Identification of biomarkers for diabetic retinopathy

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Chapter 5

Summary, discussion and future perspectives
Diabetic retinopathy (DR) is a leading cause of vision loss in diabetic patients, in particular through diabetic macular edema (DME)\(^1\). DME may develop when the leakage of fluid may exceed clearance in the retina. The pathogenesis of DME is unclear. It is thought that breakdown of the inner endothelial blood-retinal barrier (BRB) and retinal vascular hyperpermeability may lead to retinal thickening\(^2,3\), but less is known about the contribution of cytotoxic processes, neuronal damage and alterations in the vitreous gel in the etiology of DME. Retinal hyperpermeability often demonstrates a low correlation with the extent of retinal thickening\(^4\). Aiello, King and colleagues have discovered VEGF as a major mediator in DME by altering endothelial tight junctions and transcellular flow\(^5\). Although anti-VEGF therapies are effective for DME, about 50% of DME patients do not fully respond or are refractive to anti-VEGF therapy\(^6,7\). Therefore, improving the therapeutic efficacy and the development of new treatments for DME are needed. In addition, advancing knowledge in predicting treatment response in patients with DME that could help to individualize therapies and choose alternative treatments for patients with DME is required. Therefore, in this thesis, we focus on potential novel biomarkers for DR that could contribute to the definition of targets for new therapies and provide more effective management strategies for DR.

Chapter 1 reviews the role of the kallikrein kinin system (KKS) in diabetes and the mechanisms that contribute to KKS activation. The KKS has been implicated as a VEGF independent pathway of DME. Pharmacological inhibition of components of the KKS, which may be a potential new treatment option for DME, are also discussed. The effects of the KKS are primarily mediated by bradykinin (BK). Chapter 1b characterizes the effects of BK on the retinal structure and proteome of the rat retina. Intravitreal injection of BK and/or VEGF was associated with a similar increase in retinal thickening. Proteomic analysis identified pro-inflammatory molecules and plasma proteins that were elevated in BK-injected eyes, including 8 proteins that were previously reported to be increased in the human vitreous DME proteome. This study provided insight into the proteomic changes in the retina that are associated with retinal edema. The findings in chapter 1c suggested a pattern of a decreased prevalence of cardiovascular disease among people with type 1 diabetes with chronic kidney disease and without proliferative DR, suggesting that common protective factors for proliferative DR and cardiovascular disease may exist.

In Chapter 2a we aimed to explore the predictive value of specific OCT patterns and retinal features on visual outcomes and retinal thickness in patients with DME receiving anti-VEGF treatment. We analyzed the prognostic value and accuracy of specific OCT patterns and retinal features in predicting the response of 117 patients with DME to anti-VEGF therapy. Patients with DME with serous pattern at baseline were associated with a potential good response to anti-VEGF therapy. In contrast, DME patients with disorganization of retinal
inner layers (DRIL) were correlated with poorer visual outcome of anti-VEGF therapy. The prognostic value of external limiting membrane (ELM) and inner/outer segment (IS/OS) line integrities was not established. In chapter 2b, we assessed retinal layer thickness on OCT on 1413 eyes of 776 patients across a wide range of age, duration of diabetes, and DR severity. Male gender was strongly associated with increased retinal thickness, with a consistent effect across multiple retinal layers. Longer duration of diabetes was related to thinning of neuroretinal structures. When we compared eyes with no-mild DR to eyes with proliferative DR, the presence of proliferative DR had opposite effects of increased inner versus decreased outer retinal layers. In models adjusting for gender and scatter laser as possible confounders, proliferative DR remained related to increases in inner versus decreases in outer retinal layers. This study provided further insight into the neuroretinal abnormalities in advanced diabetic eye disease.

