Diabetic retinopathy (DR) is the leading cause of visual impairment and preventable blindness and represents a significant socioeconomic cost for health care systems worldwide. DR prevalence in the diabetic population is approximately one-third, and 10% have vision-threatening states, such as diabetic macular edema (DME) or proliferative DR (PDR). Because DR is the most common complication of diabetes and is expected to increase from 415 million in 2015 to 642 million by 2040, DR will become an even more serious problem in the future. The potentially substantial worldwide public health burden of DR highlights the importance of searching for new approaches beyond current standards of diabetes care.

The current available treatments for DR (laser photocoagulation, intravitreal injections of corticosteroids or anti-VEGF agents, and vitreo-retinal surgery) are applicable only at advanced stages of the disease and are associated with significant adverse effects. In early stages, the only therapeutic strategies that physicians can offer are a tight control of the risk factors for DR. The principal risk factors for developing DR are diabetes duration, glycemic control, and hypertension, but only the two latter can be druggables.

Although there is no doubt regarding the relationship between the glycemic control and the appearing and progression of DR, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group showed that HbA1c values explained up to 11% of the risk of DR, and that the unexplained 89% of variation in risk is due to elements of the diabetic milieu not captured by mean HbA1c value. Therefore, new diagnostic tools are urgently needed for early detection and monitoring the effects of treatment on DR. In this regard, retinal imaging and circulating biomarkers could be useful in detecting early disease, in identifying those diabetic patients most prone to progressive worsening who ought to be followed more often, and who could obtain the most benefit from these therapies.

A biochemical biomarker is a molecule found in blood or other biological fluid that represents a sign of abnormal process of a condition or a disease. Ideally, a biomarker should be measured in accessible tissues. Biomarkers may help to identify subjects at risk of developing a disease, patients with subclinical disease, or those patients in whom the disease will progress. In addition, they may help to monitor the therapeutic response.

Local and Systemic Inflammatory Biomarkers of Diabetic Retinopathy: An Integrative Approach

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PURPOSE. To review the usefulness of local and systemic inflammatory biomarkers of diabetic retinopathy (DR) to implement a more personalized treatment.

METHODS. An integrated research (from ophthalmologist and diabetologist point of view) of most significant literature on serum, vitreous, and aqueous humor (AH) biochemical biomarkers related to inflammation at early and advanced stages of DR (including diabetic macular edema [DME] and proliferative DR) was performed. Moreover, novel imaging retinal biomarkers of local “inflammatory condition” were described.

RESULTS. Multiple inflammatory cytokines and chemokines are increased in DR in both serum as well as in the eye (vitreous and AH). Nevertheless, local rather than systemic production of proinflammatory cytokines seems more relevant in the pathogenesis of both DR and DME. In the eye, retinal glia cells (macroglia and microglia) together with RPE are major sources of proinflammatory and angiogenic modulators. Retinal imaging allows for noninvasive clinical evaluation of retinal inflammatory response induced by diabetes mellitus.

CONCLUSIONS. Proinflammatory cytokines/chemokines play an essential role in the pathogenesis of DR. Therefore, circulating biomarkers and retinal imaging aimed at assessing inflammation have emerged as useful tools for monitoring the onset and progression of DR. In addition, “liquid biopsy” of AH seems a good option in patients with advanced stages of DR requiring intravitreous injections. This strategy may permit us to implement a more personalized treatment with better visual function outcome. Further evaluation and validation of circulating and local biomarkers, as well as multimodal imaging is needed to gain new insights into this issue.

Keywords: biomarker, inflammation, diabetic retinopathy, diabetic macular edema, aqueous humor
that the circulation biomarkers from the biomarkers obtained by
sampling an eye-related biologic fluid, such as the vitreous or
aqueous humor (AH).

As the retina constitutes a small proportion of total body
weight, a circulating biomarker for DR should be highly
specific to the retina rather than a marker of systemic vascular
disease. In addition, renal function (i.e., creatinine levels or
even better the glomerular filtration rate [GFR]) should be
taken into account when using a biomarker that is significantly
eliminated by glomerular filtration. On this basis, circulating
biomarkers could be useful to detect early disease, to identify
diabetic patients most prone to progressive worsening, in
whom intensified therapy could be prioritized, and to monitor
the effectiveness of new drugs for DR before advanced DR
stages will be developed.

