Minireviews

Cytokines and Growth Factors as Predictors of Response to Medical Treatment in Diabetic Macular Edema

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ABSTRACT

Diabetic macular edema (DME) is the most common cause of visual loss in patients with diabetes. Antivascular endothelial growth factors (anti-VEGF) agents are first-line therapy for DME. Nevertheless, up to 60% of patients (depending on the anti-VEGF drug used) have an inadequate response to anti-VEGF treatment. Several cytokines are increased in aqueous humor of patients with DME. Differences in response to treatment may be related to baseline cytokine levels. Intravitreal corticosteroids may be used as an alternative to anti-VEGF agents. Steroids have a different pharmacological profile and act on different pathophysiologic mechanisms. Their effect on aqueous humor cytokines is different from the effect of anti-VEGF therapy. This review highlights the major cytokines involved in

DME and evaluates whether baseline cytokine levels could be predictors of response to treatment in DME.

SIGNIFICANCE STATEMENT

Antivascular endothelial growth factor (anti-VEGF) agents are efficient as diabetic macular edema (DME) treatment. However, in some cases, DME fails to respond to anti-VEGF intravitreal injections. Changes in cytokine levels after treatment supported the idea that other cytokines than VEGF are implicated in DME pathogenesis and could be predictors of response to anti-VEGF treatment or corticosteroids allowing targeted and individualized therapy guided by cytokine levels.

Introduction

Diabetic macular edema (DME) is the most common cause of visual loss in patients with diabetes, representing an important health problem (Klein, 2007). It was estimated that 6.8% of individuals with diabetes had DME (Yau et al., 2012).

The worldwide prevalence of diabetes is increasing, and diabetic retinopathy (DR) is one important cause of visual loss in the working-age population. One-third of patients with diabetes have signs of DR, and one-third of patients with DR have vision-threatening disease. In type 1 diabetes, proliferative

DR is the most common vision-threatening condition. Otherwise, in type 2 diabetes, DME is the most common cause of visual loss (Bafiq et al., 2015).

DME pathogenesis is multifactorial. A chronic hyperglycemic state is the major abnormality in diabetes mellitus contributing to retinopathy (Das et al., 2015). It activates several signaling pathways leading to the expression of inflammatory markers, including vascular endothelial growth factor (VEGF), other inflammatory cytokines, and increased oxidative stress (Das et al., 2015). As a result, tight junctions breakdown and loss of pericytes and endothelial cells occur, leading to hyperpermeability of the retinal capillary network and consequently to extravasation of fluid, electrolytes, and sometimes macromolecules, increasing the retinal thickness and disruption of the normal retinal architecture (Spaide, 2016).

Although several microvascular and retinal changes in DME have been described, the underlying cellular signaling

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ABBREVIATIONS: BCVA, best-corrected visual acuity; CMT, central macular thickness; DME, diabetic macular edema; DR, diabetic retinopathy; EGF, epidermal growth factor; Flt-3L, FMS-like tyrosine kinase-3 ligand; GRO, growth-related oncogene; HGF, hepatocyte growth factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IP, inducible protein; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; MV, macular volume; PDGF, platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PIGF, placental growth factor; SAA, serum amyloid A; sVEGFR, soluble VEGFR; TGF, transforming growth factor; TNF- α , tumor necrosis factor- α ; VCAM, vascular adhesion molecule; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

pathways are not entirely known. Several cytokines are involved in the DME pathogenesis (Jonas et al., 2012). Some cytokines could be associated with advanced disease and thus may be used as biomarkers for disease severity as well as potential therapeutic targets (Hillier et al., 2017).

VEGF plays a central role in DME pathogenesis (Aiello et al., 1994), and the effect of anti-VEGF agents in the treatment of the disease has been well-demonstrated in clinical trials and validated by current clinical practice. Three anti-VEGF agents are used for the DME treatment. Bevacizumab is a full-length parent antibody and a selective VEGF-A inhibitor. Ranibizumab is a monoclonal antibody fragment that selectively inhibits VEGF-A. Aflibercept is a recombinant fusion protein that blocks VEGF-A, VEGF-B, and placental growth factor (PlGF).

Anti-VEGF agents are safe and significantly improve the visual acuity of patients with DME (Haritoglou et al., 2006; Arevalo et al., 2007; Bandello et al., 2012). Nevertheless, response to anti-VEGF treatment is variable, and many patients do not improve even after several injections. The reasons for this unpredictability are not entirely recognized, representing a significant clinical challenge (Mitchell et al., 2011; Nguyen et al., 2012). One possible explanation is that not only VEGF is implicated in the DME pathogenesis; other numerous inflammatory cytokines and signaling pathways are also implicated (Urias et al., 2017).

