

Analysis of the Results of Comparative Internal Limiting Membrane Staining with Modern Chromovitrectomy Agents

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Received: April 02, 2021; Published: June 29, 2021

Abstract

Purpose: Assess the results of comparative internal limiting membrane (ILM) staining with vital dye Membrane blue dual and the suspension Vitreocontrast in patients with idiopathic macular holes using computer colorimetry.

Materials and Methods: 15 patients with idiopathic macular holes $\geq 400 \mu\text{m}$ underwent three-port 25 Gauge vitrectomy, ILM peeling using the "inverted flap" technique followed by air tamponade. In all cases, macula region was divided into 2 equal parts, ILM within the first part was stained with Vitreocontrast suspension, the second with Membrane Blue Dual dye. After video recording of the intervention, a comparative assessment of agents staining ability was made using computer colorimetry.

Results: In a comparative colorimetric analysis, the average Euclidean distance CIELAB MembraneBlue[®] Dual between the stained ILM and the corresponding region of ILM-free retina was 15.97 ± 7.4 , for Vitreocontrast suspension 22.87 ± 6.67 . The average Euclidean distance CIELAB for Vitreocontrast suspension was significantly higher according to t-test than average Euclidean distance for MembraneBlue[®] Dual dye at $p = 0.012$. Revealed higher Euclidean distance for ILM, stained with "Vitreocontrast" suspension, suggests that when using this agent, the surgeon's eye perception of ILM staining intensity will be objectively higher than with Membrane-Blue[®] Dual solution.

Conclusion: Vitreocontrast suspension provides a more vividly perceived staining of ILM for the surgeon's eye than MembraneBlue[®] Dual, effectively and instantly settling both on the membrane itself and on possible epiretinal membranes on its surface. It does not loose adhesion throughout the entire surgical procedure. Thus, the suspension Vitreocontrast can be recommended as an alternative to existing agents for ILM staining.

Keywords: Vitreocontrast; chromovitrectomy; Peeling of the Internal Limiting Membrane; Macular Hole

Introduction

Identifying internal limiting membrane (ILM) peeling as a separate stage of endovitreous surgery can be rightfully called an ophthalmic surgical achievement of the last two decades. Such ILM properties as transparency, thinness, tendency to curling and weak reflectivity significantly complicate the membrane visualization during its grasping, attempts to separate it from the underlying retina, and the recognition of the boundaries of the maculorexis [1]. That is why initial ILM peeling was unintentional and was considered as a

postoperative complication during the retrospective histological analysis of the peeled epiretinal membranes [2]. Later, the advisability of the planned ILM-rexis was demonstrated and its theoretical justification was formulated for such pathologies as persistent macular edema, macular hole, ERM of various etiologies, myopic retinoschisis, etc. In this connection, various techniques have been proposed to improve the visualization of the ILM including modifications of endovitreous illuminators, surgical microscopes with a large field of view and depth of field, contact lenses and non-contact optical systems [3-7]. However, the most significant stage in the development of ILM peeling was the introduction of the intraoperative staining of the thin translucent structures of the vitreoretinal interface - chromovitrectomy [8]. Among many requirements to ILM vital dyes, the most important are the ability to highlight the membrane against the background of the underlying retina and maximum safety both in relation to the cellular structures of the retina and in relation to the eye as a whole. Today, agents such as Trypan blue (TB) and Brilliant blue (BB) meet the above requirements to the maximum extent. It should be noted that TB exhibits greater affinity to epiretinal membranes (ERM) than to ILM, since cells with intact cell membranes do not absorb it, in contrast to the cells that have lost their viability. BB is capable of staining both ILM and ERM, but the agent's affinity to ERM is significantly lower than that to TB. However, literature data indicate the advantage of BB as a staining agent without proven cyto- and phototoxicity [8-10]. Therefore, the MembraneBlue-Dual TM dye (DORC International, Zuidland, Netherlands) was developed that is the most convenient, effective and at the same time the safest staining agent for both the membrane and fibrous fibers on its surface. It combines 0.025% BB and 0.15% TB and being a component that increases the viscosity and density of the solution, additionally contains polyethylene glycol (PEG). This water-soluble dye provides effective staining of the ILM and ERM fibers on the blue surface of the ERM. It does not have any toxic effect on retinal structures under short-term exposure and does not cause intra- and postoperative complications [11].

