Diabetic macular edema (DME) is the most frequent cause of visual acuity loss in patients with diabetes mellitus. This statement headed the majority of publications and book chapters about diabetic retinopathy (DR) in the past 20 years and continues to do so. DME remains an unresolved problem. The Early Treatment Diabetic Retinopathy Study demonstrated that focal photocoagulation in clinically significant macular edema halved the risk of moderate VA loss in 3 years follow-up. However, in terms of VA improvement, the results were not so encouraging: of eyes with to VA of <20/40 at the beginning of the study, only 40% of those that received immediate treatment gained one line after 2 years (1). As initially the efficacy of intravitreous triamcinolone was seemingly spectacular, it led us to believe that the objective of gaining VA in these patients was feasible. However, to recent publication comparing said treatment with focal photocoagulation did not reveal VA differences with to 3-year follow-up (2).

The pathogeny of DME is complex and not entirely known. The different pathogenic pathways converge in to crucial fact: the disruption of the hemato-retinal barrier (HRB) which has 2 components: the internal HRB comprised by the retinal endothelium and a network of glial cells (astrocytes and Müller cells), and the external HRB made up by the retinal pigmentary epithelium (RPE). Although some clinical studies suggest that the alteration of the internal HRB is what most contributes to the increased patency which causes the macular edema, experimental studies suggest that the external HRB also would play an important role. The initial process in the pathogeny of diabetic retinopathy (DR) is chronic hyperglycemia and associated metabolic changes such as the formation of advanced glycation end products, increased diacylglycerol (DAG) and oxidative stress. This anomalous metabolic environment alters the structure and function of retinal vessels (forming microaneurisms, increasing leucostasis, producing vasoconstriction and ischemia) and stimulates or inhibits the synthesis of mediators by the retinal cells, endothelium and RPE. Said mediators act directly over the HRB and the vitreous. An imbalance between pro- and anti-edema mediators tilted toward the former increases the HRB patency. Doubtlessly the best known and probably most important mediator is the Vascular Endothelium Growth Factor (VEGF). This peptide has 6 isoforms: 121, 145, 165, 183, 189 and 206. The most important one in the pathogeny of DME is 165. VEGF is produced by the RPE, ganglionary cells, Müller cells, pericytes and endothelial cells. Hyperglycemia, oxidative stress, DAG through protein kinase C, hypoxia and other mediators such as interleukin-6 and -8 induce its expression. VEGF also induces conformation...
changes in the inter-endothelial tight junctions due to the phosphorilation of ocludine and ZO-1 and produces the development of fenestrations in the endothelial cells. Both events cause an increase of capillary patency and edema. Accordingly, VEGF constitutes to key therapeutic target in macular edema. Recently the results of 6 months follow-up of to prospective, randomized and multi-centre study have been published. This study compared intravitreous injection of 0.5 mg of Ranibizumab, an anti-VEGF drug, with focal photocoagulation and the combination of both treatments in patients with clinically significant macular edema. Treatment with Ranibizumab was better than laser in what concerns visual acuity and macular thickness reduction (3).

VEGF and other mediators are present in the vitreous humor and can be quantified by means of several techniques like radioimmunoanalysis or ELISA tests (Enzyme-linked immunosorbent assay). The vitreous analysis allows the identification of new pathogenic mediators and therefore potential therapeutic targets. It must be pointed out that it is crucial to take into account to number of methodological aspects when analyzing vitreous samples in order to determine and quantify peptides. In what concerns the surgical technique, to avoid diluting the samples it is necessary to obtain it with the infusion closed or under air, the latter being the safest mode because it allows to maintain the tone of the ocular globe. The volume we must obtain depends on the amount of factors to be determined, on whether we analyze other parameters such as proteins and the test to be applied. At present we have one type of ELISA, the so-called ELISA-multiplex which facilitates the identification of to large amount of mediators with very small sample sizes. As regards the design of the study, it is necessary to establish the concentration of the factor under study, also in controls. For obvious reasons it is not possible to obtain vitreous samples of patients with perfectly healthy eyes. Usually we use samples of vitrectomized patients as samples, as to treatment for retinal diseases pathogenically distant from that which is being studied.