Corticosteroids are another option to treat DME. Whereas anti-VEGF agents reduce vascular permeability by acting almost exclusively on VEGF, corticosteroids inhibit numerous inflammatory cytokines and signaling pathways, which contributes to inhibiting leukostasis, improving the integrity of endothelial cells, and decreasing vascular leakage (Tamura et al., 2005; Wang et al., 2008). For these reasons, currently corticosteroids are used as an alternative treatment in nonresponders to anti-VEGF injections. Dexamethasone intravitreal implant and fluocinolone acetonide are both approved for DME treatment (Regillo et al., 2017). Triamcinolone acetonide has also been used in clinical practice and showed efficacy in DME (Elman et al., 2010).

As previously mentioned, the efficacy of DME treatment may be related to aqueous humor concentration of some cytokines. Therefore, it is possible that the baseline levels of these molecules could be used to predict response to therapy (Shimura et al., 2017; Hillier et al., 2018; Kwon and Jee, 2018).

In this study, we review the major cytokines and growth factors involved in DME. We also analyze possible associations between baseline cytokine levels and anatomic and functional response to anti-VEGF injections and corticosteroids to guide a directed and individualized therapy according to the levels of cytokines.

Diabetic Macular Edema Pathophysiology

A chronic hyperglycemic state is the major abnormality in diabetes mellitus contributing to retinopathy. Hyperglycemia activates four major deleterious intracellular metabolic pathways implicated in DME pathogenesis: 1) polyol production, 2) hexosamine pathway, 3) synthesis of advanced glycation end products, and 4) activation of protein kinase C (Brownlee, 2001). Activation of these biochemical pathways results in proteolytic enzymatic degradation and mitochondrial dysfunction. As a consequence of the formation of reactive oxygen species, expression of inflammatory markers and

changes in the extracellular matrix occur (Romero-Aroca et al., 2016). The inner blood-retinal barrier maintains the retinal fluid electrolyte homeostasis. In DME, oxidative stress leads to pericyte loss, breakdown of cell-cell junctions, and vascular endothelial cell apoptosis, which results in blood-retinal barrier dysfunction and, consequently, extravasation of fluid, electrolytes, and possibly larger molecules (Romero-Aroca et al., 2016). Consequently, the accumulation of fluid in retinal layers leads to thickening and disruption of the normal retinal architecture (Das et al., 2015).

Inflammation results in upregulation of cytokines, chemokines, and adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule (VCAM)-1 (Miller and Fortun, 2018). Increased ICAM-1 production by the endothelial cell stimulates leukostasis. Endothelial cell death occurs by leukocyte-induced cell death. Furthermore, increased leukocyte adhesion leads to capillary obstruction and, consequently, to hypoxia. Hypoxia is an important stimulus for VEGF production (Miller and Fortun, 2018) because it increases vascular permeability and breakdown of the blood-retinal barrier (Romero-Aroca et al., 2016).

The breakdown of the blood-retinal barrier is caused by loss of the functional and/or structural integrity of tight junctions between endothelial cells (Romero-Aroca et al., 2016; Daruich et al., 2018). This will lead to the leakage of water, solutes, and proteins into the extracellular space. Several inflammatory mediators are known to interfere with the intercellular junction proteins and junctional complexes (Romero-Aroca et al., 2016; Daruich et al., 2018). For instance, tumor necrosis factor- α (TNF- α) decreases the expression of zonula occludens-1 and claudin-5 and changes their subcellular distribution in bovine retinal endothelial cells (Aveleira et al., 2010). Activation of matrix metalloproteinases (MMPs), particularly MMP-9, can also contribute to retinal pigment epithelium junction protein changes. MMP-9 degrades occludin, as shown in diabetic animal models, inducing the disruption of the tight-junction complex (Giebel et al., 2005). Interleukin (IL)-8 regulates endothelial permeability by downregulation of tight junctions (Yu et al., 2013). IL-6 also contributes to macular edema by inducing the formation of gap junctions between adjacent cells via rearrangement of actin filaments (Maruo et al., 1992). This cytokine also has a direct effect in the function of astrocytes, which give structural support to the capillaries in the retina, thus breaking the blood-retinal barrier (Romero-Aroca et al., 2016). This demonstrates that inflammatory mediators increase the inner retinal vessel permeability, potentially leading to macular edema.

Neurodegeneration also occurs in DME. Free radicals, advanced glycation end products, and inflammatory mediators lead to neuronal apoptosis and glial proliferation, increasing glutamate production and the amount of *N*-methyl-D-aspartate receptors, which constitute another important stimulus for VEGF production (Miller and Fortun, 2018).

