Chapter

Local Inflammatory Biomarkers and Potential Inflammation-Targeting Therapies in Diabetic Retinopathy

Ioana Damian and Simona Delia Nicoară

Abstract

Diabetic retinopathy (DR) is one of the most frequent microvascular complications of diabetes. A large body of evidence supports the role of inflammation in the development and progression of DR. Currently, DR is diagnosed based on the presence of morphological lesions detected on fundus examination. Yet, there are other laboratory or imaging biomarker whose alteration precede DR lesions. This chapter will first briefly explain the role of inflammation in DR pathogenesis and will analyze the molecules involved. Further, it will discuss significant and recent studies that analyzed local laboratory or imaging inflammatory biomarkers in different DR stages. It will then focus on several potential inflammation-targeting therapies which proved to be effective in animal or human studies. Validation of these reviewed biomarkers would allow the identification of patients who do not respond to the current available treatment and could benefit from an adjunctive therapy.

Keywords: diabetic retinopathy, inflammation, biomarker, retinal imaging, anti-inflammatory therapy

1. Introduction

Diabetus mellitus (DM) is an important public health issue, with a constantly growing prevalence from 4.7% in 1980 to 8.5% in 2014 [1]. In 2019, 463 million adults between 20 and 79 years had DM [2]. Diabetic retinopathy (DR) is one of the most frequent microvascular complications of diabetes. Most type 1 DM (T1DM) patients develop DR only after 5 years or even more from the onset of DM, but after 20- year history of disease, 99% present a form of DR [3]. On the contrary, for type 2 DM (T2DM) patients, DR may be present even from the diagnosis, while after 20 years of T2DM, 60% of the patients develop DR [3]. Currently, DR staging is performed depending on the presence of clinical ocular biomarkers, such as microaneurysms, hemorrhages, soft or hard exudates, macular oedema and new vessels. The treatment for DR targets especially the severe non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR) stage and diabetic macular oedema (DME). The therapeutic options are intravitreal injections with anti-VEGF agents, corticosteroids injections, retinal laser or surgery such as pars plana vitrectomy (PPV). A large body of evidence supports the role of inflammation in the development and

progression of DR. There is evidence that low-grade subclinical inflammation is responsible for many of the characteristic vascular lesions of DR [4]. In this review we provide an overview of several local inflammatory biomarkers of DR laboratory and retinal imaging together with anti-inflammatory therapeutic options.

2. Inflammation and diabetic retinopathy

Inflammation is defined as the body's mechanism of self-defense against pathogens. In this complex process, mediators such as pro-inflammatory cytokines, chemokines and adhesion molecules are involved. They initiate the interaction between the endothelium and leukocytes and consequently induce the migration of leukocytes towards the injured or infected tissue [5]. Although acute inflammation is considered beneficial, a chronic and dysregulated one produces harmful effects, being known to be involved in diseases such as rheumatoid arthritis, multiple sclerosis and atherosclerosis [5].

Lutty et al. were the first who reported an increased neutrophil number per square millimeter in both retinal and choroidal vessels in DR patients [6]. Moreover, they continued the study of neutrophils in diabetic monkeys and found an association between the accumulation of neutrophils and capillary closure [7]. Further studies found evidence that capillary occlusion and intraretinal neovascularization are associated with the attachment of neutrophils and monocytes [8], while the level of leukocytes adherence to the retinal vasculature, known as leukostasis, is associated with the progression of DR [9]. Recent findings implicate that an increase of ICAM-1 and integrins at the level of endothelial cells and leukocytes is responsible for the increase in leukostasis, while ICAM-1 blocking and CD18 deletion decreases the leukostasis induced by DM [10, 11]. Microglial cell is a resident macrophage distributed throughout the retina, which releases inflammatory cytokines such as TNF-α after being activated [12].

There are several processes in DR pathogenesis in which inflammation is involved:

1. vessel leakage: retinal endothelial permeability is induced by TNF-α, but is successfully prevented by PKCζ inhibitor [13]. After TNF-α was blocked with Etanercept, a soluble TNF-α receptor, the activation of NF-κB was inhibited and blood retinal barrier (BRB) breakdown was prevented [14]. Similarly, VEGF is directly involved in the breakdown of inner BRB, being proved that after blocking ICAM-1, the leukostasis is reduced but also VEGF induced vascular leakage is blocked [15].

2. vessel closure: IL-1β and TNF-α increase caspase 3 activity and induce endothelial cell apoptosis [16, 17].

3. pathological neovascularization: New vessels' formation is induced by leukocytes by interfering in the releasing of pro-angiogenic factors, such as VEGF, angiopoietin 1, basic fibroblast growth factor, transforming growth factor and platelet derived factor. All these agents promote recruitment of endothelial progenitor cells and enhance the migration, proliferation and survival of the pre-existing endothelial cells [17].

4. retinal neuronal death: TNF-α, IL-1β, reactive oxygen species (ROS) and excessive nitric oxide (NO) released by leukocytes during inflammation are involved in retinal neuronal cell death [5].

DM causes increased systemic and local production of numerous inflammatory molecules involved in DR onset and development.

3. Inflammatory biomarkers

A biomarker has been defined as "a biological molecule found in blood, or other bodily fluids, or tissue which represents a sign of a normal or abnormal process of a condition or disease" [18]. Due to the rapid advancement in engineering, methodology and equipment, researchers are able to generate large amounts of data analyzing only small amounts of samples, using mass spectrometry techniques. In DR studies, several kinds of samples were used, such as tear, cornea, aqueous humor (AH), lens, vitreous humor (VH), retina and serum [19]. Although VH is in direct contact with the retina, it requires a highly invasive procedure, whereas the tears can be collected using non-invasive methods but interact indirectly with the retina [19].

3.1 Local laboratory biomarkers

3.1.1 Tears

The tears are composed of an aqueous solution of water, lipids, proteins and salt. The major proteins are lactoferrin, lysozyme, serum albumin, secretory immunoglobin A, lipocalin and lipophilin [20]. One study identified 1526 proteins using protemics approaches, most of them being shown to respond to insults such as cataract surgery or diseases like DM [21, 22]. Mainly because it contains proteins in high concentration (about 8 μg/μl) and it is easy to collect, tears have been a fluid of interest for many studies [21]. Herber et al. was one of the first to demonstrate that DM patients' tear composition is different from control subjects [23]. Azzaralo et al. found increased levels of lactotransferin, also known as lactoferrin, a glycoprotein with immunomodulatory, anti-microbial and anti-tumor effects [24]. Increased levels of monocyte chemoattractant protein-1 (MCP-1) were also identified in DM with or without DR. A previous study demonstrated elevated Interleukin-1 receptor anatagonist (IL-1ra) levels, which represent an anti-inflammatory factor inhibiting pro-inflammatory activity of IL-1beta [25]. A positive correlation between IL-6 levels and DR stage was found. IL-6 is involved in synthesis and the release of acute phase reactants and matrix metalloproteinase 9 (MMP-9), decreased tear production, having also a role in the release of IL-1β [26]. TNF-α is a cytokine able to induce the disruption of BRB by loosening the tight junctions between endothelial cells and between RPE cells, with a role in the leukocytes adhesion to the endothelium [13]. TNF-α level was increased in patients with PDR (13.5 pg./mL) compared to NPDR (2.8 pg./mL) [27]. The tear sample can be obtained using two techniques: the direct one using aspiration with the help of a micropipette or a microcapillary tube placed in the inferior fornix, or the indirect one, with the help of a Schirmer test strip or cellulose sponge.

3.1.2 Aqueous humor

Aqueous humor (AH) is produced by the ciliary body epithelium and represents the fluid in the anterior chamber of the eye. It is involved in the removal of metabolic wastes, in ocular immunity, it supplies oxygen and nutrients, and has a role in refraction. AH is composed of water, electrolytes and proteins, which although are present in relatively low concentrations compared to blood serum, are vital in

the maintenance of anterior segment homeostasis. AH is not in direct contact with the retina but proteins released from it can enter AH either through disrupted BRB, cilio-retinal circulation or through diffusion from the VH and the VH-AH barrier [28]. Proteomic analysis studying AH from patients with DR compared to subjects without DM, found several proteins associated with PDR like apolipoprotein A-I (APOA1), apolipoprotein A-II (APOA2), apolipoprotein A-IV (APOA4), and α-1-acid glycoprotein 1 (ORM1) [29] as well as selenoprotein P (SELENOP), and cystathionine β-synthase (CBS) [28].

IL-23 levels were increased in PDR patients, and IL-17 was also higher in NPDR and PDR compared to control subjects as shown by Wang et al. in 625 patients with T2DM [30]. IL-17 is a powerful pro-inflammatory cytokine capable of mobilizing neutrophils and inducing the secretion of IL-6 and IL-8 prostagladin E2 [30]. Regarding IL-23, it stimulates Th17 cells to secrete IL-17, which further increases the cycle of inflammation.