Besides traditional agents for chromovitrectomy, the team of authors proposed a new agent for ILM staining - barium sulfate based suspension Vitreokontrast. Vitreokontrast is an ultrafine suspension based on the neutral non-toxic inorganic salt barium sulfate in an isotonic solution with an osmolarity of 300 - 350 mOsm, the size of particles is less than 5 microns and the density is 4.4 g/cm³. Suspension crystals exhibit high affinity to both ILM and ultrathin epiretinal membranes and residual VB fibers on the ILM surface instantly depositing on them and maintaining adhesion even under the action of irrigation flows. The authors noted the effective persistent staining of white ILM in macular hole surgery that gave high functional results and experimentally proved the safety of Vitreokontrast suspension for intraocular application [12,13].

Traditionally, the comparative assessment of the staining ability of various agents for chromovitrectomy is subjective and carried out according to the scores developed for each study. For example, in the clinical study of ILM and ERM staining intensity with the use of MembraneBlue-Dual and ILM-Blue TM solutions (DORC International) (a solution containing only 0.025% BS and 4% PEG), a subjective assessment of the efficiency of membrane visualization on the scale from 1 (weak) up to 10 (very intense) showed the value of 8 (± 2) for MembraneBlue-Dual, while for ILM-Blue, the score was 6 (± 3) [15].

However, modern advances in the field of optoelectronics made it possible to obtain diagnostic and intraoperative video and photographic images with high resolution and accurate color reproduction. And the obtained digital data representing the image can be transformed into a sequence of numbers available for analysis on a personal computer, which made it possible to introduce objective computer technology - colorimetry.

This technology allowed Russian ophthalmologists LF Linnik, EE Ioyleva to create the method of computer colorimetric analysis of the optic disc nerve, to develop statistical colorimetric standards of the main types of the optic nerve pathology and to establish criteria for predicting treatment outcomes by colorimetric parameters [16-19]. A number of foreign researchers used these criteria in the objective comparison of the intensity of ILM staining with indocyanine green (ICG) and TB and proposed modifications to the colorimetric analysis. The intensity of staining of epiretinal membranes and ILM was also investigated with the use of various models of endo-illuminators [20-22]. Thus, computer colorimetry established itself as an objective quantitative method for the comparative assessment of various dyes in ophthalmic surgery. The study of the staining ability of modern vital dyes for vitreomacular surgery in order to assess their ability to visualize vitreomacular interface structures with the use of this method is of great interest.

Purpose and Objectives

To evaluate the results of comparative staining of the internal limiting membrane with the endovitrealeal dye Membrane blue dual and the Vitreocontrast suspension in patients with through idiopathic macular ruptures using digital colorimetry method.

Materials and Methods

The study included 15 patients (15 eyes) diagnosed with stage 3 - 4 idiopathic macular hole (according to Gass). In all cases, there was no concomitant pathology or previous surgical interventions. The minimum diameter of the macular hole ranged from 414 to 1750 μm , the duration of macular hole symptoms ranged from 7 months to 7 years.

All the patients underwent a standard three-port 25 Gauge closed vitrectomy using ophthalmic unit Constellation Vision System (Alcon, USA).

At the distance of 4 mm from the limb, 3 25 Gauge ports were installed in the projection zone of the flat part of the ciliary body at 2.30, 4.00 and 9.30 o'clock. An infusion set was connected to the port at 4 o'clock. An illuminator was introduced through the port at 2.30 o'clock, a working tool was introduced through the port at 9.30 o'clock, the cutting frequency of the knife tip of the vitreotome varied from 3000 to 6500 cuts per minute at a vacuum value of 50 to 650 mm Hg.

After midline vitrectomy and PVD surgical induction by active aspiration, 0.1 ml of MembraneBlue® Dual (DORC, Netherlands) was injected into the macular region limited to the area from the inferior temporal vascular arcade to the foveola. The distance to the posterior pole at the time of insertion was approximately 3 mm. The dye exposure was 1 minute, after which the excess dye was aspirated passively. Next, 0.1 ml of Vitreocontrast suspension was injected into the macula area, while limiting the area from the superior temporal vascular arcade to the foveola (Figure 1).

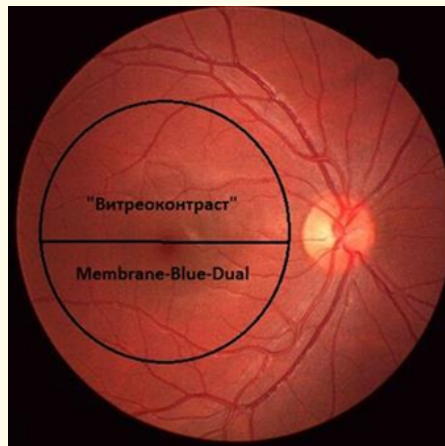


Figure 1: Dye application on the macular area.