Molecular Mediators Associated with Diabetic Macular Edema

Several inflammatory cytokines, growth factors, and metalloproteinases are increased in the aqueous humor of patients with DME.

Growth Factors. The role of VEGF in the DME pathogenesis is well-established. As we know, VEGF is an important

proangiogenic factor. Different retinal cellular types contribute to VEGF production, including the retinal pigment epithelium, pericytes, capillary endothelial cells, astrocytes, and Müller cells. VEGF induces conformational changes in the endothelial cell tight junctions, increasing microvascular permeability (Antcliff and Marshall, 1999; Antonetti et al., 1999; Funatsu et al., 2006).

Platelet-derived growth factor (PDGF), which is expressed in platelets, fibroblasts, and other cell types, promotes cellular proliferation and guides cellular movement (Yu et al., 2018).

Interleukins. IL-6 contributes to the regulation of immune responses and initiation of acute phase reactions (Funatsu et al., 2003). It is produced by endothelial cells, monocytes, B or T lymphocytes, fibroblasts, and glial cells. IL-6 also increases angiogenesis and vascular permeability by promoting VEGF expression (Funatsu et al., 2003).

IL-10 promotes an antigenic response to hypoxia in the eye and is produced by T cells and activated macrophages (Dace et al., 2008; Ghasemi et al., 2012).

Chemokines. IL-8 attracts and activates neutrophils and T lymphocytes because it is also proangiogenic. It is produced by endothelial and glial cells in response to ischemia (Taub et al., 1996; Jo et al., 2003).

Monocyte chemoattractant protein (MCP)-1 stimulates monocyte migration and infiltration of injured vessel walls (Romero-Aroca et al., 2016). Activated monocytes differentiate into macrophages, which secrete cytokines and growth factors, such as VEGF, angiopoietins, TNF- α , ILs, MMP-2, and MMP-9, contributing to the loss of electrolyte retinal homeostasis (Das et al., 2015). In addition, MCP-1 induces angiogenesis due to upregulation of VEGF-A gene expression (Hong et al., 2005). MCP-1 is expressed by microglial cells that are present in increased number around vessels in DME (Romero-Aroca et al., 2016).

Interferon- γ -inducible protein (IP)-10 stimulates monocytes and T lymphocytes into the retina and is produced in a variety of cells in response to interferon- γ and lipopolysaccharide (Ide et al., 2008).

Adhesion Molecules. ICAM-1 potentiates leukostasis, mediates neutrophil adhesion, increases vascular permeability, and promotes capillary closure in response to elevated VEGF levels. ICAM-1 is expressed in the retinal vasculature (Miyamoto et al., 2000).

Levels of Cytokines in Diabetic Macular Edema

Several studies have compared the cytokine levels in the aqueous humor of patients with DME (study group) with that of patients undergoing cataract surgery (control group) (Table 1).

Aqueous humor levels of VEGF, IL-8, and MCP-1 were consistently increased in patients with DME (Roh et al., 2009; Funk et al., 2010; Sohn et al., 2011; Jonas et al., 2012; Yu et al., 2018), whereas results regarding other cytokines are more variable. IL-6 is also increased in patients with DME (Roh et al., 2009; Jonas et al., 2012; Wen et al., 2015), although some studies did not show a statistically significant difference (Sohn et al., 2011; Yu et al., 2018).

Other cytokines significantly elevated in patients with DME are PlGF (Jonas et al., 2012; Kwon and Jee, 2018), ICAM-1 (Jonas et al., 2012), hepatocyte growth factor (HGF) (Jonas et al., 2012; Wen et al., 2015), epidermal growth factor (EGF), monokine induced by interferon (IFN)- γ , plasminogen activator inhibitor 1, VCAM (Jonas et al., 2012), transforming growth

factor (TGF)- β , serum amyloid A (SAA) (Wen et al., 2015), and MMP-9 (Kwon et al., 2016). Results regarding IP-10, MMP-1, and IL-2 were inconsistent.

VEGF, ICAM-1, IL-6, and MCP-1 levels in vitreous samples collected by vitrectomy were significantly higher in patients with DME than in patients with diabetes without DME or in patients who are nondiabetic. Additionally, soluble vascular endothelial growth factor receptor (sVEGFR)-2 and pentraxin-3 levels are significantly higher in patients with DME (Noma et al., 2014). In contrast, pigment epithelium-derived factor (PEDF) levels were reduced (Funatsu et al., 2003, 2005, 2006).

Cytokine Levels as Biomarkers of Disease Severity

As several cytokines were increased in DME, some studies started to evaluate the possibility of cytokines as biomarkers of severity.