In Chapter 3a, we studied the prognostic value of selected candidate circulating retina-specific mRNAs, including messenger RNA (mRNA) of retinoschisin, RPE65, rhodopsin, and endothelial progenitor cell (EPC) markers CD34 and CD133 for patients with DME that receive anti-VEGF therapy. Blood samples were collected from 89 patients with DME according to the study protocol of the multicenter, prospective Bevacizumab and Ranibizumab in Diabetic Macular Edema (BRDME) study. Plasma mRNA levels of retinoschisin were negatively correlated with visual acuity and plasma mRNA levels of rhodopsin were positively correlated with visual acuity in patients with DME. Change in retinal thickness between baseline and months 1, 2, and 3 during anti VEGF treatment was associated with mRNA levels of retinoschisin and rhodopsin. However, we found no significant association between mRNA levels of retinoschisin and rhodopsin and outcomes between baseline and month 6. We did not find detectable amounts of plasma EPC CD133 and RPE 65 mRNA in the circulation of DME patients. The studies in chapter 3b discovered that elevated photoreceptor-secreted Retinol Binding Protein 3 (RBP3) in the retina and vitreous of individuals with extreme duration of type 1 diabetes is associated with long-term protection from advanced DR, independent of glycemic control. This represents the first neuroretinal protein identified with potential protective activity against the toxic effects of hyperglycemia on the retinal vasculature, a major cause of DR onset and worsening. Intravitreal injection of RBP3 and its specific overexpression in rodents inhibited actions of VEGF and normalized diabetes-induced capillary permeability, abnormal neural retinal function, retinal thinning and acellular capillaries. These findings identify the first neuroretinal protein to act on retinal vasculature, suggesting numerous therapeutic and diagnostic possibilities.
DISCUSSION AND FUTURE PERSPECTIVES

Optimizing current imaging in DR

In this thesis, we evaluated specific retinal patterns and features in predicting the response of patients with DME that received anti-VEGF therapy using OCT imaging. OCT imaging is widely used in the diagnosis of DME. We have shown that several OCT patterns are associated with different treatment responses of DME patients to anti-VEGF therapy. We found that the presence of subretinal fluid and disorganization of retinal inner layers are predictive OCT parameters of treatment response of DME patients to anti-VEGF therapy. In contrast, we found no statistically significant associations between treatment response external limiting membrane (ELM) and inner/outer segments (IS/OS) integrities. The identification of the integrities of these structures may be influenced by the resolution of the OCT systems. Therefore, identifying these retinal features using next-generation OCT systems for prognostic purposes may be more accurate.

Other imaging modalities in the management of DR include fundus photography and fluorescein angiography (FA). FA has been useful for decades in ophthalmologic practice for evaluating, diagnosing and treating retinal diseases. FA has also been used to classify eyes with DME by proportion of fluorescein leakage as focal or diffuse\textsuperscript{14,15}. However, the use of these classifications in predicting treatment response is limited since the terms are often used without clear definitions and the reproducibility of grading FA has been only moderate\textsuperscript{16}. In addition, there are important side-effects and complications of fluorescein injection, including nausea, vomiting and vasovagal reaction. There seems to be a trend toward decreasing use of FA in the management of DME\textsuperscript{17,18}. In contrast to FA, OCT is a noninvasive imaging modality without significant side effects or complications. Next-generation OCT devices will be developed with higher resolution and sensitivity. While OCT is superior in imaging retinal structures, FA provides essential information about retinal vasculature status and leakage. Optical coherence tomography angiography (OCTA) is a new imaging modality, which has the potential to show retinal structures and retinal vascular plexus. This promising imaging technique visualizes retinal structures at the retinal capillary layer level\textsuperscript{19}. Future studies using OCTA will need to be conducted to better understand pathogenic mechanisms of DME and to identify specific OCTA patterns and retinal features that may help in predicting treatment response in DME.

