cases the hole was undetectable. Mean visual acuity improved from 0.12 to 0.31 in the whole group, but from 0.15 to 0.46 in the 13 successful cases. Final visual acuity was 0.5 or more in 5 eyes, and 0.4 or more in 10 eyes. Visual acuity improved by two lines or more in 12 of the 13 successfully operated eyes, and by three lines or more in 11 eyes (Table 2). The pre- and post-operative results of SLO examination in the 13 cases of anatomic success were compared. At presentation, the line was seen as broken in 10 cases despite the presence of an absolute scotoma with the 5′ test in all the patients. On final examination the line was still seen as broken in only 1 case. An absolute central scotoma with the 5′ test, though of smaller size, was still detectable in 1 case.

The difference between the two groups in terms of anatomic success (flattening of the edges of the hole) is statistically significant ($P<0.05$). If patients with a macular hole presenting with symptoms for less than 2 years are considered, the anatomic success rates are 19/19 in group 1 and 12/17 in group 2, a difference which is also statistically significant ($P<0.05$).

At the 6-month examination there was no recurrence of the macular hole, no additional case of retinal detachment, and no eye from either group had been operated on for cataract. Visual acuity remained the same, except in three eyes in which it had slightly improved.

Discussion

The operative treatment of macular holes, consisting of the ablation of the cortical vitreous and long-acting gas tamponade, had an anatomic success rate of 58% in the initial series published by Kelly and Wendel in 1991 [11]. This rate increased to 86% in a series of 191 eyes

Table 3 Results of full-thickness macular hole surgery reported in the literature

Authors	Year	No. of eyes	Adjuvant	Anatomic success rate
Kelly and Wendel [11]	1991	52	None	52%
Glaser et al. [7]	1992	23	TGF-β2 (330–1330 ng)	100 %
Smiddy et al. [21]	1993	30 58	None (placebo) TGF-β2 (660–1330 ng)	53% 91%
Patel and Wendel [18]	1994	191 156 <1 year	None None	86% 93%
Liggett et al. [15]	1994	11	Autologous serum	100%
Garcia-Arumi et al. [5]	1994	24	Autologous serum	83%
Tilanus and Deutman [23]	1994	19	Tissucol	84%
Blumenkranz et al. [2]	1994	26	Autologous plasma-thrombin mixture	77%

reported in 1994, and even 93% in the eyes with symptom duration of less than 1 year [18]. By comparison, the placebo series of Smiddy et al., who operated without attempting to remove occasionally associated epiretinal membranes, had a success rate of only 53% [21]. The use of TGF-β2 raised Smiddy and colleagues' anatomic success rate to 91% [21], which was very close to the results obtained by Patel and Wendel without healing modulator [18]. However Coleman et al, were unable to reproduce such good results with TGFβ [3].

In this situation, is there a need for any biologic or synthetic glue to improve the healing of the hole? This question has not yet been resolved, because of the absence of a convincing comparative prospective study (Table 3).

The first clinicopathologic studies of successfully operated macular holes, which were published recently [4, 16], showed not only the apposition of the edge of the hole, but also that the hole was sealed by Müller cells or astrocytes in two of the three cases examined. It is therefore justifiable to try to enhance this process by use of a healing stimulator.

We chose to use an autologous platelet concentrate because it was easy and safe to prepare extemporaneously from the patient's blood, and because platelet concentrates have already been used for stimulation of dermal and nerve healing in animal models [9, 12, 22], and for cutaneous ulcer healing in humans.

The α granules of platelets contain various growth factors of which at least platelet-derived growth factor, platelet derived angiogenesis factor, TGF-β and platelet factor 4 [12] are known to be involved in promoting the wound healing process. Whereas the platelet extracts used in dermal cicatrization were prepared from several donors, we only used the patient's own platelets for surgery of macular holes, thus avoiding any riks of vital infection. The autologous platelet concentrate may be prepared by a hematology laboratory in the hour before the operation.

We obtained a success rate of 95% for the group operated on with autologous platelet concentrate, compared to 65% for the conventionally treated group. This difference is statistically significant ($P<0.05$) but is no more than an indication that the use of platelets improves the closing of macular holes, because this pilot study included two consecutive groups of patients, and not randomized cases. Nevertheless, it is remarkable that group 2 (operated on without platelets), had the less favorable results, despite being operated on after the platelet group and therefore after greater surgical experience. Finally, like others, we were unable to reproduce the high anatomic success rate obtained by Patel and Wendel without any addition of a biologic substance [18].

These results strongly suggested that autologous platelet concentrate could significantly improve the success rate in macular hole surgery and led us to begin a multicenter, prospective, randomized trial in order to compare the results of surgery using platelet concentrate and placebo.

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