VEGF, PlGF- β , ICAM-1, IL-6, IL-8, IL-10, VCAM-1, and MCP-1 aqueous levels were evaluated in patients with DME associated with a central macular thickness (CMT) ≥ 310 μ m. CMT, best-corrected visual acuity (BCVA), and macular volume (MV) were used as outcome measures of disease severity at baseline. CMT and MV were evaluated through spectral domain optical coherence tomography. This study demonstrated that increased levels of ICAM-1 were significantly correlated with superior MV measurements (Hillier et al., 2017). Higher levels of IL-10 were significantly associated with worse visual acuity (Hillier et al., 2017). Although some correlations were established between cytokines and MV and BCVA, the absence of association between cytokines and CMT is possibly a result of the challenging measurement of CMT. In fact, the correct evaluation of CMT requires the identification of the foveal center, which is particularly difficult in diffuse DME (Hillier et al., 2017). Another previous study reported that only ICAM-1 levels were associated with CMT (Jonas et al., 2012). Nevertheless, another study suggested that IL-10 could be associated not only with CMT but also with BCVA (Kwon and Jee, 2018).

A possible limitation pointed to by these studies resides in the fact that analyzed samples were collected from aqueous humor and not from the vitreous humor. In fact, when considering studies that analyzed only vitreous humor samples, a statistically significant correlation between other cytokines, including VEGF, ICAM-1, IL-6, MCP-1, and PEDF levels, and CMT were demonstrated (Funatsu et al., 2009). Another study also demonstrated an association between VEGF and ICAM-1 and CMT, but sVEGFR-2 and pentraxin-3 vitreous levels were not significantly correlated with CMT (Noma et al., 2014).

Cytokine and Growth Factor Level Variation in Response to Anti-VEGF and Corticosteroids Intravitreal Injections

Bevacizumab, ranibizumab, and aflibercept intravitreal injections are the first-line therapy for DME. These agents have been proven to be safe and effective with anatomic and function improvement (Wells et al., 2015). Intravitreal corticosteroids, such as triamcinolone, dexamethasone, and fluocinolone, are effective in reducing the macular edema and improve visual acuity (Gillies et al., 2014); however, they are mostly used as second-line therapy in DME. Dexamethasone intravitreal implant is useful in the treatment of DME resistant to anti-VEGF injections and in vitrectomized eyes.

TABLE 1
Cytokines associated with diabetic macular edema

Cytokine	Kwon et al.	Jonas et al.	Roh et al.	Sohn et al.	Yu et al.	Wen et al.	Funatsu et al.	Kwon et al.	Funk et al.
Interleukins									
IL-1 α		=	=						
IL-1 β	=	=	=						
IL-2	↑	=	=						
IL-3		↑							
IL-4		=	=						
IL-5		=							
IL-6		↑	↑	=	=	↑	↑		=
IL-10	=	=	=						
IL-17	=								
IL-12p70, IL-12p40		=							
Growth factors									
VEGF	↑	↑	↑	↑	↑	↑	↑		=
EGF		↑	=						
HGF		↑				↑			
PlGF	↑	↑							
TGF- α		=							
TGF- β		↑				↑			
PDGF-AA				=	↑				
Chemokines									
MCP-1		↑	↑	↑	↑	↑	↑		↑
MCP-3		=							
IL-8	↑	↑	↑	↑	↑				↑
IP-10		↑		↑	↑				
Adhesion molecules									
ICAM-1		↑					↑		
VCAM		↑							
Others									
IFN- α , IFN- β		=							
IFN- γ		=	=						
TNF- α			=						
PEDF							↓		
MMP-1		=						↑	
MMP-9		↑						↑	
PAI-1		↑							
MIG		↑							
SAA						↑			

(=) No significant differences in cytokine levels between control and DME group; (↑): Cytokine levels increased in DME compared to control group; (↓): Cytokine levels decreased in DME compared to control group. Abbreviations: EGF, epidermal growth factor; HGF, human growth factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IP-10, interferon-inducible protein; MCP, monocyte chemoattractant protein; MIG, monokine induced by IFN- γ ; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor 1; PDGF, Platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PlGF, placental growth factor; SAA, serum amyloid A; TGF, transforming growth factor; TNF, tumour necrosis factor; VCAM, vascular intercellular adhesion molecule; VEGF, vascular endothelial growth factor.¹¹

It also can be considered as first-line option in pseudophakic eyes, in patients with contraindications to anti-VEGF therapy, pregnant women, and patients who are incapable of returning for frequent evaluation (Bafiq et al., 2015).