New biomarkers for DR

Besides characteristics on imaging techniques regularly used in the management of DME, such as OCT, other predictors of treatment response may be found in the peripheral blood of patients with DME. As discussed previously, about half of patients with DME do not fully respond to anti-VEGF therapy\textsuperscript{7,20}. Identifying a biomarker for DME could lead to a better understanding of potential underlying pathogenic mechanisms in non-responders and contribute to
the optimization of the best treatment strategy for patients with DME. Therefore, we explored
the most strongly associated DR biomarkers in DME with a focus on predicting treatment
response. We have demonstrated that circulating retinoschisin and rhodopsin mRNA levels
may have value as biomarkers in patients with DME. Retinoschisin is primarily expressed in
rod and cone photoreceptors and bipolar cells, and maintains the integrity of the inner nuclear
layer and outer plexiform layer\textsuperscript{21}. Loss of retinoschisin function may lead to splitting of the
retina and cystic cavities radiating from the central retina\textsuperscript{22}. Rhodopsin is found in rod photo-
receptor cells and is involved in visual phototransduction\textsuperscript{23}. Prognostic studies are needed to
further elucidate the value of these biomarkers in predicting treatment response in DME. In
addition, it is unknown whether circulating levels of retinoschisin and rhodopsin mRNAs are
general markers of visual acuity or specific for patients with DME. Further research is required
to explore the association between the plasma mRNA levels, corresponding protein levels and
pathogenic mechanisms in DME. The Kallikrein Kinin System (KKS) has been discovered by
Feener and colleagues as a potential VEGF-independent mechanism of DME\textsuperscript{24-29}. Therefore,
the KKS may be an interesting candidate pathway for the identification of non-responders of
anti-VEGF therapy in DME. Previous reports also have suggested that several components
of the KKS may be potential biomarkers in DME\textsuperscript{27,28,30}. Further studies are needed to study
the potential of the KKS as a potential biomarker for DR and DME. Advancing knowledge
in genetics is another field to find new biomarkers for DR. In addition to genetic variation,
epigenetics may mediate the relationship between genotype and environmental factors. The
most important mechanisms of epigenetic changes are DNA methylation, modifications of
histone tails and micro-RNAs\textsuperscript{32-34}. Exploring epigenetics in diabetes has just begun. DME
may develop and progress even after improved glycemic control\textsuperscript{35,36}. Further studies need
to explore the role of epigenetic modifications in DR and DME. Epigenetic modifications are
reversible, thereby enabling the development of new therapeutic targets for DR and DME\textsuperscript{27}.

\textbf{Need for new treatments for DME}

The effects of the KKS pathway are primarily mediated by bradykinin (BK). We showed that
intravitreal injection of BK and VEGF increase retinal thickness, vasodilation and tortuosity
in a similar magnitude and time manner. To date, there is no representative animal model
available for DME. For example, rats lack a macula. Thus, the mechanisms that contribute
to BK-and VEGF-induced retinal thickening and translation to human studies will require
additional studies. Another finding of our study regarding the efficacy of anti-VEGF therapies
in DME was that the improvement in mean visual-acuity letter score of all patients with DME
improved to a lower extent than reported in previous studies. Our study excluded patients
that may confound the interpretation of these study results, such as hypertension or ocular
treatment within 3 months of initiation of the study. The difference in improvement of anti-
VEGF therapy between studies may be related to differences in population, study design,
treatment protocol, and anti-VEGF therapy among other factors. However, these findings
suggest the need for new treatments in patients with DME.


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CURRICULUM VITAE

Ward Fickweiler was born in 1985 in Apeldoorn, the Netherlands. After completing his pre-university education in Schiermonnikoog and Leeuwarden (1997-2003), he moved to the University of Groningen in 2003 to study Medicine. He received his Bachelor’s degree in 2006 and graduated with a Doctor of Medicine (M.D.) in 2009, distinguished with a nomination for Best Scientific Thesis from the University of Groningen, where he subsequently specialized in Ophthalmology. During his clinical years, he developed specific interest in the area of diabetic eye disease and started working on research which resulted in this thesis under supervision of Dr. Anneke Hooymans, Dr. Bruce Wolffenbuttel, and Dr. Leonie Los. In 2013, he spent a year performing basic science research in the laboratory of Dr. Edward Feener at Joslin Diabetes Center, Harvard Medical School, Boston, USA. After finishing the ophthalmology residency training program in the Netherlands in 2016, he returned to Joslin Diabetes Center to further develop his research in diabetic eye disease in the laboratories of Dr. Jennifer Sun, Dr. Lloyd P Aiello and Dr. George King. He received the Mary K. Iacocca Junior Fellowship Award, which is designed to recognize Joslin fellows with an important project and with high potential for future impact in a career in diabetes. The author is married to Suzanne Bergenhenegouwen. Together they have one child (Wout, born in 2018).