Local biomarkers reflect more directly the pathogenic
events that are taking place in the retina. However, to obtain
a sample of AH, and in particular vitreous fluid, is quite
invasive but could be useful for selecting the best option of
intravitreal treatment (see below). In recent years, the
everging technologies in imaging have opened up a new
concept of “imaging biomarker,” which permits us a better
understanding of the main pathogenic process involved in DR
in a particular patient. The combined information obtained by
imaging and the assessment of local biomarkers is a promising
area that fully connects with the precision medicine.

Most of reported biomarkers are based on the assessment of
molecules involved in the pathogenesis of DR (i.e., advanced
glycation endproducts [AGEs], molecules reflecting basal
membrane turnover, oxidative stress, inflammation and angio-
genesis). Because inflammation has a crucial role in the
pathogenesis of DR, the present review will be focused on
this issue.

**SERUM BIOMARKERS RELATED TO INFLAMMATION**

**Circulating Proinflammatory Cytokines**

Several inflammatory molecules have been proposed as serum
biomarkers of DR. TNF-α has been the proinflammatory
cytokine that has been more consistently related to DR. TNF-α
is a cytokine that promotes the irreversible adhesion of
leukocytes to the endothelium (leukostasis), increases the
production of reactive oxygen species, and is implicated in the
blood-retinal barrier breakdown. Baseline circulating TNF-
α was a predictor of DR incidence, as well as of diabetic
complications progression. In addition, a strong correlation
was observed between serum levels of TNF-α and PDR as
well as a reduction of elevated serum RBP4 in obese children
by lifestyle intervention, have been reported. In addition,
TNF-α has been implicated in the pathogenesis of DR.

**Retinol-Binding Protein 4**

Retinol-binding protein 4 (RBP4) is an adipokine that is
secreted by hepatocytes and adipose tissue and acts as a
transport protein for vitamin A. Circulating levels of RBP4
have been found related to body mass index (BMI), waist-to-hip
ratio, triglyceride levels, systolic blood pressure, and decreased
-high-density lipoprotein cholesterol levels; all of them compo-
nents of the metabolic syndrome. In addition, RBP4 levels have
been associated with insulin resistance in subjects with
obesity, impaired glucose tolerance, or type 2 diabetes mellitus
(T2DM). Furthermore, it has been postulated that elevated
RBP4 contributes to insulin resistance by downregulating
-glucose transporter type 4. There is a close relationship between RBP4 and inflammation. In this regard, a correlation between RBP4 and inflammatory factors, such as C-reactive protein and IL-6, as well as a reduction of elevated serum RBP4 in obese children by lifestyle intervention, have been reported. Moreover, in an animal model overexpressing RBP4, an early-onset microglia activation followed by a progressive retinal
degeneration mediated by an increased expression of pro–IL-18
was observed. Therefore, RBP4 could be involved in the
-inflammatory process of DR and could be considered a
biomarker of early stages of DR. Takebayashi et al. found
elevated RBP4 levels in T2DM patients in comparison with
-nondiabetic subjects, and significant increased levels in patients with PDR versus no DR or nonproliferative DR.
However, this relation was reduced after adjusting for urinary
albumin excretion (UAEx). Moreover, Li et al. found that both
UAEx and serum RBP4 levels were significantly higher in PDR
patients. Nevertheless, other studies have not found any
association between RBP4 levels and DR. There are some factors that could influence RBP4 levels, such as BMI, some drugs (i.e., antidiabetic and hypolipidemic agents), vitamin A deficiency, and GFR. Therefore, further research taking into account these confounding factors
are needed.

In patients with T2DM, there is a low grade of systemic
inflammation and both adipose tissue and macrophages are the
main responsibility of the proinflammatory cytokine produc-
tion. Plasma diffusion favored by the breakdown of the blood-
retinal barrier could participate in the development and
progression of DR but the local synthesis is the most important
source of proinflammatory cytokines/chemokines. In other
words, the proinflammatory cytokines produced by RPE and
glial cells (macrogia and microglia) play a primary role in the
pathogenesis of DR (Fig. 1).