Concerning IL-10, the cytokine with immunosuppresive properties, capable of attenuating the antigen-presenting ability of APC, studies found contradictory results: decreased concentrations in DR compared to NPDR by Cheung et al. and Zhang et al., contrary to Hu et al. who reported increased levels [30]. It was also suggested that IL-10 levels could be correlated with central subfield thickness (CST), one study reporting higher IL-10 concentration in PDR (224,79 pg./mL) that in the control group (160/14 pg./mL) [31]. TGF-beta is involved in the differentiation of activated $CD4^{+}Th_0$ cells into Treg cells, but it is also involved in promoting differentiation of $CD4^{+}Th_0$ cells into Th_{17} cells in combination with IL-23 [32]. Zhang et al. found elevated and stable TGF-beta levels in DM compared to control [30].

Myonectin inhibits the inflammatory response triggered by lipopolysaccharide in macrophages and in myonectin-knockout mices, in which the proinflammatory gene expression was identified. Previous studies demonstrated decreased myonectin concentrations in AH belonging to T2DM especially with PDR but also in NPDR compared to control subjects [33, 34]. In refractory DME, IL-2, IL-8, PIGF and VEGF were found elevated but only IL-8 was correlated with responsiveness, exhibiting higher levels in the refractory group [31]. This result is supported by Jeon et al., who observed that after injecting triamcinolone intravitreally to patients unresponsive to IVB, the effect was positive while the efficacy was associated with IL-8 levels in the AH [35].

IL-6, another proinflammatory cytokine was found elevated in PDR and DME patients. Additionally, it induces VEGF expression, being involved in the breakdown of BRB and thus being involved in the pathogenesis of earlier stages of NPDR [36]. IL-6 is considered as a driving force because it changes the overall cytokine profile in the AH of DM patients. Moreover, the level of VEGF in AH was strongly correlated with VEGF level from the VH [37].

IFN-α is a known angiogenic inhibitor, with a protective role against retinal damage in diabetes. Cheung et al. found reduced IFN-α levels in DM patients compared to control subjects [38].

MCP-1 level in AH is believed to be a predictor for a worse visual acuity outcome of PDR patients after vitrectomy, while there was no significant correlation between VEGF levels and BCVA, as noted by Lei et al. [39]. MCP-1 stimulates the recruitment and activation of monocytes and macrophages which further leads to higher microvascular permeability or ischemia from vessel occlusions. A few studies have investigated the effect of intravitreal triamcinolone [40] or dexamethasone implant [41] on the intraocular inflammatory cytokines and concluded that both drugs could significantly decrease the level of MCP-1 in the AH.

Samples from AH are collected through a paracenthesis from patients undergoing cataract surgery, trabeculectomy or from post-mortem eyes. The volume collected ranges from 100 to 150 μL. The samples from living patients are more useful since the profile from post-mortem eye is different due to the accumulation of metabolic waste [26].

3.1.3 Vitreous humor

The vitreous humor is the gelatinous component of the posterior segment of the eye that gives its spherical shape. In addition to its role in structural support, the transparent nature of the vitreous body aids in light transmission to the retina. Because of its avascular nature, much of the proteins found in VH are derived from the retina itself [28]. Several proteins in the VH have been identified as biomarkers for different stages of DR, like components of the acute phase response, coagulation pathway, complement system and other inflammatory pathways (e.g., VEGF, amyloid-β A4 protein, metalloproteinase inhibitor 1, kininogen-1) [19].

Pigment epithelium-derived factor (PEDF) is a negative regulator of inflammatory processes, with lower levels in VH from DR patients compared to NDR. This finding suggest that beside an increase in proinflammatory cytokines, there is also a decrease in the anti-inflammatory ones [42]. Clusterin, a protein involved in the regulation of complement cascade, was found decreased in DR patients [43]. One of its function consists in an anti-inflammatory protection for the BRB [44]. Kallikrein, a known central component of pro-inflammatory kallikrein-kinin system, was found elevated in the VH from DR patients [45].

Anti-VEGF agents such as bevacizumab did not have a significant effect on VH inflammatory proteins as shown by Loukovaara et al. [46], as opposed to Zou et al. who studied VH proteome from patients treated with anti-VEGF agent ranibizumab and found decreased VEGF levels as well as a decrease in complement activation protein, acute inflammatory response and platelet degranulation [47]. Similarly, photocoagulation reduced the levels of pro-inflammatory protein osteopontin (SPP1), as shown by Wei et al. [48].

When exposed to intravitreal injection of IL-1β, retinal capillary endothelial cells degenerate [49], while co-administration of IL-1β, together with high concentrations of glucose, disrupt the RPE cells [50]. Increased levels of IL-1β were found in the VH collected from diabetic patients compared to control [51], underlining its role it the DR-related inflammatory processes.

IL-8, responsible for activating neutrophils and T Lymphocytes, together with IL-6, a chemokine responsible for increased endothelial cell permeability by rearranging actin filaments and changing the shape of endothelial cells, were both found elevated in VH of PDR patients [52]. Their origin does not seem to be the serum, since higher levels were found in the vitreous, suggesting that macrophages, RPE cells, glial cells or monocytes from the vitreous of PDR patients, could produce citokines [53]. IL-10, another anti-inflammatory cytokine, with the ability of deactivating macrophages, was not found to be increased in PDR patients, the author of the study suggesting the lack of a counter balanced by the anti-inflammatory cytokines [54].

MCP-1, with its known role in promoting leukocyte recruitment and inducer of angiogenesis and fibrosis, was found elevated in DR VH, with levels being higher than in the serum [55]. Hyperglycemia seems to increase the formation of MCP-1 from RPE cells, retinal vascular endothelial cells and Muller's cells [56]. This finding further encouraged that MCP-1 gene polymorphism to be proposed as a potential risk factor for DR [57].

Stromal cell-derived factor-1 (SDF-1) is upregulated in damaged tissues and as a response, it mobilizes progenitor cells to promote repair [58]. Together with VEGF, it promotes endothelial progenitor cells from the bone marrow to the ischemic retina [59]. Butler et al. found increased SDF-1 concentrations in VH from DME or PDR patients, and moreover the increase was correlated with disease severity [60].

Extracellular High-Mobility Group Box-1 Protein (HMGB1) as a proinflammatory cytokine, leads to overexpression of TNF-α, MCP-1, ICAM-1, through activation of NF-β [61]. In addition, El-Asrar reported elevated levels of HMGB1 in VH belonging to PDR patients. They further reported that HMGB1 and its receptor for advanced glycation products (RAGE) are expressed by vascular endothelial cells and stromal cells in PDR fibrovascular epiretinal membranes [61].

Tumor Necrosis Factor-α (TNF-α) was found elevated in VH from DM patients, with a higher vitreous/serum ratio [51]. In addition, TNF-α was also found expressed in vascular endothelial and stromal cells in epiretinal membranes belonging to PDR eyes [62].

ICAM-1 levels in PDR were found increased, especially in active PDR compared to inactive PDR [61]. ICAM-1 was highly expressed in retinal and choroidal blood vessels, as well as in fibrovascular membranes, and its expression seems to correlate with the number of migrated neutrophils in the retina and choroid [6]. When ICAM-1 blocking was obtained, the leukostasis, endothelial cell death and also the vascular leakage in the retinal vessels was diminished.

When retinal capillary endothelial cells were exposed to high concentration of glucose, TNF-α or IL-1β, they produce the membrane-bound form of vascular adhesion protein-1(VAP1) and release the soluble vascular adhesion protein 1 (sVAP-1) [63]. Its role consists in leukostasis and leukocyte entrapment. High levels of sVAP-1 were found in the serum and VH of patients with PDR [64].

Samples from VH are collected through vitreous taps from patients that underwent PPV or after intravitreal injections. Simo et al. raised the problem of confounding factors that could lead to misinterpretation of result. Vitreous hemorrhage could induce a massive influx of serum proteins. Similarly, the disruption of BRB also leads to increased protein levels in VH. The first problem could be solved by rejecting samples with hemoglobin >5 mg/mL, while the second one, by correcting the intravitreal concentration of peptide under study for total vitreal proteins or calculating the vitreous/plasma ratio [56].

3.2 Imaging retinal biomarkers

3.2.1 Hyperreflective retinal foci

These lesions are found under the name of hyperreflective foci, dots or spots. Hyperreflective retinal foci (HRF) have been first described in wet AMD but they were also noted in retinal vein occlusion, MacTel type 2, central serous retinopathy, retinitis pigmentosa, Stargardt disease and Best disease. Different pathogenetic origins were hypothesized: initially they were considered precursor of hard exudates or lipid-laden macrophages [65], representing subclinical features of lipoprotein extravasation that were not observed on clinical examination, fundus photography, or fluorescein angiography, due to their small size [66]. In contrast to the previous studies, Lee et al. proposed that HRF represent activated microglial cells since they determined the presence of increased soluble CD14 in the AH. Soluble CD14 seems to be released by activated microglial cells [67, 68]. Other theories assumed that HRF represent debris of photoreceptors or RPE hyperplasia [65, 66].

HRF are initially present in the inner retinal layers, where resting retinal microglia are normally located and after they are activated, they subsequently migrate to

the outer retinal layers [66, 69]. Another argument that HRF are in fact microglia is that both are primarily found in the inner retina in DR without edema and show an extended distribution into the outer retina in DME [68]. By contrast, Bolz et al. described that HRF are distributed throughout all retinal layers in eyes with different types of diabetic macular edema (DME), diffuse or cystoid [70]. Uji et al. reported the presence of HRF in the outer retina in 53.7% cases while in the inner retina in 99.1% cases in eyes with DME [71]. Uji et al. further suggested an origin from degenerated photoreceptors or macrophages engulfing the former, since HRF were found in the outer retina closely associated with disrupted external limiting membrane or IS/OS line [71].