Immediately after the suspension precipitation the on the ILM surface, passive aspiration of excess agent was performed. After the visual examination of the macular region, the formation of the ILM fragments started using a 25G end-gripping forceps Constellation Grieshaber Revolution (Alcon, USA). The formation of the ILM flap was started at 2.0 - 2.5 mm to the superior temporal arcade from the

edge of the hole. First a pinch was made by microtweezers at the indicated point to separate an ILM part from the retina. Then, grasping the ILM end with tweezers the membrane was separated along 2-3 o'clock meridians with a movement directed along the arc of an imaginary circle with the macular hole in the center, while controlling so that no separation of the ILM occurred from the edge of the hole. Then, the ILM separated along the arc was grasped at the end and the ILM separation was continued. Successive interceptions of the edges of the ILM portion under the separation were made with tweezers performing a circular separation of the ILM around the macular hole without this fragment complete detachment. Then, using a vitreotome with the "shave" mode the edges of the separated annular ILM fragment facing the vitreous cavity were trimmed, after which the ILM flap was placed in an inverted manner inside the macular hole and gently pressed towards the foveola center.

At the final stage of the surgery, the air tamponade of the vitreous cavity was started. When a small layer of liquid remained above the hole a cannula was inserted for passive aspiration and the fluid was removed: first above the rupture area, then above the optic disc surface. The ports were removed and the scleral approaches were sealed. At the end of the surgery, a subconjunctival injection of 0.5 ml of 0.4% dexamethasone solution was administered.

All the stages of the surgical procedure including ILM peeling were recorded by Panasonic LQ-MD800E digital video camera (Panasonic Corporation, Osaka, Japan) connected to an OMS-800 OFFISS operating microscope (Topcon, Japan). Before each recording routine exposure and calibration were performed adjusting the recording system balance of the white to a standardized reference (Xpo-Balance; Lastolight Ltd., Coalville, Leicestershire, UK). After the surgery, the operating surgeon reviewed and evaluated all video samples for the gross inconsistency of the resulting image visible through the microscope eyepieces. Those video recording samples were selected that had the highest image quality and the maximum ILM contrast within the vascular arcades. The image samples were transferred for analysis to the Image J computer program for the study of biomedical images (National Institutes of Health, USA). Then, in these images, the regions of interest were identified using the method of analyzing one agent based on a series of images (Multiple image method) for each of the agents, that is:

1. Highlighting of the same ILM area stained with Membrane Blue Dual before and after membrane peeling in the images obtained at different time points.
2. Highlighting of the same ILM area stained with the Vitreokontrast suspension. That is, one and the same region of interest was analyzed at different time points - before and after membrane peeling.

For comparative colorimetric analysis, we used the method developed by MacAdam in 1942. It is based on his systematic empirical analysis of the human visual perception of color differences and MacAdam ellipse developed on the basis of these observations that allows calculating of the difference in the intensity of the perceived color contrast. This chart was later adapted and became applicable as the CIELAB color space (CIE 1976L*, a*, b*), in which colors are represented according to their recognition by the human eye. To determine the color sensations of an individual and the perception of their differences as required for the assessment of various agents for chromovitrectomy, it is necessary to convert the device hardware values (RGB models) into the CIE L*, a*, b* color coordinate system (where CIE stands for Commission internationale de l'éclairage [French] - International Commission on Illumination) and to calculate the difference between the obtained values of colors. This color space reflects the universal perception and changes of equal visual expression at an equal distance within the color space (Euclidean distance). Euclidean distance may be considered as the direct measurement of the strength of the contrast perceived.

Thus, in order to reveal the color contrast between the two selected ROIs in each of the methods, first, the Image J software calculated the average color of all the pixels within each of the two restricted ROIs. The average color was obtained as the average RGB value of all the pixels of the selected region of interest where computer color coding in the RGB system (from 0 to 256) based on the principle of decomposing each area into color components (red, blue and green) in 256 luminous gradations was used.

Further, the obtained indicators were normalized to the standard CIE 1976 L * a * b scale using the MATLAB software (version R2011b, Mathworks, Natick, MA). When translating images into the CIELAB color space, values were obtained expressed in terms of L, a, b.

To calculate the color difference, the formula was used:

$$\Delta E_{ab}^* = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

Where:

ΔE_{ab}^* - Euclidian distance of the two regions of interest

L_1^* - The indicator of the illumination of the first region of interest

a_1^* - The indicator of the axis the red-green of the first region of interest

b_1^* - The indicator of the axis the blue-yellow of the first region of interest

L_2^* - The indicator of the illumination of the second region of interest

a_2^* - The indicator of the axis the red-green of the second region of interest

b_2^* - The indicator of the axis the blue-yellow of the second region of interest.

