CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMTATOGENOUS RETINAL DETACHMENT

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CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMTATOGENOUS RETINAL DETACHMENT (Abstract): Proliferative vitreoretinopathy (PVR) is one of the most frequent causes of failure of rhegmatogenous retinal detachment (RRD) surgery. Aim: To measure the vitreous levels of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein 1 (MCP-1) in eyes with RRD and in a control group. Material and methods: A prospective study of 40 patients operated for RRD (study group) and 20 patients with epiretinal membrane or macular holes (selected as control group since they needed vitrectomy but had attached retinas). Vitreous samples were collected during vitrectomy and were assessed for the presence of cytokines using a fluorescent bead-based multiplex assay. Results: The concentration of G-CSF (8.59 pg/ml) and MCP-1 (1615.2 pg/ml) were significantly increased in the study group, when compared to the control group (0 and 469.13 pg/ml, respectively). MCP-1 was also significantly increased in the subgroup of patients with PVR compared to the patients with uncomplicated RRD. Conclusions: The levels of these biomarkers support the idea that proliferative vitreoretinopathy has an inflammatory component. Keywords: RHEGMTATOGENOUS RETINAL DETACHMENT, PROLIFERATIVE VITREORETINOPATHY, VITREOUS CYTOKINES.

INTRODUCTION
Proliferative vitreoretinopathy (PVR) is a complication of rhegmatogenous retinal detachment (RRD) characterized by fibrocellular, contractile, epiretinal or subretinal membranes that prevent retinal reattachment. It is one of the most frequent causes of RRD surgery failure (1). Retinal break and subsequent retinal detachment are believed to allow macrophages, retinal pigment epithelial cells, glial cells and fibroblasts to migrate into the vitreous, where they proliferate, form extracellular matrix and assemble into membranes (2).

Several models of the pathogenesis of this disease have been described, some models focusing on the role of inflammatory cells, others on the role of growth factors and cytokines (2). Numerous authors have identified elevated levels of a variety of growth factors, cytokines and chemokines in the vitreous of eyes with PVR (3).

Cytokines serve as signals between cells and are involved in cell proliferation and
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migration, inflammation, angiogenesis and fibrosis. Chemokines are mediators that may promote recruitment of leukocytes to the sites of inflammation, enhance immune responses, and also participate in angiogenesis and fibrosis (4).

Multiplex bead-based flow cytometric assays have the major advantage of assessing the presence of numerous different biomarkers in a small quantity of biological fluid. Several authors believe that multiplex immunoassays show similar performance to ELISA in terms of accuracy and reproducibility and have superior sensitivity. (5).

In the present study we have measured the vitreous concentrations of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein-1 (MCP-1) in eyes with RRD and in a control group, using a multiplex fluorescent bead-based flow cytometric assay.

MATERIAL AND METHODS

The design of this study was prospective, controlled and observational. The study group consisted of 40 eyes of 40 patients with RRD who underwent 23-G pars plana vitrectomy in the Ophthalmology Department of the Iasi University Hospital, Romania, between March and September 2015. All patients were aged over 18 years and had a primary RRD. There were 30 patients with uncomplicated RD and 10 patients with RD and PVR (4 stage B, 5 stage C and 1 stage D, according to the 1983 Retina Society classification) (6). The exclusion criteria were: history of intraocular surgery in the past 6 months, history of intraocular inflammation, diabetic retinopathy or retinal vascular occlusions, general or intraocular steroids or anti-vascular endothelial growth factor in the past 3 months, impossibility to visualize the causative retinal break preoperatively.

The control group consisted of 20 patients with macular hole or epiretinal membrane who also underwent pars plana vitrectomy. We have selected these conditions because the retinas are attached but they also require a surgical approach, thus providing an opportunity for the sampling of vitreous.

All patients and controls underwent an ophthalmological examination by the recruiting surgeon, including measurement of visual acuity and intraocular pressure, biomicroscopy of the anterior segment of the eye, biomicroscopy or indirect ophthalmoscopy of the posterior segment.

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Iasi University of Medicine and Pharmacy. Informed consent was obtained from each patient prior to study initiation.