HRF appears on OCT as small discrete, well-circumscribed, dot-shaped hyperreflective intraretinal lesions (**Figure 1**) [69]. Their size is less than 30 μm, and as opposed to hard exudates they do not induce back-shadowing, while their reflectivity is similar to retinal nerve fiber layer and equal or greater than the retinal pigment epithelium (RPE) band [67, 72].

As opposed to HRF, hard exudates are thought to form from microaneurysms, which is the major source of leakage in focal edema [68]. Other theories even suggested that HRF can occur even before DR development, representing a neurodegenerative process [69]. Both the presence and number of HRF was increased in diabetics versus control subjects, especially in those with signs of DR, but interestingly, none of the patients had signs of DME, hard exudates nor subclinical signs of DME on OCT examinations [68].

The size and also the number of HRF seem to decrease after treatment with anti-VEGF or corticosteroids [71]. The decreased microglial activity following anti-VEGF therapy could contribute to the reduced loads of inflammatory cytokines, which further translates into decreased number of HRS and reduced CMT [68]. Moreover, HRS was the first feature to disappear or reduce after anti-VEGF therapy [66]. One study revealed that an initial presence of larger number of HRF responded better to dexamethasone implants than anti-VEGF therapy but a higher number of HRF is associated with early recurrence of DME after steroid implant [67].

One study found that eyes with HRF in the outer retinal layers presented a greater disruption in EZ (IS/OS) lines at baseline but they improved more than the eyes without the foci at 12 months. Taking into account this result, the authors further suggested that greater VA improvement could be partially explained by foveal photoreceptor restoration in eyes with HRF in the outer retinal layers [65].

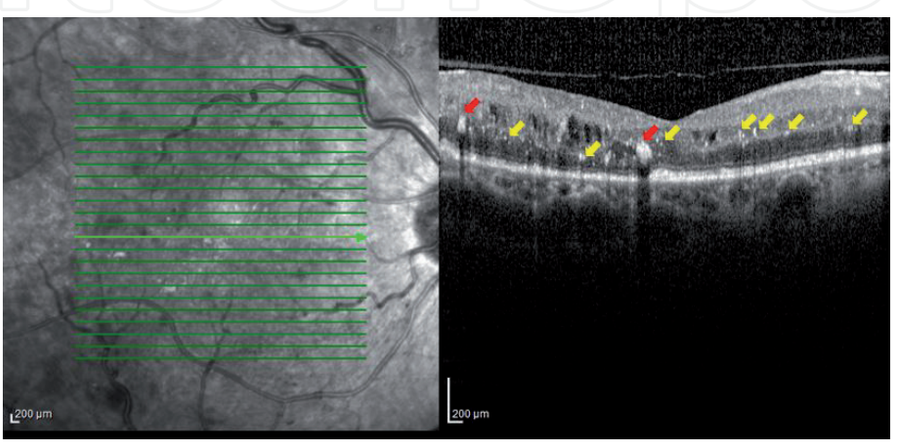

Figure 1.
Spectral domain OCT linear scan in the macula of a diabetic patient with PDR. The yellow arrows indicate HRF while red arrows indicate hard exudates.

3.2.2 Sub-foveal neurosensory detachment

Hypoalbuminemia lowers the intravascular osmotic pressure and together with increased hydrostatic pressure can cause retention of fluid in the subretinal space. The role of subretinal fluid (SRF) in final visual and anatomical outcomes is still unclear. There is a number of studies which have shown that the presence of SRF is associated with good anatomical and functional gains. Contrary to this, there are other studies which have reported the association between the presence of SRF with poor visual gains [73]. Results from RESTORE study [74] and post hoc analysis from RISE/RIDE study [75] proved the protective role of SRF. These studies revealed the correlation between the presence of baseline SRF with better visual gains at the end of 1 year. These studies also showed a positive impact from SRF in response to ranibizumab therapy. Eyes with SRF seem to have increased IL-6 levels, which signifies active inflammation in these eyes, suggesting that inflammation has an important role in the development of this phenotype of DME [76]. Sonoda et al. found that eyes with subretinal detachment (SRD) presented increased intravitreal levels of IL-6, IL-8 and VEGF [77]. Discontinuation of the external limiting membrane in the SRD type of DME induces cellular damage, which further attracts scavenger cells to the retina, producing IL-6 (**Figure 2**) [78]. The migration of activated microglia in the outer segments of the retinal layer could support the production of IL-6 and subsequently the accumulation of SRF. The SRD type of DME is associated with an increased number of HRF and significant functional impairment of the macula [78]. The presence of numerous HRF is also associated with poorer glycemic control in patients without significant DME [79]. The levels of ICAM 1 are associated with the height of SRF, indicating that increased vascular permeability by inflammatory mediators results in SRF [80]. An important finding was that DME eyes with SRF respond significantly better to dexamethasone implants and the authors consequently supported the use of dexamethasone implants in patients with DME with SRF [81]. Interestingly, a recent study assessing the change of parameters in DME with SRD after treatment, reported that intravitreal injection of steroid like dexamethasone implant showed greater decrease in number of RHF compared to anti-VEGF agent such as ranibizumab [82].

3.2.3 Hyperreflective choroidal foci

The analysis of the choroid could be an important tool in the assessment of progressing DR. Choroidal parameters such as luminal area (LA), stromal area (SA) and total choroidal area (TCA) were found decreased in diabetic patients compared to control by a recent study [83]. Hyperreflective choroidal foci (HCF) is a recently described entity in DR. It is postulated that HCF represent HRF migrated from the retina, into the choroidal layers, which was permitted by ELM and EZ disruption (**Figure 3**). The hypothesis that HCF are actually migrated HRF, is supported by the study of Roy and al. where all 59 eyes with HCF had HRF as well and there was no eye which had HCF in the absence of HRF on SD-OCT [84]. Presence of HCF could be used as a biomarker of disease severity in eyes with DR [84]. HCF were found to be present significantly more in PDR eyes than NPDR eyes [67].

3.2.4 Intraretinal cystoid spaces

Inner BRB is affected by elevated levels of VEGF leading to increased vascular permeability, which will result in a decreased osmotic gradient, extracellular fluid accumulation, and cyst formation [67]. VEGF is also responsible for the depletion of the occludin in RPE which will further disrupt the tight junction in the outer

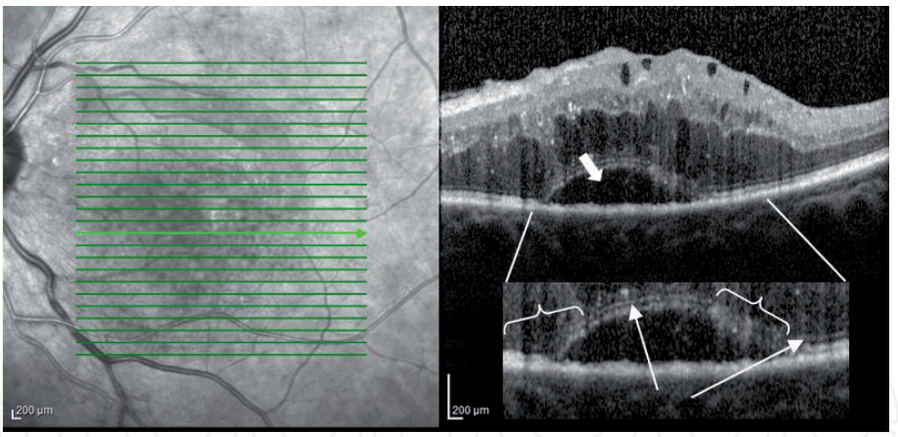

Figure 2.
Spectral domain OCT linear scan in the macula of a diabetic patient with PDR. The small arrow indicates sub-foveal detachment. In the magnified segment of the image, the tall arrows indicate intact external limiting membrane (ELM) while the curly brackets indicate a discontinuous ELM.

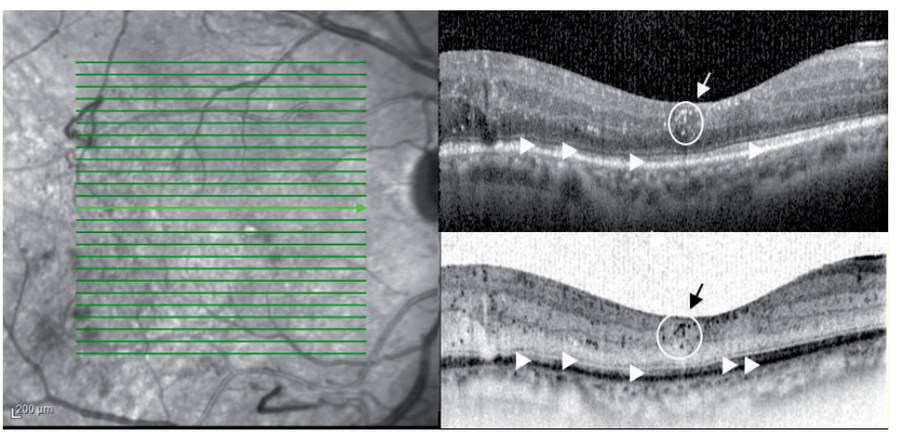

Figure 3.
Spectral domain OCT linear scan in the macula of a diabetic patient with PDR. Right upper image: White-on-black OCT scan. Right lower image: Black-on-white OCT scan. The white arrows head indicate hyperreflective choroidal foci. The white and black arrow highlights a group of hyperreflective retinal foci.