**LOCAL BIOMARKERS RELATED TO INFLAMMATION**

**Proinflammatory Cytokines**

Müller cells are the principal retinal macroglia cells and are
considered to be “the communicator” cells between vessels
and neurons, participating in regulation of neuronal nutrition,
development, and metabolism. Besides their crucial role in
structural neuronal support and signaling, Müller cells regulate
potassium and water homeostasis (by expression of aquaporins,
and in particular aquaporin 4 [AQP4]), participate in the
Local and Serum Inflammatory Biomarkers in DR

In situations such as insulin resistance, obesity, and T2DM, there is a low grade of systemic inflammation with an increase of serum levels of proinflammatory cytokines, which are mainly produced by adipose tissue and macrophages. These circulating cytokines could play a role in the pathogenesis of DR in T2DM. However, the main contributor to the developing of both DR and DME is the intraocular production of proinflammatory cytokines.

**Figure 1.** In situations such as insulin resistance, obesity, and T2DM, there is a low grade of systemic inflammation with an increase of serum levels of proinflammatory cytokines, which are mainly produced by adipose tissue and macrophages. These circulating cytokines could play a role in the pathogenesis of DR in T2DM. However, the main contributor to the developing of both DR and DME is the intraocular production of proinflammatory cytokines.

Microglia cells are the major resident immunocompetent cells in the central nervous system and they share the phenotypic markers of monocytes and macrophages. In DM, microglia cells undergo a shift in the activity phenotype (from so-called “surveying microglia” to “activated microglia”) and change their location in the retina, migrating from the inner to the outer retinal layers. Many signals and modulators can trigger a transformation of microglial cells to the activated (alerted or reactive) state, including complement; antibodies; cytokines; chemokines; neurotrophic factors; surface structures; and DNA/RNA of viral, bacterial, or fungal origin, abnormal endogenous proteins, plasma components, proteins and peptides, neurotransmission-related compounds, ions, and so forth. Recently, a general increase in retinal glia cells of proinflammatory cytokines was documented in the AH in diabetic patients without DR and with mild DR, compared with nondiabetic subjects. In particular, in patients with diabetes with no clinical signs of DR, IFN-γ, IL-1β, IL-6, TNF-α, TGF-β, monocyte chemotactic protein (MCP-1), β-catenin, nitric oxide (NO), cyclooxygenase (COX) 2, prostaglandin E2 (PGE2), inducible NO synthase (iNOS), AGE receptor (RAGE), calcium-binding protein B (S100B), glutamate, D-serin, adenosine triphosphate (ATP), Müller cells react to hyperglycemia by facing a reactive gliosis, with an increase in gli fibrillary acidic protein (GFAP), nestin and vimentin, functional activation, and cellular proliferation. An increase in GFAP has been documented in experimental studies and in diabetic donors. In human ocular fluids, specifically in the AH, GFAP increase has been documented in patients with diabetes with no clinical signs of DR and with nonproliferative DR compared with healthy controls. Moreover, an increase in AQP4 (a channel protein that allows the flow of free water through the cell membrane, regulated by Müller cells) has been documented by histology in animal models in diabetes mellitus (DM). Recently, an increase in AQP4 has been reported in human ocular fluids, specifically in the AH, even in patients with no clinical signs of DR. Therefore, GFAP and AQ4 can be considered as AH biomarkers of Müller cell (activation). Retina macroglial cell activation in DR has also been confirmed by clinical studies using optical coherence tomography (OCT).

Hemopexin

Apart from proinflammatory cytokines, hemopexin has been found overexpressed in diabetic retina, and in vitro studies have shown that increases the permeability of the outer blood-retinal barrier. It is worth mentioning that this effect was detected using a hemopexin dosage in the range detected in the vitreous fluid of patients with DME (50 µg/mL). Hemopexin is the best characterized permeability factor in steroid-sensitive nephrotic syndrome and its infusion induces experimental proteinuria. In addition, hemopexin could be involved in either causing or perpetuating enhanced glomerular permeability in minimal change nephrotic syndrome. T-cell-associated cytokines, like TNF-α, are able to enhance...
hemopexin production in mesangial cells in vitro, and this effect is prevented by corticosteroids. Therefore, it could be postulated that the increase of hemopexin induced by diabetes plays a similar role in the retina, thus contributing to the vascular leakage (hyperpermeability) that is the main pathogenic factor of DME. In fact, dexamethasone significantly reduced the hyperpermeability induced by hemopexin.