All in all, 100 measurements were carried out.

Data analysis was carried out using the Microsoft Excel computer program and Statistica 6 software. The distribution normality was determined using the Kolmogorov-Smirnov formula, and all measurements were characterized by a normal distribution. P value ≤ 0.05 was considered to be statistically significant for all t-tests of the study. The data was presented in the form of a box-and-whisker interval chart.

Results and Discussion

In all the cases, after the introduction of vital dyes, there was a visible change in the ILM color. When staining the ILM with Vitreokontrast suspension a more pronounced color border between the stained and nonstained areas of the ILM was observed. After ILM peeling, the border between the retinal area without ILM and with intact ILM was discernible with each agent. During the manipulation with the ILM according to the inverted flap technique, the membrane stained with Membrane Blue dual was mobile, and in some positions facing the vitreous cavity its edge was not visualized clearly enough, which complicated spatial orientation and grasping of the edge. This also made the surgeon to turn off the feed irrigation fluid in order to reduce the mobility of the ILM fragment. When Vitreocontrast suspension was used as a staining agent the edge of the separated ILM fragment was brightly stained and its grasping was not difficult in all the cases. During the colorimetric analysis according to the Multiple image method after the videoregistration of the surgeries pictures for each patient were obtained. In the pictures regions of interest with the size of 60X60 pixels were designated first, before the ILM peeling with its most intense staining with each of the agents (Figure 2), then the corresponding areas of the retina after ILM peeling (Figure 3). In a comparative colorimetric analysis, the average Euclidean distance CIELAB for the MembraneBlue® Dual agent between the stained ILM and the corresponding area of the retina without the ILM was 15.97 ± 7.4 , for the Vitreocontrast suspension it was 22.87 ± 6.67 . Based on the data obtained, a swing chart was plotted with the median (Chart 1) as the midpoint value.

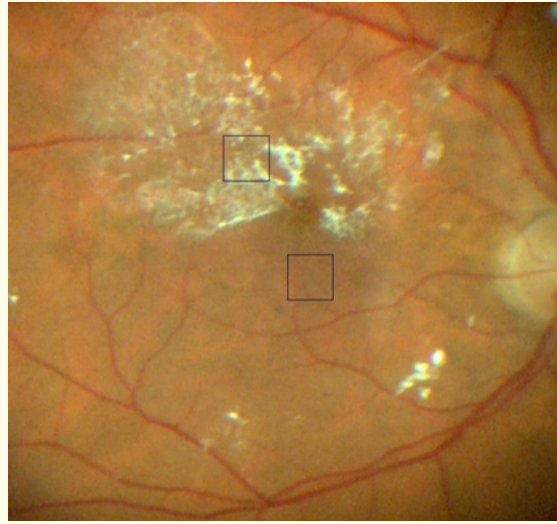


Figure 2: Intraoperative fundus videoregistration of a patient with a macular hole after injection of MembraneBlue® Dual solution and “Vitreocontrast” suspension prior to ILM peeling. A continuous line indicates regions of interest for colorimetric analysis. The upper square corresponds to the staining site with “Vitreocontrast” suspension, the lower one corresponds to MembraneBlue® Dual solution.

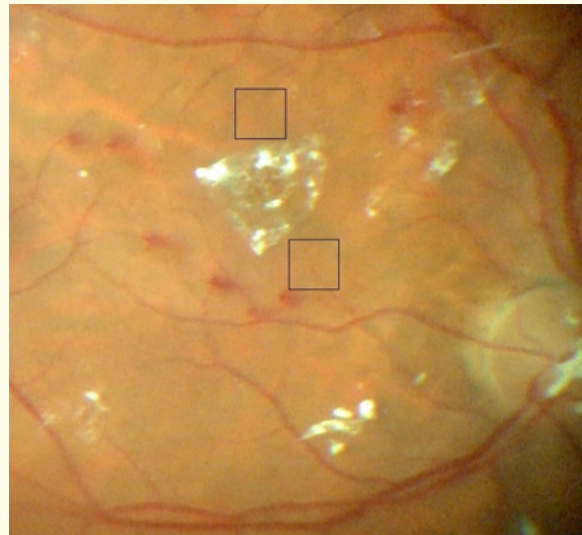


Figure 3: Intraoperative fundus videoregistration of a patient with macular hole after ILM peeling. A continuous line indicates regions of interest for colorimetric analysis. The upper square corresponds to the area previously stained with “Vitreocontrast” suspension, the lower one - with MembraneBlue® Dual solution.