BRB [85]. Damian and al. found decreased RPE thickness in almost all quadrants in the PDR-DMO group as opposed to NPDR-DMO group, where the quadrants number was lower [86]. This finding was explained by a disruption of the RPE-photoreceptors complex due to ischemia and level of inflammation that varies according to the stage of DR. Cysts larger than >200 µm in the outer nuclear layer (ONL) are seen in advanced stages of DME and may cause irreversible damage to visual functions. Due to their location, they damage photoreceptor cells and disrupt inner segment–outer segment (IS/OS) junction (**Figure 4**). Regarding their content, there is paucity of information in the literature. Liang and al. hypothesize that fibrin and other inflammatory cells may fill these spaces [87]. Treatment with anti-VEGF therapy leads to a decrease in the number and size of ONL cysts by decreasing the permeability of the inner BRB. This was associated with improvement in BCVA and microperimetric retinal sensitivity. Hyperreflective signals within the cyst are associated with severe disruption of the BRB [67].

3.2.5 Foveal hyperautofluorescence

Fundus autofluorescence (FAF) is a rapid, noninvasive imaging technique that may give new insights into the evaluation of DME. Short wavelength FAF derives its signal mainly from lipofuscin in the RPE. Long wavelength autofluorescence

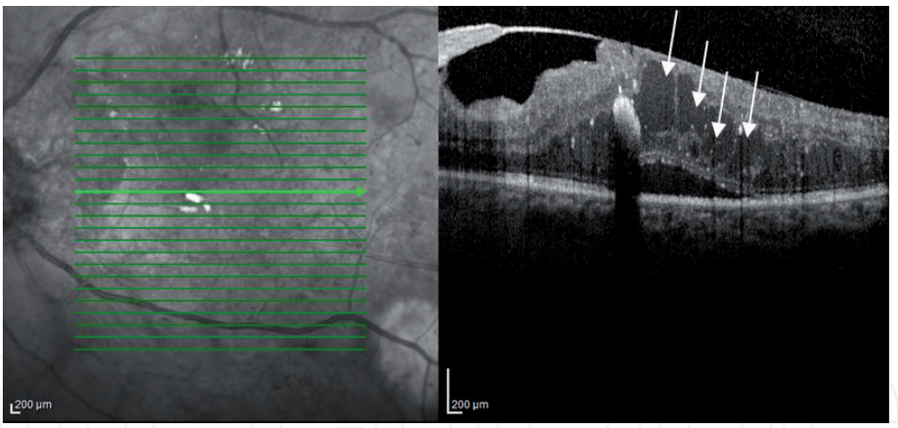

Figure 4.
Spectral domain OCT linear scan in the macula of a diabetic patient with PDR. The white arrows indicate intraretinal cystoid spaces.

or near-infrared (NIR) FAF derives its signal from melanin which is present in RPE and choroid. Melanin accumulates in the apical parts of the RPE cells and is thought to protect the RPE. In DR, local ocular inflammation and oxidative stress lead to increased amount of lipofuscin and decreased amount of lutein and zeaxanthin in the macula. This is responsible for increased FAF signal in subjects with DME. In addition, activation of microglia in DM could cause oxidation of proteins and lipids. Histologic studies have found lipofuscin to accumulate in microglia [67].

4. Potential inflammation-targeting in diabetic retinopathy

Over the years, significant effort has been made to develop new strategies for the treatment of DR by targeting inflammation. Clinical and pre-clinical trials have tested a variety of anti-inflammatory therapeutics.

4.1 Steroids

Intravitreal injection of crystalline cortisone was first reported in 2001 by Jonas et al. [88]. Their mechanism of action consist in repressing pro-inflammatory transcription factors. Several routes of administration were tried but the oral steroids were avoided because of the high risk of DM exacerbation and systemic complications [89]. Tamura et al. found that intravitreal injection of dexamethasone suppresses the leukostasis, the up-regulation of ICAM-1 and also prevents retinal vascular leakage [90]. Since it seems to target different pathways than those targeted by anti-VEGF agents, corticosteroids may improve DME [91]. Especially DME with SRD, no HRF and a continuous ellipsoid zone at the fovea seems to have a better response to injectable dexamethasone implant, as shown by previous studies [92], explained by the increased concentrations of inflammatory cytokines and IL-6 found in the AH and VH of eyes with SRD. In a similar manner, intravitreal triamcinolone has proved to be effective in the resolution of cyst in the CME type of DME [93]. The source of intraretinal cyst is the liquefaction and necrosis of Müller cells which subsequently induces the production of prostaglandins and inflammatory cytokines. The better outcome of steroid might be explained by the reduction of Müller cells' swelling [94]. One study found better results for intravitreal injection of triamcinolone than with anti-VEGF therapy in reducing macular thickness and improving BCVA in patients with SRD but the authors requested

cautious interpretation of the results because of the short follow-up (24 weeks) and the increased risk of associated long-term complications like cataract and elevated intraocular pressure [95].

4.2 NSAIDs

Their anti-inflammatory activity is characterized by inhibiting the production of the two isoforms of cyclooxygenase enzyme-mediated eicosanoid (COX): COX-1 and COX-2. While COX-1 is involved in homeostatic processes, COX-2 produces pro-inflammatory Prostaglandins (PGE), like PGE2 and PGF2α [96]. In the retina of diabetic animal models, increased expression of COX-2 and PGE2 was found, and it seems that COX-2 has a pivotal role in DR pathogenesis since the inhibition of COX-2 but not COX-1 reduced the levels of PGE2 [97]. Retinal inflammatory reactions such as ICAM-1 expression and leukostasis were blocked after COX-2 inhibition [98]. Aspirin, sodium salicylate and sulfasalazine prevented capillary cell apoptosis and vessel degeneration while COX-2 inhibitors reduced vessel degeneration, vascular leakage and capillary cell apoptosis [99–101]. Moreover, retinal microaneurysm development was decreased by high doses of aspirin (900 mg/day), as shown by the Early Treatment DR study and the Dipyridamole Aspirin Microangiopathy of Diabetes Study [102, 103]. While COX-2 inhibitors have the potential to increase the incidence of strokes and heart attacks, their use in clinical trial being discouraged [104], local COX-2 inhibitors have shown reduction of DR sigs similar to systemic COX-2 [105].

4.3 SAR 1118

It is a small-molecule antagonist of leukocyte function-associated antigen (LFA)-1 a cell surface adhesion molecule of the β_2 (CD18) family of integrin receptors expressed on leukocytes, and intercellular adhesion molecule (ICAM)-1, expressed by endothelial cells. SAR 1118 inhibits the binding of LFA-1, to ICAM1–1, being capable of preventing leukocytes adhesion to endothelial cells in vivo [106]. Rao et al. found that SAR 1118 eye drops significantly reduced BRB breakdown and leukostasis in a dose dependent manner [106].

4.4 Etanercept, infliximab

Both drugs block TNF-α induced inflammation. High dose of etanercept reduced leukocyte adhesion and suppressed BRB breakdown, reduced ICAM-1 expression but it did not reduced the expression of CD11a, CD11b and CD18, and neither changed the retinal vascular endothelial growth factor levels [14]. After Infliximab administration, BCVA improved and CMT decreased [107]. But caution should be taken since intravitreal use of TNF-α inhibitors has proved to induce intraocular inflammation [56].

4.5 Anti-CD49a neutralizing antibody

Integrin alpha 4 (CD49d), in complex with integrin beta1, forms very late antigen-4 (VLA-4), which interacts with vascular cell adhesion molecule-1, being involved in leukocyte adhesion. Iliaki et al. showed that injection of an anti-alpha 4 integrin/CD49d neutralizing antibody reduced the diabetes induced upregulation of NF- kappaB activation, VEGF, and TNF-alpha protein levels and significantly reduced diabetes-induced leukocyte adhesion and vascular leakage [108].

4.6 Vitamins C and E

Another key mediator in inflammation is oxidative stress. Vitamin C attenuated the development of acellular capillaries [109] and vitamin E reversed some changes in the retinal vessels of diabetic patients [110].

4.7 Soluble RAGE, LR-90

The receptor for advanced glycation end products (AGEs) has been implicated in the pathogenesis of diabetic complications. When soluble RAGE, a competitor of cellular RAGE for its ligands was administered, neuronal dysfunction and reduced development of capillary lesions was proved by Barile et al. in mouse diabetic models [111].

4.8 Apocynin, statin

NAPDH oxidase activity blocking by apocynin reduces oxidative stress. Previous studies showed that by blocking NADPH oxidase, oxidative stress, retinal inflammation, vessel leakage as well as neovascularization are prevented [5].