As far as we know, there are five different studies comparing cytokine levels after treatment with bevacizumab (Roh et al., 2009; Funk et al., 2010; Sohn et al., 2011; Wen et al., 2015; Yu et al., 2018), two studies with ranibizumab (Shiraya et al., 2017; Hillier et al., 2018), and only one study analyzing cytokine levels after treatment with intravitreal aflibercept (Mastropasqua et al., 2018). Furthermore, one study evaluated cytokine level variation after treatment with triamcinolone and another study after combined treatment with triamcinolone plus bevacizumab (Table 2). A recent study evaluated the variation in the following cytokine levels after dexamethasone implant: IL-1 β , IL-3, IL-6, IL-8, IL-10, MCP-1, IP-10, TNF- α , and VEGF (Figueras-Roca et al., 2019). In this study, an anterior chamber sampling was performed at baseline at dexamethasone implant injection time, at cataract surgery 8 weeks afterward, and whenever DME relapsed (Figueras-Roca et al., 2019). IP-10 and MCP-1 aqueous humor levels seem to be related to dexamethasone intraocular action because they decreased after injection and increased when DME relapsed. In addition,

IL-6 and IL-8 may play a role in DME late evolution and clinical relapse beyond dexamethasone effect (Figueras-Roca et al., 2019).

Clinical data about the variation of cytokines and growth factors levels after fluocinolone implant were lacking.

Cytokine Response to Anti-VEGF Therapy. After bevacizumab intravitreal injections, VEGF levels were consistently decreased (Roh et al., 2009; Funk et al., 2010; Wen et al., 2015). However, other cytokines, including IL-6, MCP-1 (Roh et al., 2009; Funk et al., 2010; Wen et al., 2015), IL-8 (Roh et al., 2009; Funk et al., 2010), TGF- β , HGF, basic fibroblast growth factor, and SAA (Wen et al., 2015), did not change significantly.

After ranibizumab treatment with monthly intravitreal injections, ICAM-1, VEGF, PlGF, IL-6, and MCP-1 levels decreased. VEGF levels were reduced by 97%, which was followed by IL-6, PlGF, ICAM-1, and MCP-1 (63%, 51%, 15%, and 6% lower, respectively) (Hillier et al., 2018). Eotaxin-1 levels after two monthly injections of ranibizumab were also significantly decreased (Shiraya et al., 2017).

Additionally, after five monthly injections of aflibercept (2 mg) a significant decrease in VEGF, IP-10, IL-1 β , IL-1RA, IL-5, IL-6, eotaxin, FMS-like tyrosine kinase-3 ligand (Flt-3L), IL-12p40, IL-12p70, and growth-related oncogene (GRO) levels

TABLE 2

Aqueous humor cytokine level changes in response to anti-VEGF and triamcinolone intravitreal injections

Cytokine	Bevacizumab					Ranibizumab		Aflibercept	Triamcinolone	Bevacizumab + Triamcinolone
	Roh et al.	Wen et al.	Funk et al.	Sohn et al.	Yu et al.	Hillier et al.	Shiraya et al.	Mastropasqua et al.	Sohn et al.	Yu et al.
Interleukins										
IL-1 α							=			
IL-1 β							=			
IL-1RA								↓		
IL-4								↓		
IL-5							=	↓		
IL-6	=	=	=	=	=	↓	=	↓	↓	=
IL-10						=		=		
IL-12p70, IL-12p40								↓		
GM-CSF								↑		
Growth factors										
VEGF	↓	↓	↓	↓	↓	↓		↓	↓	↓
EGF								=		
HGF		=								
PIGF						↓				
TGF- β		=				=				
PDGF-AA				=	=				↓	↓
Chemokines										
MCP-1	=	=	=	=	=	↓	=	=	↓	↓
IL-8	=		=	=	=	=	=	=	=	↑
IP-10				=	=		=	↓	↓	=
Eotaxin-1							↓	↓		
MIP-1 α , MIP-1 β							=	=		
Flt-3L								↓		
Fractalkine								↑		
Adhesion molecules										
ICAM-1						↓				
VCAM						=				
Others										
TNF- α							=			
SAA		=								
GRO								↓		

(=) No significant differences in cytokine levels after treatment; Cytokine levels decreased after treatment (↓); Cytokine levels increased after treatment (↑). Abbreviations: EGF, epidermal growth factor; Flt-3L, fms-like tyrosine kinase-3 ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; GRO, growth related oncogene; HGF, human growth factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IP-10, interferon-inducible protein 10; MCP, monocyte chemoattractant protein; MIG, monokine induced by IFN- γ ; MIP - macrophage inflammatory protein; MMP, matrix metalloproteinase; PAI 1, plasminogen activator inhibitor 1; PDGF, Platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PIGF, placental growth factor; SAA, serum amyloid A; TGF, transforming growth factor; TNF, tumour necrosis factor; VCAM, vascular intercellular adhesion molecule; VEGF, vascular endothelial growth factor.

was observed. On the contrary, fractalkine and granulocyte macrophage colony-stimulating factor, two chemokines, increased after therapy (Mastropasqua et al., 2018).