**Noninvasive Imaging Retinal Biomarkers of Inflammation in DR and DME**

Recently there has been an increasing interest in determination and validation of noninvasive imaging retinal parameters, as possible biomarkers of local retinal “inflammatory condition” in DR and DME (both prognostic and predictive of treatment response) by using different imaging modalities, but mostly spectral-domain (SD)-OCT and fundus autofluorescence. These imaging biomarkers include subfoveal neuroretinal detachment (SND) and hyperreflective retinal spots/foci (HRS) evaluated on SD-OCT, and foveal hyperautofluorescence (FAF) evaluated on fundus autofluorescence.

DME associated with SND is a specific pattern of DME associated with higher concentration of inflammatory cytokines, specifically IL-6 in the vitreous, when compared with DME without SND. HRS have been recently evaluated in different retinal and choroidal conditions, such as early stages of DR, DME, AMD, and macular edema due to retinal vein occlusion. HRS with specific characteristics, such as dimension <30 μm, reflectivity similar to nerve fiber layer, absence of back-shadowing, and location in both inner and outer retina, were suggested to represent activated aggregates of microglial cells, thus can be considered an imaging biomarker of retinal inflammatory response. An increase in number of HRS was reported in preclinical and early clinical DR, as well as in DME. A higher number of HRS was present in DME with SND versus DME without SND. Also, an increased area of FAF was described in eyes with DME. As HRS correlate inversely with retinal sensitivity determined with microperimetry in DME, it was suggested that HRS may become a new OCT parameter for evaluation of functional efficacy of treatments in center-involving DME.

Apart from these potential biomarkers, a significant increase in the thickness of the inner nuclear layer on SD-OCT (mostly formed by the nuclei of bipolar and Müller cells) has been reported in patients with nonproliferative DR, indicating that this finding may represent a clinical sign of Müller cell activation due to hypertrophy of these cells.

Further evaluation and validation of multimodal imaging biomarkers is needed to gain new insights in DME. Prognostic and predictive biomarkers evaluating both morphology and function, which are minimally invasive, or even better noninvasive, may help in choosing more personalized treatment with better visual function outcome.

**Therapeutic Implications**

The identification of reliable biomarkers of the development and progression of DR will permit us to obtain different phenotypes based on the primary pathogenic mediators and will provide a valuable information that could modulate the therapeutic strategy. In addition, both circulating biomarkers and new imaging techniques can give us useful information for monitoring the response to a specific treatment in an individualized manner. At present, one of the most important therapeutic implications derived from the measurement of molecular biomarkers relies on advanced stages of DR and DME (see below).

**Personalized Treatment Using Intravitreal Injections Based on “Liquid Biopsy”**

VEGF plays a crucial role in the pathogenesis of both DME and PDR. However, it should be noted that in the study by Aiello et al., 36% of diabetic patients with PDR had undetectable levels of VEGF in the vitreous fluid. This finding explains why intravitreal anti-VEGF treatments fail in a significant proportion of patients and reveals that VEGF-independent pathways play a primary role in these patients. Therefore, the development of therapeutic strategies for blocking other growth factors and/or...
proinflammatory cytokines/chemokines seems to be necessary. The use of AH during the first injection could be useful for examining the predominant pathogenic pathway in a personalized manner ("liquid biopsy"). This approach would permit us to select a more rational and probably a more cost-effective treatment (Fig. 3).

**CONCLUSIONS**

The assessment of reliable biomarkers of DR or DME is a challenge to be met. Because inflammation plays an essential role in the pathogenesis of DR, serum biomarkers and retinal imaging aimed at assessing the presence of inflammation have emerged as useful tools for monitoring the appearance and progression of DR. However, further evaluation and validation of serum and multimodal imaging biomarkers is needed to gain new insights into this issue.

Prognostic and predictive biomarkers evaluating both morphology and function may help in choosing more personalized treatment with a better visual function outcome. In this regard, growing evidence suggests the reliability and usefulness of AH humor samples in characterizing the intraocular phenotypes. This approach based on "liquid biopsy" will permit us to optimize the treatment, thus resulting not only in a more efficient treatment but also in one less expensive for health care providers.

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