4.9 Captopril, Telmisartan, Talsartan, Olmesartan, candesartan, Enalapril

The RAS system is involved in different DR pathways, such as oxidative stress and AGEs. Captopril proved to inhibit capillary degeneration in early stages of DR, while Losartan (an AT1R blocker) and Enalapril (angiotensin converting enzyme inhibitor) reduced the progression of DR by 70% and 65% respectively [112]. Retinal adherent leukocyte was significantly suppressed by telmisartan or valsartan [113].

4.10 Plasma kallikrein inhibitors: KVD001 and THR-149

Plasma kallikrein is involved in both VEGF-mediated and VEGF-independent mechanisms of DME. In a Phase 1B study, KVD001, a novel intravitreous plasma kallikrein selective competitive inhibitor was administered in DME eyes [114]. The authors of the previous study reported that KVD001 was safe and well tolerated while a significant number of eyes experienced a reduction of CRT (central retinal thickness) and BCVA improvement. Another recent Phase 2A study evaluated patients who received either a sham, a high dose or a low dose of 4 intravitreal injections of KVD001 at monthly intervals [115]. There were no statistical differences between the number of gained letters, neither regarding the central subfield thickness (CST) nor the diabetic retinopathy severity scale (DRSS) but only 32.5% of patients from the 6 μg dose group experienced a reduction in vision compared to 54.5% of patients from the sham group ($p = 0.042$) [116]. THR-149 is a reversible peptide inhibitor of plasma kallikrein, by inhibiting the release of bradykinin in the vitreous. Since it is VEGF-independent, anti-VEGF nonresponding patients could benefit from its effect. A Phase 1 study in which 12 patients were followed, proved THR-149 is safe and well tolerated while BCVA improved since day 1 and maintained until month 3 [117].

5. Conclusions

In accordance with the presented studies, multiple local laboratory and imaging biomarkers are involved in the onset and progression of DR, which could support and improve the screening, treatment and follow-up of these patients.

The encouraging positive effects of several tested drugs on BCVA but also on anatomical outcomes are likely to provide a background for further research.

Conflict of interest

The authors declare no conflict of interest.

Author details

Ioana Damian* and Simona Delia Nicoară
University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania

*Address all correspondence to: ioana.damian@umfcluj.ro

References

[1] Diagnosis and Management of Type 2 Diabetes. World Health Organization [Internet]2020. Available from: https://www.who.int/publications/i/item/who-ucn-ncd-20.1.[Accessed: 2021-06-10]

[2] International Diabetes Federation. Diabetes Atlas 9th edition 2019. Available at: https://www.diabetesatlas.org/en/.

[3] Jenkins, A.J., Joglekar, M.V., Hardikar, A.A. et al. Biomarkers in Diabetic Retinopathy. Rev Diabet Stud, 12(1-2): 159-195. DOI: 10.1900/RDS.2015.12.159

[4] Joussen, A. M., Poulaki, V., Le, M. L., Koizumi, K., Esser, C., Janicki, H., et al. A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB J.* 18, 1450-1452. DOI: 10.1096/fj.03-1476fje.

[5] Zhang W, Liu H, Rojas M, Caldwell RW, Caldwell RB. Anti-inflammatory therapy for diabetic retinopathy. Immunotherapy. 2011 May;3(5):609-628. DOI: 10.2217/imt.11.24

[6] McLeod DS, Lefer DJ, Merges C, Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. Am J Pathol. 1995 Sep;147(3):642-53. PMID: 7545873; PMCID: PMC1870979.

[7] Kim SY, Johnson MA, McLeod DS, Alexander T, Hansen BC, Lutty GA. Neutrophils are associated with capillary closure in spontaneously diabetic monkey retinas. Diabetes. 2005 May;54(5):1534-1542. DOI: 10.2337/diabetes.54.5.1534.

[8] Schröder S, Palinski W, Schmid-Schönbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am J Pathol. 1991 Jul;139(1):81-100. PMID: 1713023; PMCID: PMC1886150.

[9] Miyamoto K, Khosrof S, Bursell SE, Rohan R, Murata T, Clermont AC, Aiello LP, Ogura Y, Adamis AP. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. Proc Natl Acad Sci U S A. 1999 Sep 14;96(19):10836-10841. DOI: 10.1073/pnas.96.19.10836.

[10] Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J. 2004 Sep;18(12):1450-1452. DOI: 10.1096/fj.03-1476fje.

[11] Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP, Luscinskas FW, Adamis AP. Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. Invest Ophthalmol Vis Sci. 2000 Apr;41(5):1153-1158. PMID: 10752954.

[12] Yang LP, Sun HL, Wu LM, Guo XJ, Dou HL, Tso MO, Zhao L, Li SM. Baicalein reduces inflammatory process in a rodent model of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009 May;50(5):2319-2327. DOI: 10.1167/iovs.08-2642.

[13] Aveleira CA, Lin CM, Abcouwer SF, Ambrósio AF, Antonetti DA. TNF-α signals through PKCζ/NF-κB to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes. 2010 Nov;59(11):2872-2882. doi: 10.2337/db09-1606.

[14] Joussen AM, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Döhmen S, Adamis AP. Nonsteroidal

anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J. 2002 Mar;16(3): 438-440. DOI: 10.1096/fj.01-0707fje

[15] Miyamoto K, Khosrof S, Bursell SE, Moromizato Y, Aiello LP, Ogura Y, Adamis AP. Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). Am J Pathol. 2000 May;156(5):1733-1739. DOI: 10.1016/S0002-9440(10)65044-4.

[16] Kowluru RA, Odenbach S. Role of interleukin-1beta in the pathogenesis of diabetic retinopathy. Br J Ophthalmol. 2004 Oct;88(10):1343-1347. DOI: 10.1136/bjo.2003.038133

[17] Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? Angiogenesis. 2007;10(3):149-166. DOI: 10.1007/s10456-007-9074-0

[18] Simo-Servat O., Simo, R., Hernandez, C. Circulating Biomarkers of Diabetic Retinopathy: An Overview Based on Physiopathology. Journal of Diabetes Research. 2016. https://DOI.org/10.1155/2016/5263798

[19] Youngblood H, Robinson R, Sharma A, Sharma S. Proteomic Biomarkers of Retinal Inflammation in Diabetic Retinopathy. Int J Mol Sci. 2019 Sep 25;20(19):4755. DOI: 10.3390/ijms20194755.

[20] Kijlstra A, Kuizenga A. Analysis and function of the human tear proteins. Adv Exp Med Biol. 1994;350:299-308. DOI: 10.1007/978-1-4615-2417-5_51

[21] de Souza GA, Godoy LM, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. Genome Biol. 2006;7(8):R72. DOI: 10.1186/gb-2006-7-8-R72.

[22] Aass C, Norheim I, Eriksen EF, Thorsby PM, Pepaj M. Single unit filter-aided method for fast proteomic analysis of tear fluid. Anal Biochem. 2015 Jul 1;480:1-5. DOI: 10.1016/j.ab.2015.04.002

[23] Herber S, Grus FH, Sabuncuo P, Augustin AJ. Two-dimensional analysis of tear protein patterns of diabetic patients. Electrophoresis. 2001 May;22(9):1838-1844. DOI: 10.1002/1522-2683(200105)22:9<1838::AID-ELPS1838>3.0.CO;2-7.

[24] Azzarolo, AM, Brew, K, Kota, S, Ponomareva, O, Schwartz, J, Zylberberg, C. Presence of tear lipocalin and other major proteins in lacrima fluid of rabits. Comparative biochemistry and physiology Part B: Biochemistry and Molecular Biology. 2004. 138(2): 111-117. https://DOI.org/10.1016/j.cbpc.2004.02.012

[25] Eizirik DL, Tracey DE, Bendtzen K, Sandler S. An interleukin-1 receptor antagonist protein protects insulin-producing beta cells against suppressive effects of interleukin-1 beta. Diabetologia. 1991 Jun;34(6):445-448. DOI: 10.1007/BF00403185.

[26] López-Contreras AK, Martínez-Ruiz MG, Olvera-Montaño C, Robles-Rivera RR, Arévalo-Simental DE, Castellanos-González JA, Hernández-Chávez A, Huerta-Olvera SG, Cardona-Muñoz EG, Rodríguez-Carrizalez AD. Importance of the Use of Oxidative Stress Biomarkers and Inflammatory Profile in Aqueous and Vitreous Humor in Diabetic Retinopathy. Antioxidants (Basel). 2020 Sep 20;9(9):891. DOI: 10.3390/antiox9090891. PMID: 32962301;

[27] Costagliola C, Romano V, De Tollis M, Aceto F, dell'Omo R, Romano MR, Pedicino C, Semeraro F. TNF-alpha levels in tears: a novel biomarker to assess the degree of diabetic retinopathy. Mediators Inflamm. 2013;2013:629529. DOI: 10.1155/2013/629529.

[28] Chiang SY, Tsai ML, Wang CY, Chen A, Chou YC, Hsia CW, Wu YF, Chen HM, Huang TH, Chen PH, Liu HT, Shui HA. Proteomic analysis and identification of aqueous humor proteins with a pathophysiological role in diabetic retinopathy. J Proteomics. 2012 Jun 6;75(10):2950-2959. DOI: 10.1016/j.jprot.2011.12006.