Cytokine Response to Corticosteroid Therapy. One study compared the differences in aqueous humor cytokine concentrations in two groups of patients with DME. The first group was treated with 4-mg intravitreal injection of triamcinolone, whereas the second group was treated with one injection of 1.25 mg of bevacizumab. As expected, a decrease in VEGF levels in both groups was observed. On the other hand, IL-6, IP-10, MCP-1, and PDGF-AA decreased significantly after treatment with triamcinolone but not with bevacizumab. No significant differences were observed in IL-8 levels after both treatments (Sohn et al., 2011).

A different study compared cytokine level variation after intravitreal bevacizumab and after combined treatment with intravitreal bevacizumab plus subtenon triamcinolone injection. Aqueous humor samples were obtained immediately before and 4 weeks after intravitreal injection of 1.25 mg bevacizumab isolated or in association with 20 mg of subtenon triamcinolone. Cytokine levels prior to injection were similar. In the bevacizumab group, only VEGF levels decreased. VEGF level reduction was higher in the bevacizumab group when compared with the bevacizumab plus triamcinolone group

(Yu et al., 2018). No significant differences were found in IL-6, IL-8, IP-10, MCP-1, and PDGF-AA after treatment. Nevertheless, a decrease of MCP-1, PDGF-AA, and VEGF levels and an increase in IL-8 levels were observed 4 weeks after combined therapy. IL-6 and IP-10 levels remained stable after bevacizumab plus triamcinolone group treatment (Yu et al., 2018).

Cytokines and Growth Factors as Predictors of Response to ANTI-VEGF or Corticosteroids Intravitreal Treatment

Although the great majority of patients improved DME after anti-VEGF treatment, some patients fail to respond to this therapy. To identify which patients will not respond to anti-VEGF injections and will benefit of treatment with corticosteroids, recent studies started to evaluate the hypothesis of cytokines and growth factors as predictors of response to anti-VEGF or corticosteroid intravitreal injections (Shimura et al., 2017; Hillier et al., 2018; Kwon and Jee, 2018).

Anti-VEGF Therapy. Hillier et al. (2018) measured VEGF, PIGF, TGF- β 2, ICAM-1, IL-6, IL-8, IL-10, VCAM, and MCP-1 levels in patients with DME. Participants

received ranibizumab, monthly 0.5-mg intravitreal injections for 3 months. Response to treatment was evaluated after the three injections based on MV, CMT, and BCVA (Hillier et al., 2018). This study demonstrated that superior baseline ICAM-1 levels were associated with higher reduction of MV. For instance, MV decreased 0.0379 mm^3 for every additional 100 pg/ml of baseline aqueous ICAM-1. On the contrary, increased VEGF levels at baseline were associated with poor response to treatment. MV increased 0.0731 mm^3 for every additional 100 pg/ml of baseline VEGF (Hillier et al., 2018). At baseline, no other cytokine was associated with MV. Considering possible associations between cytokine levels and CMT, the authors demonstrated that baseline VEGF levels were associated with poor anatomic response measured using CMT. Also, baseline ICAM-1 and CMT 3 months after therapy were not correlated. Besides, BCVA was not associated with any of the analyzed cytokines (Hillier et al., 2018).

Another study demonstrated that increased baseline levels of VEGF, PlGF, sVEGFR-1, MCP-1, ICAM-1, IL-6, and IP-10 were associated with better response to treatment with ranibizumab. In this study, patients were treated with monthly intravitreal injections of ranibizumab (0.5 mg) until CMT was inferior to $300 \text{ }\mu\text{m}$, and for 6 months after, this anatomic point was achieved. The number of injections performed to reach a CMT inferior to $300 \text{ }\mu\text{m}$ ranged from one to six (avg. 2.8 ± 1.9). Besides this, the total number of injections necessary to achieve and maintain this condition averaged 3.2 ± 1.3 over the course of 6 months (Shimura et al., 2017). VEGF, PlGF, VEGFR1 and VEGFR2, MCP-1, ICAM-1, PDGF-AA, IL-6, IL-8, and IP-10 were measured. Patients were separated in six different groups according to the total number of injections necessary to achieve a CMT inferior to $300 \text{ }\mu\text{m}$. No significant differences in BCVA and CMT among the six groups were observed at baseline, but significant differences were detected after 6 months. When comparing the group that received one single injection (good responders) with the group that received six injections (poor responders), significant differences in baseline VEGF, sVEGFR1, PlGF, MCP-1, ICAM-1, IP-10, and IL-6 were observed (Shimura et al., 2017).