Surgeries were performed by a single surgeon (C.D.), using local anesthesia, 3-port transconjunctival vitrectomy system (Alcon Constellation, Alcon Laboratories, Alcon, Fort Worth, Texas, USA) and a wide-angle viewing system (EIBOS, Moeller-Wedel GmbH & Co. KG, Wedel, Germany).

The vitreous samples were obtained undiluted by manual suction in a sterile syringe connected to the vitreous cutter, before opening the infusion line. If suction became impossible because of blocking with vitreous gel, the cutter function was activated and the suction continued. The content of the syringe was immediately transferred into labelled 1.5 ml Eppendorf Protein LoBind tubes and frozen at -20° C. The tubes were then stored at -150° C. In all cases, the infusion line was opened
immediately after sample collection and vitrectomy was carried on uneventfully.

All vitreous samples were thawed and centrifuged in September 2015. The concentrations of biomarkers (cytokines, chemokines and growth factors) were measured using a multiplex microbead-based immunoassay designed for cytometry. (Cytometric Bead Array®, BD Biosciences, New Jersey, USA). The capture beads corresponding to each analyte to be measured were mixed together in wash buffer, centrifuged, the supernatant was discarded and the beads were resuspended in diluent to a final concentration of 50 µl/test. 50 µl of the supernatant from the centrifuged samples were incubated in labelled tubes with 50 µl mixed capture beads for 1 hour at room temperature. 50 µl of phycoerythrin (PE) detection reagent were added in each tube and then incubated for 2 hours. After washing away the unbound PE and resuspension in wash buffer, the samples were acquired on a FACSAria™ flow cytometer platform. Each bead population has distinct fluorescence intensity, permitting simultaneous measurements of multiple protein concentrations. The median fluorescence intensity of each biomarker was correlated with the dilution of standard biomarkers, the resulting correlation enabling us to quantify the concentration of biomarkers in the study samples. The sensitivity range of each assay was 0-2500 pg/ml.

The statistical analysis was performed using MedCalc (MedCalc Software bvba, Ostend, Belgium). The significance of the difference in concentration of biomarkers between groups was assessed by the Wilcoxon test. A p value of less than 0.05 was considered significant.

**RESULTS**

The mean granulocyte colony stimulating factor (G-CSF) concentration in the study group was $8.59 \pm 28.9$ pg/ml (between 0-161.9 pg/ml), whereas all the eyes in the control group had undetectable levels of G-CSF (0 pg/ml). Since the found values did not have normal distribution, a Wilcoxon test was used to study the difference between groups. The difference was statistically significant, $p=0.02$.

![Fig 1. Concentrations of cytokines in the study and control group (pg/ml)](image-url)
The mean monocyte chemoattractant protein-1 (MCP-1) concentrations in the study and control group were 1615.2 ± 790.8 pg/ml (range 270.1-2500) and 469.13 ± 263.08 pg/ml (range 118.48-990.49), respectively. The Wilcoxon test showed a highly significant difference, p=0.0001 (fig. 1).

When comparing the concentrations of MCP-1 and G-CSF between the PVR and non-PVR group we found a significant increase of MCP-1 in the PVR group (but not of G-CSF). (tab. I)

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Concentration of G-CSF and MCP-1 in retinal detachment – comparison between eyes with or without proliferative vitreoretinopathy.</th>
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<tbody>
<tr>
<td></td>
<td>Mean concentration, RRD without PVR (pg/ml)</td>
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<tr>
<td>G-CSF</td>
<td>4.09 ± 14.31 (0-71)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>1451.22 ± 795.15 (270.16-2500)</td>
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</tbody>
</table>

DISCUSSION

The course of PVR is the result of the balance (or disruption of balance) between the destructive and protective mechanisms triggered by the occurrence of a retinal break.

The clinical features of PVR correspond to the histopathological findings, represented by fibrocellular (and, in evolution, contractile) membranes on the anterior or posterior retinal surface (3).