[29] Balaiya S, Zhou Z, Chalam KV. Characterization of Vitreous and Aqueous Proteome in Humans With Proliferative Diabetic Retinopathy and Its Clinical Correlation. Proteomics Insights. 2017 Mar 15;8:117864181 6686078. DOI: 10.1177/11786418166 86078.

[30] Zhang H, Liang L, Huang R, Wu P, He L. Comparison of inflammatory cytokines levels in the aqueous humor with diabetic retinopathy. Int Ophthalmol. 2020 Oct;40(10):2763-2769. DOI: 10.1007/s10792-020-01463-9

[31] Kwon JW, Jee D. Aqueous humor cytokine levels in patients with diabetic macular edema refractory to anti-VEGF treatment. PLoS One. 2018 Sep 11;13(9):e0203408. DOI: 10.1371/journal.pone.0203408

[32] Kajdaniuk D, Marek B, Borgiel-Marek H, Kos-Kudła B. Transforming growth factor β1 (TGFβ1) in physiology and pathology. Endokrynol Pol. 2013;64(5):384-396. DOI: 10.5603/EP.2013.0022.

[33] Sun, H., Li, Z., Hu, W. *et al.* Association of serum and aqueous humor myonectin concentrations with diabetic retinopathy. Sci Rep **11,** 7215 (2021). https://DOI.org/10.1038/s41598-021-86677-2

[34] Zhang J, Hu W, Lin P, Wang R. Decreased serum myonectin concentrations in diabetic nephropathy patients. Clin Exp Med. 2020 Nov;20(4):601-607. DOI: 10.1007/s10238-020-00654-z.

[35] Jeon S, Lee WK. Effect of intravitreal triamcinolone in diabetic macular edema unresponsive to intravitreal bevacizumab. Retina. 2014 Aug;34(8):1606-1611. DOI: 10.1097/IAE.0000000000000109.

[36] Moriarty AP, Spalton DJ, Moriarty BJ, Shilling JS, Ffytche TJ, Bulsara M. Studies of the blood-aqueous barrier in diabetes mellitus. Am J Ophthalmol. 1994 Jun 15;117(6):768-771. DOI: 10.1016/s0002-9394(14)70320-4.

[37] Funatsu H, Yamashita H, Noma H, Mimura T, Nakamura S, Sakata K, Hori S. Aqueous humor levels of cytokines are related to vitreous levels and progression of diabetic retinopathy in diabetic patients. Graefes Arch Clin Exp Ophthalmol. 2005 Jan;243(1):3-8. DOI: 10.1007/s00417-004-0950-7

[38] Cheung CM, Vania M, Ang M, Chee SP, Li J. Comparison of aqueous humor cytokine and chemokine levels in diabetic patients with and without retinopathy. Mol Vis. 2012;18:830-7. Epub 2012 Apr 4. PMID: 22511846; PMCID: PMC3327438.

[39] Lei J, Ding G, Xie A, Hu Y, Gao N, Fan X. Aqueous humor monocyte chemoattractant protein-1 predicted long-term visual outcome of proliferative diabetic retinopathy undergone intravitreal injection of bevacizumab and vitrectomy. PLoS One. 2021 Mar 5;16(3):e0248235. DOI: 10.1371/journal.pone.0248235.

[40] Sohn HJ, Han DH, Kim IT, Oh IK, Kim KH, Lee DY, Nam DH. Changes in aqueous concentrations of various cytokines after intravitreal triamcinolone versus bevacizumab for diabetic macular edema. Am J Ophthalmol. 2011 Oct;152(4):686-694. DOI: 10.1016/j.ajo.2011.03.033

[41] Podkowinski D, Orlowski-Wimmer E, Zlabinger G, Pollreisz A,

Mursch-Edlmayr AS, Mariacher S, Ring M, Bolz M. Aqueous humour cytokine changes during a loading phase of intravitreal ranibizumab or dexamethasone implant in diabetic macular oedema. Acta Ophthalmol. 2020 Jun;98(4):e407-e415. DOI: 10.1111/aos.14297.

[42] Gao BB, Chen X, Timothy N, Aiello LP, Feener EP. Characterization of the vitreous proteome in diabetes without diabetic retinopathy and diabetes with proliferative diabetic retinopathy. J Proteome Res. 2008 Jun;7(6):2516-2525. DOI: 10.1021/pr800112g

[43] Hernández C, Garcia-Ramírez M, Simó R. Overexpression of hemopexin in the diabetic eye: a new pathogenic candidate for diabetic macular edema. Diabetes Care. 2013 Sep;36(9):2815-2821. DOI: 10.2337/dc12-2634.

[44] Kim JH, Kim JH, Jun HO, Yu YS, Min BH, Park KH, Kim KW. Protective effect of clusterin from oxidative stress-induced apoptosis in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci. 2010 Jan;51(1):561-566. DOI: 10.1167/iovs.09-3774

[45] Kita T, Clermont AC, Murugesan N, Zhou Q, Fujisawa K, Ishibashi T, Aiello LP, Feener EP. Plasma Kallikrein-Kinin System as a VEGF-Independent Mediator of Diabetic Macular Edema. Diabetes. 2015 Oct;64(10):3588-3599. DOI: 10.2337/db15-0317

[46] Loukovaara S, Nurkkala H, Tamene F, Gucciardo E, Liu X, Repo P, Lehti K, Varjosalo M. Quantitative Proteomics Analysis of Vitreous Humor from Diabetic Retinopathy Patients. J Proteome Res. 2015 Dec 4;14(12):5131-5143. DOI: 10.1021/acs.jproteome.5b00900

[47] Zou C, Han C, Zhao M, Yu J, Bai L, Yao Y, Gao S, Cao H, Zheng Z. Change of ranibizumab-induced human vitreous protein profile in patients with proliferative diabetic retinopathy based on proteomics analysis. Clin Proteomics. 2018 Mar 9;15:12. DOI: 10.1186/s12014-018-9187-z.

[48] Wei Q, Zhang T, Jiang R, Chang Q, Zhang Y, Huang X, Gao X, Jin H, Xu G. Vitreous Fibronectin and Fibrinogen Expression Increased in Eyes With Proliferative Diabetic Retinopathy After Intravitreal Anti-VEGF Therapy. Invest Ophthalmol Vis Sci. 2017 Nov 1;58(13):5783-5791. DOI: 10.1167/iovs.17-22345.

[49] Kowluru RA, Odenbach S. Role of interleukin-1β in the pathogenesis of diabetic retinopathy British Journal of Ophthalmology 2004;**88:**1343-1347. http://dx.DOI.org/10.1136/bjo.2003.038133

[50] Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüssmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH. ARB: a software environment for sequence data. Nucleic Acids Res. 2004 Feb 25;32(4):1363-1371. DOI: 10.1093/nar/gkh293

[51] Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (Lond). 2006 Dec;20(12):1366-1369. DOI: 10.1038/sj.eye.6702138

[52] Murugeswari P, Shukla D, Kim R, Namperumalsamy P, Stitt AW, Muthukkaruppan V. Angiogenic potential of vitreous from Proliferative Diabetic Retinopathy and Eales' Disease patients. PLoS One. 2014 Oct 13;9(10):e107551. DOI: 10.1371/journal.pone.0107551

[53] El-Ghrably IA, Dua HS, Orr GM, Fischer D, Tighe PJ. Intravitreal invading cells contribute to vitreal cytokine milieu in proliferative vitreoretinopathy. Br J Ophthalmol. 2001 Apr;85(4):461-70. DOI: 10.1136/bjo.85.4.461. PMID: 11264138; PMCID: PMC1723908.

[54] Hernández C, Segura RM, Fonollosa A, Carrasco E, Francisco G, Simó R. Interleukin-8, monocyte chemoattractant protein-1 and IL-10 in the vitreous fluid of patients with proliferative diabetic retinopathy. Diabet Med. 2005 Jun;22(6):719-722. DOI: 10.1111/j.1464-5491.2005.01538.x

[55] Abu El-Asrar AM, Struyf S, Kangave D, Geboes K, Van Damme J. Chemokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Eur Cytokine Netw. 2006 Sep;17(3):155-165. PMID: 17194635.

[56] Simó-Servat O, Hernández C, Simó R. Usefulness of the vitreous fluid analysis in the translational research of diabetic retinopathy. Mediators Inflamm. 2012;2012:872978. DOI: 10.1155/2012/872978. Epub 2012 Sep 17. PMID: 23028204; PMCID: PMC3457631

[57] Rangasamy S, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. Middle East Afr J Ophthalmol. 2012 Jan;19(1):52-59. DOI: 10.4103/0974-9233.92116

[58] Heather M. Hatch, Donghang Zheng, Marda L. Jorgensen, and Bryon E. Petersen.Cloning and Stem Cells.Dec 2002.339-351.http://DOI.org/10.1089/153623002321025014

[59] Grant MB, Afzal A, Spoerri P, Pan H, Shaw LC, Mames RN. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opin Investig Drugs. 2004 Oct;13(10):1275-1293. DOI: 10.1517/13543784.13.10.1275.