Kwon et al. (2018) analyzed baseline aqueous humor IL-1 β , IL-2, IL-8, IL-10, IL-17, PlGF, and VEGF levels after three monthly intravitreal bevacizumab injections. Response to treatment was defined as either CSMT $<300 \text{ }\mu\text{m}$ or a CMT reduction of $50 \text{ }\mu\text{m}$ or more after 1 month. Baseline IL-8 levels were significantly superior in the nonresponders group.

Corticosteroid Therapy. One study evaluated the response to treatment with intravitreal triamcinolone injections in patients who failed to respond to bevacizumab (Jeon and Lee, 2014). Patients enrolled in this study had at least three previous monthly injections with bevacizumab and less than 11% reduction in CMT. Triamcinolone (4-mg) injections were performed at least 2 months after the last bevacizumab injection. Although CMT decreased significantly in the first 2 months of treatment, after 3 months, the difference was not statistically significant. In contrast, BCVA improved significantly at 2 and 3 months of treatment (Jeon and Lee, 2014). The baseline levels of several cytokines, including VEGF, TGF- β 2, IL-2, IL-6, IL-8, and TNF- α , were measured. IL-8 appeared to be an independent factor to better anatomic response after triamcinolone treatment (Jeon and Lee, 2014).

Discussion

Several inflammatory cytokines and growth factors are increased in the aqueous and vitreous humor of patients with DME, suggesting that these molecules play an important role in DME pathogenesis and may be potential therapeutic targets.

Clinical data already demonstrated that anti-VEGF agents induce changes in numerous cytokines and growth factors. However, cytokine level variation is different according to the anti-VEGF therapy used, and this may explain the different efficacy among anti-VEGF agents. After treatment with bevacizumab, only VEGF significantly decreased (Roh et al., 2009; Funk et al., 2010; Wen et al., 2015). By opposition, and although ranibizumab selectively inhibits VEGF-A, several cytokines decreased after its treatment, including VEGF, IL-6, PlGF, ICAM-1, and MCP-1 (Hillier et al., 2018). This highlights the complex interplay between VEGF and other cytokines. The different influence in cytokine levels is a possible explanation for ranibizumab higher potency when compared with bevacizumab. In fact, ranibizumab is superior and effective in patients with poor response after bevacizumab (Hanhart and Chowers, 2015; Wells et al., 2015; Ehrlich et al., 2016, 2018). In turn, aflibercept not only inhibits VEGF-A but also VEGF-B and PlGF. Many studies reported a significant reduction of VEGF, IL-6, IL-5, IL-18, eotaxin, GRO, IL-12p70, IL-12p40, IP-10, Flt-3L, and IL-1RA levels and an increase of fractalkine after aflibercept (Mastropasqua et al., 2018). This intricate relationship between aflibercept and DME signaling molecules is even more complex, which is the reason for its efficacy in previous patients who were nonresponders.

Corticosteroids act in numerous pathways involved in DME pathophysiology. They may be effective in patients who are nonresponders after anti-VEGF treatment (Regillo et al., 2017). In fact, the MEAD study demonstrated the efficacy of dexamethasone intravitreal implant in patients previously treated with anti-VEGF agents (Augustin et al., 2015). Similarly, the FAME study showed that fluocinolone acetonide had better results in terms of BCVA gains than ranibizumab in DME with more than 3 years of persistence (Cunha-Vaz et al., 2014). Results showed a significant decrease in VEGF, MCP-1, PDGF-AA, IL-6, and IP-10 levels after intravitreal triamcinolone injections, but IL-8 levels did not change significantly (Sohn et al., 2011). After combining intravitreal bevacizumab and subtenon triamcinolone, a decrease of VEGF, MCP-1, and PDGF-AA were also observed. On the contrary, IL-6 and IP-10 levels did not change significantly, and interestingly, an increase of IL-8 was observed. This increase in IL-8 levels may be a compensatory response to VEGF suppression (Yu et al., 2018). Interestingly, PDGF-AA was reduced by corticosteroids but not affected by bevacizumab. As far as we know, PDGF-AA levels after ranibizumab or aflibercept injection were not evaluated.

Regarding the eventual hypothesis of cytokines and growth factors as predictors of disease severity, current clinical data demonstrated that only ICAM-1 consistently appeared to be associated with DME severity (Funatsu et al., 2009; Jonas et al., 2012; Hillier et al., 2017). Besides this, elevated baseline ICAM-1 levels were associated with better response to treatment (Shimura et al., 2017; Hillier et al., 2018). For this reason, ICAM-1 baseline levels could be used as a possible predictor of response to anti-VEGF.