In the case of DME we utilize as controls patients with idiopathic macular hole or epiretinal membrane. It is important to reject cases exhibiting vitreous hemorrhage or hemoglobin is detected in the sample. The fact that there is blood in the vitreous means that the intravascular content has accessed the vitreous cavity. Thus, we could find high concentrations of to factor for that reason only. The vitreous concentration of to factor could be influenced by its plasmatic concentration. To avoid this bias, it is necessary to carry out case-control studied in which both groups are paired per plasmatic concentrations of the factor to be studied. Another option is to calculate the factor vitreous concentration/plasmatic concentration quotient. Finally, the disruption of HRB which occurs in DR produces an increase of proteins in the vitreous. Therefore, finding high concentrations of to factor in the vitreous could signify to non-specific increase of proteins through diffusion. Accordingly, we must correct the vitreous concentrations of the factor by the total proteins. By taking into account these aspects we will be able to adequately assess the results of our studied. At this point, it is also very important to understand the pathogenic meaning of finding to high or reduced concentration of to factor in vitreous samples of patients with to given disease. By applying this methodology we have assessed the role which can be played by two mediators, erithropoietin (EPO) and somatostatin (SST), in the pathogeny of DME (4,5).

EPO is to glycoprotein related to erithropoiesis synthesized by the fetal liver and the adult kidney. Its expression has also been demonstrated in the brain and the fetal and adult retina. Recent research suggest it could play to neuroprotective role in said tissues. Hypoxia is to strong stimulant of systemic and intra-ocular synthesis of EPO. In fact, high EPO concentrations have been found in patients with proliferative DR and some studies show it has to pro-angiogenic activity similar to that of VEGF. In said study we found high concentrations in patients with DME and patients with proliferative DR in comparison with to control group without differences between the diabetic groups. The increase of EPO in the case of proliferative DR could contribute to retinal neovascularization. However, the relationship between the increase of EPO and macular edema is not clear. In fact, there is evidence that this hormone counteracts the increased patency of the blood-brain barrier (analogous to HRB) induced by VEGF and restoring the tight junctions in an in vitro model of said structure. Cases of improvement in DME in patients treated with systemic EPO due to kidney insufficiency have been published. Therefore, it is possible that the increase of intravitreous EPO in patients with DME is part of to protective self-regulating mechanism.
against vascular hyper-permeability. SST is known for its inhibiting action of the growth hormone but it must be pointed out that it is ubiquitous, with its retinal expression having been demonstrated. In our study we found lower SST concentrations in patients with DME in comparison with control patients. In the same study we found lower concentrations of SST in patients with proliferative DR in comparison with controls, without finding differences with macular edema patients. There are data which point to the possibility that the SST deficit could contribute to increases in vascular patency. This hormone has an anti-secretion activity in the digestive tube. Expression of SST receptors has been found in the apical part of RPE, where several fluid and ion-transporting systems are located. Accordingly, SST could regulate this transport. In addition, cases of patients with macular edema of inflammatory etiology have been published. These were successfully treated with systemic somatostatin analogs. In other words, the SST deficit could have to direct relationship with the pathogeny of DME.

There are many aspects yet to be research in the pathogeny of DME. Vitreous analyses allow to infer what is happening in the retina of diabetic patients, but we must take into account some methodological considerations in order to make an adequate assessment of the study results, rejecting samples with blood or where hemoglobin was detected, carry out case-control studies with groups paired by plasmatic concentration of the peptide in question and correct the vitreous concentration of the fact by the total protein concentration. In addition, the results must be interpreted on to biologically plausible basis and be consistent with the knowledge about the topic at the time. EPO and SST could be related with the pathogeny of DME. The former as to protective factor against disease and the deficit of the latter as to factor which favors the increase of vascular patency.

REFERENCES