[60] Butler JM, Guthrie SM, Koc M, Afzal A, Caballero S, Brooks HL, Mames RN, Segal MS, Grant MB, Scott EW. SDF-1 is both necessary and sufficient to promote proliferative retinopathy. J Clin Invest. 2005 Jan;115(1):86-93. DOI: 10.1172/JCI22869.

[61] El-Asrar AM, Nawaz MI, Kangave D, Geboes K, Ola MS, Ahmad S, Al-Shabrawey M. High-mobility group box-1 and biomarkers of inflammation in the vitreous from patients with proliferative diabetic retinopathy. Mol Vis. 2011;17:1829-38. Epub 2011 Jul 6. PMID: 21850157; PMCID: PMC3137555.

[62] El-Asrar AM. Role of inflammation in the pathogenesis of diabetic retinopathy. Middle East Afr J Ophthalmol. 2012 Jan;19(1):70-74. DOI: 10.4103/0974-9233.92118.

[63] Noda K, Nakao S, Ishida S, Ishibashi T. Leukocyte adhesion molecules in diabetic retinopathy. J Ophthalmol. 2012;2012:279037. DOI: 10.1155/2012/279037. Epub 2011 Nov 2. PMID: 22132315; PMCID: PMC3216271.

[64] Murata M, Noda K, Fukuhara J, Kanda A, Kase S, Saito W, Ozawa Y, Mochizuki S, Kimura S, Mashima Y, Okada Y, Ishida S. Soluble vascular adhesion protein-1 accumulates in proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci. 2012 Jun 26;53(7):4055-4062. DOI: 10.1167/iovs.12-9857.

[65] Yoshitake, T., Murakami, T., Suzuma, K. *et al.* Hyperreflective Foci in the Outer Retinal Layers as a Predictor of the Functional Efficacy of Ranibizumab for Diabetic Macular Edema. Sci Rep **10,** 873 (2020). https://DOI.org/10.1038/s41598-020-57646-y

[66] Vujosevic, S., Bini, S., Midena, G., Berton, M., Pilotto, E, Midena, E. Hyperreflective Intraretinal Spots in Diabetics without and with Nonproliferative Diabetic Retinopathy:

An *In Vivo* Study Using Spectral Domain OCT. Journal of Diabetes research. 2013. https://DOI.org/10.1155/2013/491835

[67] Markan A, Agarwal A, Arora A, Bazgain K, Rana V, Gupta V. Novel imaging biomarkers in diabetic retinopathy and diabetic macular edema. Ther Adv Ophthalmol. 2020 Sep 4;12:2515841420950513. DOI: 10.1177/2515841420950513. PMID: 32954207; PMCID: PMC7475787.

[68] Lee H, Jang H, Choi YA, Kim HC, Chung H. Association Between Soluble CD14 in the Aqueous Humor and Hyperreflective Foci on Optical Coherence Tomography in Patients With Diabetic Macular Edema. Invest Ophthalmol Vis Sci. 2018 Feb 1;59(2):715-721. DOI: 10.1167/iovs.17-23042.

[69] Liu S, Wang D, Chen F, Zhang X. Hyperreflective foci in OCT image as a biomarker of poor prognosis in diabetic macular edema patients treating with Conbercept in China. BMC Ophthalmol. 2019 Jul 23;19(1):157. DOI: 10.1186/s12886-019-1168-0.

[70] Bolz M, Schmidt-Erfurth U, Deak G, Mylonas G, Kriechbaum K, Scholda C; Diabetic Retinopathy Research Group Vienna. Optical coherence tomographic hyperreflective foci: a morphologic sign of lipid extravasation in diabetic macular edema. Ophthalmology. 2009 May;116(5):914-920. DOI: 10.1016/j.ophtha.2008.12.039.

[71] Uji A, Murakami T, Nishijima K, Akagi T, Horii T, Arakawa N, Muraoka Y, Ellabban AA, Yoshimura N. Association between hyperreflective foci in the outer retina, status of photoreceptor layer, and visual acuity in diabetic macular edema. Am J Ophthalmol. 2012 Apr;153(4):710-7, 717.e1. DOI: 10.1016/j.ajo.2011.08.041.

[72] Midena E, Pilotto E, Bini S. Hyperreflective Intraretinal Foci as an OCT Biomarker of Retinal Inflammation in Diabetic Macular Edema. Invest Ophthalmol Vis Sci. 2018 Nov 1;59(13):5366. DOI: 10.1167/iovs.18-25611.

[73] Giocanti-Aurégan A, Hrarat L, Qu LM, Sarda V, Boubaya M, Levy V, Chaine G, Fajnkuchen F. Functional and Anatomical Outcomes in Patients With Serous Retinal Detachment in Diabetic Macular Edema Treated With Ranibizumab. Invest Ophthalmol Vis Sci. 2017 Feb 1;58(2):797-800. DOI: 10.1167/iovs.16-20855.

[74] Bianca Gerendas, Christian Simader, Gabor Gy Deak, Sonja Gudrun Prager, Jan Lammer, Sebastian M Waldstein, Michael Kundi, Ursula Schmidt-Erfurth; Morphological parameters relevant for visual and anatomic outcomes during anti-VEGF therapy of diabetic macular edema in the RESTORE trial. Invest. Ophthalmol. Vis. Sci. 2014;55(13):1791

[75] Sophie R, Lu N, Campochiaro PA. Predictors of Functional and Anatomic Outcomes in Patients with Diabetic Macular Edema Treated with Ranibizumab. Ophthalmology. 2015 Jul;122(7):1395-1401. DOI: 10.1016/j.ophtha.2015.02.036.

[76] Sonoda S, Sakamoto T, Yamashita T, Shirasawa M, Otsuka H, Sonoda Y. Retinal morphologic changes and concentrations of cytokines in eyes with diabetic macular edema. Retina. 2014;34(4):741-748. DOI: 10.1097/IAE.0b013e3182a48917.

[77] Sonoda S, Sakamoto T, Shirasawa M, Yamashita T, Otsuka H, Terasaki H. Correlation between reflectivity of subretinal fluid in OCT images and concentration of intravitreal VEGF in eyes with diabetic macular edema. Invest Ophthalmol Vis Sci. 2013 Aug 9;54(8):5367-5374. DOI: 10.1167/iovs.13-12382.

[78] Vujosevic S, Torresin T, Berton M, Bini S, Convento E, Midena E. Diabetic

Macular Edema With and Without Subfoveal Neuroretinal Detachment: Two Different Morphologic and Functional Entities. Am J Ophthalmol. 2017 Sep;181:149-155. DOI: 10.1016/j.ajo.2017.06.026.

[79] De Benedetto U, Sacconi R, Pierro L, Lattanzio R, Bandello F. Optical coherence tomographic hyperreflective foci in early stages of diabetic retinopathy. Retina. 2015 Mar;35(3):449-453. DOI: 10.1097/IAE.0000000000000336

[80] Zhu D, Zhu H, Wang C, Yang D. Intraocular soluble intracellular adhesion molecule-1 correlates with subretinal fluid height of diabetic macular edema. Indian J Ophthalmol. 2014 Mar;62(3):295-298. DOI: 10.4103/0301-4738.111184.

[81] Moon BG, Lee JY, Yu HG, Song JH, Park YH, Kim HW, Ji YS, Chang W, Lee JE, Oh J, Chung I. Efficacy and Safety of a Dexamethasone Implant in Patients with Diabetic Macular Edema at Tertiary Centers in Korea. J Ophthalmol. 2016;2016:9810270. DOI: 10.1155/2016/9810270

[82] Vujosevic S, Toma C, Villani E, Muraca A, Torti E, Florimbi G, Leporati F, Brambilla M, Nucci P, De Cilla' S. Diabetic macular edema with neuroretinal detachment: OCT and OCT-angiography biomarkers of treatment response to anti-VEGF and steroids. Acta Diabetol. 2020 Mar;57(3):287-296. DOI: 10.1007/s00592-019-01424-4.

[83] Damian I, Nicoara, SD. Optical coherence tomography biomarkers of the outer blood-retina barrier in patients with diabetic macular oedema. Journal of Diabetes research. 2020; article ID 8880586. DOI: 10.1155/2020/8880586.

[84] Roy R, Saurabh K, Shah D, Chowdhury M, Goel S. Choroidal Hyperreflective Foci: A Novel Spectral Domain Optical Coherence Tomography Biomarker in Eyes With Diabetic Macular Edema. Asia Pac J Ophthalmol (Phila). 2019 Jul-Aug;8(4):314-318. DOI: 10.1097/APO.0000000000000249

[85] E. M. Hartnett, A. Lappas, D. Darland, J. R. McColm, S. Lovejoy, and P. A. D'Amore, "Retinal pigment epithelium and endothelial cell interaction causes retinal pigment epithelial barrier dysfunction via a soluble VEGF-dependent mechanism," Experimental Eye Research, vol. 77, no. 5, pp. 593-599,2003.)

[86] Damian I, Roman G, Nicoara, SD. Analysis of the choroid and its relationship with the outer retina in patients with diabetes mellitus using binarization techniques based on spectral-domain optical coherence tomography. J. Clin. Med. 2021: 10,210. DOI: 10.3390/jcm10020210.