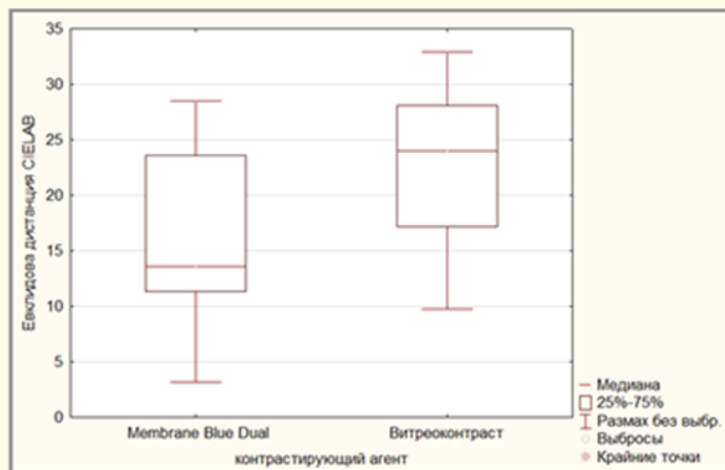


Chart 1: A span chart (box-and-whisker "box with mustache") with a median as the midpoint value. The CIELAB (Euclidean distance) indicator is plotted on the ordinate axis.

The average CIELAB Euclidean distance for Vitreokонтраст suspension was statistically significantly higher measured by Student’s t-test than this indicator for MembraneBlue® Dual at $p = 0.012$. In this study, all surgical interventions were performed using the same mercury illuminator, which allowed comparison of the obtained values.

The higher value of the Euclidean distance for the ILM stained with the Vitreokонтраст suspension allows us to assert that when this agent is used the perception of the ILM staining intensity by the surgeon’s eye will be objectively higher than when using the MembraneBlue® Dual solution.

Additionally, when analyzing video recordings for colorimetric analysis, we carried out the analysis of changes in the intensity of ILM staining with Vitreokонтраст. Besides, the same area of the ILM was assessed in the part not subjected to peeling in different video images, first, immediately after the injection of the staining agent (Figure 4) and then before the air tamponade (Figure 5).

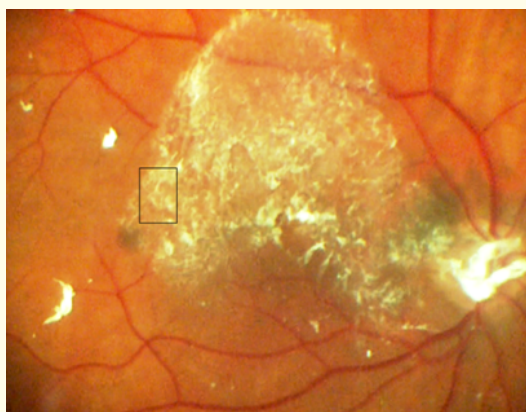


Figure 4: On the intraoperative fundus videoregistration, the ILM area after administration of the Vitreokонтраст suspension was defined.

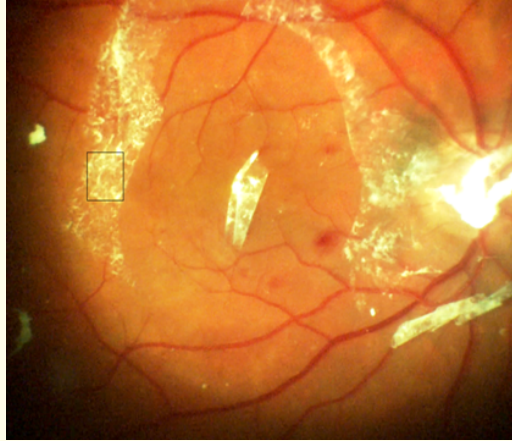


Figure 5: On the intraoperative fundus videoregistration, the ILM area after administration circumferential membrane peeling is defined.

And in all the cases the area of the ILM stained by Vitreocontrast preserved the original color luminance at all the stages of the surgical intervention.

Conclusion

An objective analysis of the staining properties of modern agents for ILM staining by computer colorimetry showed a significant advantage of using Vitreocontrast suspension in comparison with the traditional water-soluble Membrane blue dual dye. Suspension Vitreocontrast provides more clearly perceived by the eye of the surgeon pronounced staining of the ILM effectively and instantly precipitating both on the membrane itself and on the possible ERMs on its surface. It does not lose adhesion during the entire period of surgical intervention and allows the surgeon to perform precision manipulations with the ILM and other vitreomacular interface structures. Thus, Vitreocontrast suspension can be recommended as an alternative to existing staining agents for the ILM.

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Volume 12 Issue 7 July 2021

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