

SHORT COMMUNICATION

OUR EXPERIENCE IN THE FLOWCYTOMETRIC DNA ANALYSIS OF SUBRETINAL FLUID IN THE REGMATOGENOUS RETINAL DETACHMENT AND PROLIFERATIVE VITREORETINOPATHY

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ABSTRACT

Studies of the subretinal fluid in two groups of patients - with retinal detachment and retinal detachment and preoperative proliferative vitreoretinopathy to level C₁, are done. By the possibilities of FC-DNA analysis, some parameters are used: total cell count and percentage of proliferating cells (percent of S-phase+G₂/M-phase). The results, obtained in our study show a higher proliferative activity and a higher total cell count in the group with PVR. Additional studies with larger groups of patients are needed.

Introduction

Proliferative vitreoretinopathy /PVR/ is a polyethiological process, characterized by cell proliferation and forming of two membranes on both sides of the retina, leading to its tractional detachment (1,2). It is considered the most frequent reason for the failure of operative treatment of regmatogenous retinal detachment (RD). (3)

The tearing of the retina and its consequent detachment lead to the collapse of the blood-retinal barrier (BRB) and switch on a cascade of complicate interactions, which end with a reparative process and forming of new tissue (4). Most of the contemporary studies are directed to the search of reliable clinical and laboratory parameters, allowing to make

a prognosis for the disease progress, as well as elaborating possible methods for therapeutic influence of the main pathogenetic stages of its development (5,6,7). The potential possibilities to understand the mechanism of the processes and the ways for their control, motivate our interest to make a survey about some parameters of the subretinal fluid (SRF), cell structure and their dynamics in the process of inner proliferation in the retinal detachment.

Purpose. Our goal is to examine some indexes of the SRF cell population in patients with RD and RD with PVR to level C₁, by using the possibilities of flowcitometric DNA analysis (FC DNA). (8)

Materials and Methods

In the process of our research are included results for the treatment and SRF DNA-analysis of 50 persons with regmatogenous retinal detachment with or without existence of

Abbreviations: PVR-proliferative vitreoretinopathy; BRB-blood-retinal barrier; SRF-subretinal fluid; RD -retinal detachment; PEC-pigment epithelium cells; FC DNA - flowcitometric DNA - analysis

PVR (22 persons with RD and 28-with RD and preoperative PVR to level C_1). All the patients have a standard surgical treatment, including sclerage, episcleral filling, punctation and evacuation of SRF.

The analysis of the SRF aspirates is made on laser flowcytometer FACSort (Becton Dickinson). Cycle TEST PLUS DNA Reagent Kit- a product of the same firm, is used. A previous calibration with DNA QC Particles Kit (Becon Dickinson) is made (9).

All the procedures at the preparation of SRF specimens and FC DNA- analysis are completed according to the requirements of the protocol. Accepting, keeping, analysis and processing of the results are performed using the program products Cell FIT v.2.01.2 and ModFit v.2.0 (Mac).

This methodological approach for SRF analysis gives the possibility, by designing of a DNA- histogram, to define and present graphically the cell concentration as well as the percentage distribution of the cells in the separate phases of the cell cycle.

Results and Discussion

As it was already pointed out, in retinal detachment, in the corpus vitreum cavity, there are some cell elements passed from the blood circulation through the disturbed BRB as well as cells from the retinal pigment epithelium. Going to the new microenvironment, activated by the immune proteins situated there, these cells undergo a specific process of dedifferentiation and are included actively in the development and recovering of the injured tissues.

We have chosen parameters, giving some characteristics of the SRF cell population in the two groups of patients, as follows:

- total cell number - gives a quantitative characteristic of the cell structure;
- percentage of the proliferating cells (S-phase + G_2/M phase) of the cell's cycle-

gives a characteristic of the process of cell proliferation.

The analysis of the results among the examined 50 persons, presented on **Fig. 1**, shows that in the patients with RD the index "total number of cell elements in SRF" is considerably smaller (average value - 1653) than in the patients with RD and PVR (average value -3148).

The results for the proliferating activity (percentage of S- phase + G_2/M phases) in the two groups of patients show that the quantity of the proliferating cells in the patients with RD plus PVR (average value - 10,74) is two times higher than in the patients with uncomplicated RD (average value 5,32). (**Fig.2**).