[87] Liang MC, Vora RA, Duker JS, Reichel E. Solid-appearing retinal cysts in diabetic macular edema: a novel optical coherence tomography finding. Retin Cases Brief Rep. 2013 Summer;7(3):255-258. DOI: 10.1097/ICB.0b013e31828eef49

[88] Jonas JB, Hayler JK, Söfker A, Panda-Jonas S. Intravitreal injection of crystalline cortisone as adjunctive treatment of proliferative diabetic retinopathy. Am J Ophthalmol. 2001 Apr;131(4):468-471. DOI: 10.1016/s0002-9394(00)00882-5.

[89] Cunningham MA, Edelman JL, Kaushal S. Intravitreal steroids for macular edema: the past, the present, and the future. Surv Ophthalmol. 2008 Mar-Apr;53(2):139-149. DOI: 10.1016/j.survophthal.2007.12.005

[90] Tamura H, Miyamoto K, Kiryu J, Miyahara S, Katsuta H, Hirose F, Musashi K, Yoshimura N. Intravitreal injection of corticosteroid attenuates leukostasis and vascular leakage in

experimental diabetic retina. Invest Ophthalmol Vis Sci. 2005 Apr;46(4):1440-4. DOI: 10.1167/iovs.04-0905.

[91] Amoaku WM, Saker S, Stewart EA. A review of therapies for diabetic macular oedema and rationale for combination therapy. Eye (Lond). 2015 Sep;29(9):1115-1130. DOI: 10.1038/eye.2015.110

[92] Zur D, Iglicki M, Busch C, Invernizzi A, Mariussi M, Loewenstein A; International Retina Group. OCT Biomarkers as Functional Outcome Predictors in Diabetic Macular Edema Treated with Dexamethasone Implant. Ophthalmology. 2018 Feb;125(2):267-275. DOI: 10.1016/j.ophtha.2017.08.031

[93] Shimura M, Yasuda K, Nakazawa T, Hirano Y, Sakamoto T, Ogura Y, Shiono T. Visual outcome after intravitreal triamcinolone acetonide depends on optical coherence tomographic patterns in patients with diffuse diabetic macular edema. Retina. 2011 Apr;31(4):748-754. DOI: 10.1097/IAE.0b013e3181f04991.

[94] Reichenbach A, Wurm A, Pannicke T, Iandiev I, Wiedemann P, Bringmann A. Müller cells as players in retinal degeneration and edema. Graefes Arch Clin Exp Ophthalmol. 2007 May;245(5):627-636. DOI: 10.1007/s00417-006-0516-y

[95] Liu Q, Hu Y, Yu H, Yuan L, Hu J, Atik A, Guan M, Li D, Li X, Tang S. Comparison of intravitreal triamcinolone acetonide versus intravitreal bevacizumab as the primary treatment of clinically significant macular edema. Retina. 2015 Feb;35(2):272-279. DOI: 10.1097/IAE.0000000000000300.

[96] Wang D, DuBois RN. Pro-inflammatory prostaglandins and progression of colorectal cancer. Cancer Lett. 2008 Aug 28;267(2):197-203. DOI: 10.1016/j.canlet.2008.03.004. Epub 2008 Apr 11. PMID: 18406516; PMCID: PMC2553688.

[97] Ayalasomayajula SP, Amrite AC, Kompella UB. Inhibition of cyclooxygenase-2, but not cyclooxygenase-1, reduces prostaglandin E2 secretion from diabetic rat retinas. Eur J Pharmacol. 2004 Sep 13;498(1-3):275-278. DOI: 10.1016/j.ejphar.2004.07.046.

[98] Ayalasomayajula SP, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. Eur J Pharmacol. 2003 Jan 5;458(3):283-289. DOI: 10.1016/s0014-2999(02)02793-0

[99] Sun W, Gerhardinger C, Dagher Z, Hoehn T, Lorenzi M. Aspirin at low-intermediate concentrations protects retinal vessels in experimental diabetic retinopathy through non-platelet-mediated effects. Diabetes. 2005 Dec;54(12):3418-3426. DOI: 10.2337/diabetes.54.12.3418

[100] Zheng L, Howell SJ, Hatala DA, Huang K, Kern TS. Salicylate-based anti-inflammatory drugs inhibit the early lesion of diabetic retinopathy. Diabetes. 2007 Feb;56(2):337-345. DOI: 10.2337/db06-0789.

[101] Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. Invest Ophthalmol Vis Sci. 2006 Mar;47(3):1149-1160. DOI: 10.1167/iovs.05-0531

[102] Effects of aspirin treatment on diabetic retinopathy. ETDRS report number 8. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology. 1991 May;98(5 Suppl):757-65. PMID: 2062511

[103] Effect of aspirin alone and aspirin plus dipyridamole in early diabetic retinopathy. A multicenter randomized controlled clinical trial. The DAMAD Study Group. Diabetes. 1989 Apr;38(4):491-8. PMID: 2647556.

[104] Kim SJ, Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. Surv Ophthalmol. 2010 Mar-Apr;55(2):108-133. DOI: 10.1016/j.survophthal.2009.07.005

[105] Kern TS, Miller CM, Du Y, Zheng L, Mohr S, Ball SL, Kim M, Jamison JA, Bingaman DP. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. Diabetes. 2007 Feb;56(2):373-379. DOI: 10.2337/db05-1621.

[106] Rao VR, Prescott E, Shelke NB, Trivedi R, Thomas P, Struble C, Gadek T, O'Neill CA, Kompella UB. Delivery of SAR 1118 to the retina via ophthalmic drops and its effectiveness in a rat streptozotocin (STZ) model of diabetic retinopathy (DR). Invest Ophthalmol Vis Sci. 2010 Oct;51(10):5198-204. DOI: 10.1167/iovs.09-5144. Epub 2010 May 5. PMID: 20445119; PMCID: PMC3066602.

[107] Sfikakis PP, Markomichelakis N, Theodossiadis GP, Grigoropoulos V, Katsilambros N, Theodossiadis PG. Regression of sight-threatening macular edema in type 2 diabetes following treatment with the anti-tumor necrosis factor monoclonal antibody infliximab. Diabetes Care. 2005 Feb;28(2):445-447. DOI: 10.2337/diacare.28.2.445.

[108] Iliaki E, Poulaki V, Mitsiades N, Mitsiades CS, Miller JW, Gragoudas ES. Role of alpha 4 integrin (CD49d) in the pathogenesis of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009 Oct;50(10):4898-4904. DOI: 10.1167/iovs.08-2013.

[109] Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes. 2001 Aug;50(8):1938-1942. DOI: 10.2337/diabetes.50.8.1938

[110] Bursell SE, King GL. Can protein kinase C inhibition and vitamin E prevent the development of diabetic vascular complications? Diabetes Res Clin Pract. 1999 Sep;45(2-3):169-182. DOI: 10.1016/s0168-8227(99)00047-9.

[111] Barile GR, Pachydaki SI, Tari SR, Lee SE, Donmoyer CM, Ma W, Rong LL, Buciarelli LG, Wendt T, Hörig H, Hudson BI, Qu W, Weinberg AD, Yan SF, Schmidt AM. The RAGE axis in early diabetic retinopathy. Invest Ophthalmol Vis Sci. 2005 Aug;46(8):2916-2924. DOI: 10.1167/iovs.04-1409

[112] Mauer M, Zinman B, Gardiner R, Suissa S, Sinaiko A, Strand T, Drummond K, Donnelly S, Goodyer P, Gubler MC, Klein R. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med. 2009 Jul 2;361(1):40-51. DOI: 10.1056/NEJMoa0808400.

[113] Nagai N, Izumi-Nagai K, Oike Y, Koto T, Satofuka S, Ozawa Y, Yamashiro K, Inoue M, Tsubota K, Umezawa K, Ishida S. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. Invest Ophthalmol Vis Sci. 2007 Sep;48(9):4342-4350. DOI: 10.1167/iovs.06-1473. PMID: 17724226.

[114] Sun JK, Maturi RK, Boyer DS, Wells JA, Gonzalez VH, Tansley R, Hernandez H, Maetzel A, Feener EP, Aiello LP. One-Time Intravitreal Injection of KVD001, a Plasma Kallikrein Inhibitor, in Patients with

Central-Involved Diabetic Macular Edema and Reduced Vision: An Open-Label Phase 1B Study. Ophthalmol Retina. 2019 Dec;3(12):1107-1109. DOI: 10.1016/j.oret.2019.07.006

[115] Study of the Intravitreal Plasma Kallikrein Inhibitor, KVD001, in Subjects With Center-involving Diabetic Macular Edema (ciDME). [Internet]. 2020. Available from: https://clinicaltrials.gov/ct2/show/results/NCT03466099. [Accessed: 2021-07-27]

[116] KalVista for DME. [Internet] Available from: https://www.kalvista.com/products-pipeline/kalvista-dme [Accessed: 2021-07-27

[117] Results of a Phase 1, Open-Label, Dose-Escalation Study of THR-149 for the Treatment of DME. [Internet]. 2019. Available from: https://www.oxurion.com/sites/default/files/THR-149-001_%20Retina%20Society%20PDugel_15Sep2019.pdf[Accessed: 2021-07-27]