It should be noted that the only agent that was found to decrease ICAM-1 was ranibizumab (Hillier et al., 2018), since no other study analyzed the effects of bevacizumab, aflibercept, or triamcinolone on ICAM-1 levels.

Two of the three studies previous described identified an association between higher baseline ICAM-1 levels and a favorable response to intravitreal anti-VEGF injections. All three studies evaluated baseline IL-8 levels, but only one showed that higher levels of IL-8 were correlated with poor response. Results regarding VEGF levels were not consistent.

Even though all studies that evaluated MCP-1 and IL-8 levels before treatment showed an upregulation, the role of these molecules in macular edema is still not clear. MCP-1 may lead to edema by inducing VEGF production as a result of vascular damage induced by hyperglycemia. IL-8 is a neutrophilic chemotactic factor and T-cell activator in the innate immune system; it is increased in the aqueous humor of patients, but it is not clear how it contributes to edema formation. Some theories suggest that IL-8 inflammation induced damage to the blood-retinal barrier, contributing to macular edema (Jonas et al., 2012). Others suggest that the contribution of IL-8 to DME may represent an immune pathophysiology (Owen and Hartnett, 2013). In support of this hypothesis, IL-8 has been shown to be positively correlated with severity of macular edema in the setting of DME but not macular edema resulting from branch retinal vein occlusion (Lee et al., 2012). Thus, IL-8 appears to play a role in the development of DME specifically (Owen and Hartnett, 2013). It is interesting that the known cytokine that does not demonstrate a response to intravitreal steroid or anti-VEGF is IL-8 (Owen and Hartnett, 2013). This suggests that our current therapies are not effectively reducing IL-8 function. In a rabbit model of endotoxin-induced uveitis, the anti-IL-8 antibody decreased the clinical and histologic grade of inflammation (Verma et al., 1999).

IL-6 levels were increased in the majority of the studies. Its levels have been shown to be increased in several number of other retinal diseases, such as retinal vein occlusions and uveitic macular edema. In uveitic macular edema, systemic inhibition of IL-6 has shown benefits (Mesquida et al., 2019). However, an in vitro model has shown that IL-6 can lead to breakdown of the outer retinal blood brain barrier but not of the inner blood-retinal brain barrier (Mesquida et al., 2019). However, even though both barriers are affected in DME, the latter is the main one involved. The impact of IL-6 in DME pathophysiology needs further study. In addition, IL-6 also promotes angiogenesis, specifically via induction of VEGF expression (Funatsu et al., 2003). There may be interplay between these critical pathways, leading to breakdown of the blood-retinal barrier and subsequent DME.

Two studies analyzed IL-10 and showed an association between IL-10 baseline levels and worse BCVA (Hillier et al., 2017) and CMT (Kwon and Jee, 2018); however, a third study failed to show any correlation (Jonas et al., 2012) leaving the question, whereas IL-10 could be used as a disease severity biomarker unanswered. IL-10 levels did not change after treatment with ranibizumab (Hillier et al., 2018) and aflibercept (Mastropasqua et al., 2018), and no study analyzed IL-10 levels after other treatment options.

The role of VEGF as predictor of response to treatment is not completely clarified. Although some authors suggested that increased levels of VEGF are associated with poor response to

ranibizumab, (Hillier et al., 2018) others demonstrated an association between increased baseline VEGF levels and good response to treatment with this agent (Shimura et al., 2017). Given the importance of VEGF in DME pathogenesis, we could expect that patients with increased baseline VEGF levels should have better results after anti-VEGF treatment. However, it is important to notice that elevated baseline VEGF levels could represent a more severe disease and therefore lead to a poorer response.

Further studies are required to understand the relationship between baseline VEGF levels and response to anti-VEGF therapy.

Some limitations to the studies referred in this review must be considered. First, the large number of cytokines analyzed may have led to statistical errors related to multiple testing. Second, almost all studies analyzed samples from aqueous humor since they are easily obtained using a minimally invasive technique. However, vitreous fluid samples would be the ideal method to evaluate the cytokine and growth factor variation after treatment.

Despite this, currently there are no studies that establish with certainty the predictive factors of the therapeutic response to anti-VEGF or corticosteroids.

In conclusion, currently there are not enough clinical data to define which molecules could be related to DME severity and to predict response to anti-VEGF or corticosteroids treatment. In the future, new clinical trials to evaluate these questions would be useful to determine the best option for first-line therapy and to define the criteria for the drug switch.

Authorship Contributions

Participated in research design: Torres-Costa, Alves Valente, Falcão-Reis, Falcão.

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