We come to the conclusion that patients with RD and some level of preoperative PVR to C_1 are presented with higher values of the total cell concentration and proliferative activity.

The first event after the retinal detachment is the formation of fluid in the subretinal area, containing a great number of cells. Initially these cells are derived from the pigment epithelium of the retina, leucocytes and macrophages from the blood circulation. The abrupt disturbance of the BRB, appearing in RD, creates conditions for a sharp change in the local homeostasis and accelerates these processes (10).

It is well known that in the healthy corpus vitreum, the existence of any kind of cells is not normal (except for hyalocytes). It could mean that a very small quantity of such cells is enough to destabilize the environment. Nevertheless, some authors prove that this conclusion is not quite correct. Experiments with transplantation of pigment epithelium cells (PEC) in the subretinal environment show that the epithelial phenotype of the transplanted cells is preserved when the BRB is intact, otherwise there will be a

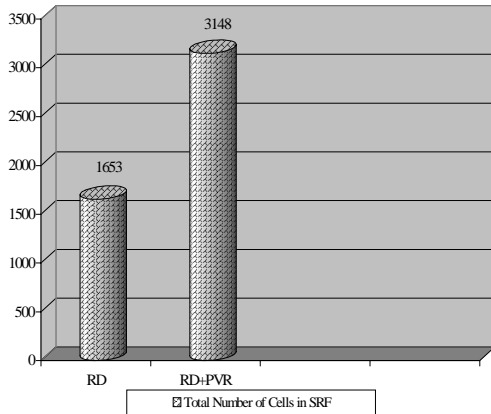


Fig. 1

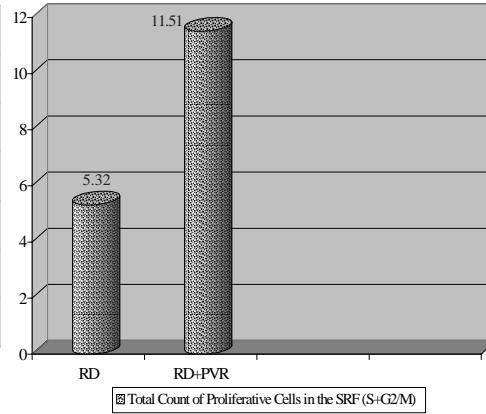


Fig. 2

dedifferentiation of PEC in a fibroblast-like or a macrophage-like phenotype (11). At the beginning of the retinal rupture and the consequent RD, the processes of BRB collapse and dispersion of PEC, cell elements and proteins from the serum go simultaneously. Once gotten into the new vitreal environment, these cells switch on a process of proliferation under the activity of the serum immune proteins and locally synthesized by the macrophages cytokines (12).

Shown that way, the development of PVR in RD is a very complicated complex of processes which requires combination of various influences, caused by the critical levels of different factors, originating from serum, macrophages and hyaline body (2).

Stimulation of the PEC proliferation as a result of all these influences leads to a natural growth of the cell's number. In the process of proliferation cells go through different phases of cell cycle from G_0 , the phase of peace, to the M-phase- the phase of total split of the cell. Every one of the cell cycle stages responds to an exact level of the cell preparation for mitosis, where the S-phase index, that we have examined, presents an intermediate stage in which normal diploid

cells have already ended their DNA replication.

Our results once again prove that there is a dependency between cell and humoral correlations in RD. The following conclusion is taken into consideration: the smallest changes in the homeostasis would have a reciprocal influence on the existing cell population, which from its side changes additionally the local environment. It has to be pointed out that the mandatory participation of the disturbed by the pathological process BRB, is a necessary condition to change the homeostasis.

The limited number of examined patients cannot give us a reason for statistically reliable conclusions. The received results however, are an indication for the necessity of continuing examinations in the direction of FC DNA analysis, which could be used as an objective prognostic method, in addition to the clinical evaluation.

The possibility for widening of therapeutic approaches by using medicines, controlling effectively the PVR, could lead to a new behavior in the treatment.

The great medical and social significance of the problem justifies our efforts, directed

to search new methods for getting optimal results.

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