The primordial element seems to be partial de-differentiation, migration and proliferation of retinal pigment epithelial (RPE) cells, creating areas of hyperplasia, first at the limit between detached and attached retina and at the margins of retinal breaks. This process may start 3 days after retinal detachment begins. The next step is the activation of glial cells, with proliferation of astrocytes, Muller cells, microglia and capillary endothelial cells (1).

The role of growth factors, cytokines and chemokines is highlighted by an ever-growing body of literature, the sampling of vitreous and subretinal fluid being relatively easy during modern vitreoretinal surgery. The measuring of different factors is also made easier by tests that use minute quantities of biological material.

In our study group of eyes with rhegmatogenous retinal detachments we found a significant increase in the concentrations of G-CSF and MCP-1 (when compared with a control group of eyes with macular holes or idiopathic epiretinal membranes).

Our findings support the data published by Kunikata et al. (8) who have also found that MCP-1/CCL2 and G-CSF were significantly elevated in eyes with RRD, while TNF was not. They have reported that intravitreal triamcinolone is able to suppress elevated levels of intraocular MCP-1 in eyes with RRD. MCP-MCP-1 (also known as C-C motif ligand 2) is a chemokine that recruits monocytes, memory T cells and dendritic cells to sites of tissue injury (4).

Yoshimura et al. (4) have also reported that MCP-1 was significantly elevated in
eyes with diabetic macular edema, proliferative diabetic retinopathy, vein occlusion or rhegmatogenous retinal detachment. They have found that MCP-1, IL6 as well as IL8 are increased.

G-CSF is a cytokine that stimulates the survival, proliferation and function of neutrophil precursors and mature neutrophils. In an experimental setting, vitreous from eyes with proliferative vitreoretinopathy stimulated G-CSF and IL-17A production by ARPE-19 cells (10). G-CSF has been shown to have a neuroprotective effect after retinal ganglion cell axotomy (11)

In a retrospective case-control study, Ricker et al. (12) have compared the concentration of 50 biomarkers in the vitreous of patients with uncomplicated RRD and patients who developed PVR within the first 3 months after surgery. They have found a significant increase of IL2, MCP-1/CC12 and other 15 biomarkers in the vitreous of patients that developed PVR. They found no differences for the values of IL4, IL10, IL12p70, VEGF, bFGF, TNF and other 17 biomarkers (however, they did not provide values for the biomarkers that did not differ between the study and control group). Banerjee et al. (13) promoted the idea that the detectable levels of interleukins and chemokines support the role of an inflammatory response in PVR.

**CONCLUSIONS**

In our study group of patients with rhegmatogenous retinal detachment, the concentrations of G-CSF and MCP-1/ CCL2 are significantly increased when compared with a control group. A significant increase of MCP-1 in eyes with proliferative vitreoretinopathy compared to eyes with uncomplicated retinal detachments was also found, supporting the hypothesis of the implication of this soluble factor in the pathogeny of PVR.

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**REFERENCES**

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**NEWS**

**DREAM OR REALITY? VIDEO HEAD IMPULSE TEST READY TO BE USED**

Looking in the past, over the history of vestibulogy, the aim was always to measure the function of all the vestibular receptors. Vestibular evoked miogenic potentials (cervical and ocular) show the activity of the saccule and the utricle. Now, using video Head Impulse Test is possible quantify the semicircular canals function by measuring the eye rotation response to an abrupt head rotation in the plane of the canal. The main measure of canal adequacy is the ratio of the eye movement response to the head movement stimulus i.e. the gain of the vestibulo-ocular reflex (VOR). However there is a need for normative data about how VOR gain is affected by age and also by head velocity, to allow the response of any particular patient to be compared to response of healthy subjects in their age range. This study held on helathy subjects divided in decade age groups. VOR gain decreased at high head velocities, but was largely unaffected by age (McGarvie LA, MacDougall HG, Halmagyi GM, Burgess AM, Weber KP, Curthoys IS. The Video Head Impulse Test (vHIT) of Semicircular Canal Function – Age-Dependent Normative Values of VOR Gain in Healthy Subjects. *Frontiers in Neurology* 07/2015; 6:154.)

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