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IDENTIFICATION OF BIOMARKERS FOR DIABETIC RETINOPATHY

Ward Fickweiler

Thesis of the University of Groningen

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university of
 groningen

Identification of biomarkers for diabetic retinopathy

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

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Chapter 1

Introduction

DIABETIC RETINOPATHY

Diabetes mellitus is a metabolic disorder that is characterized by high glucose levels which may result in damage to various organs, including the heart, kidneys, and eyes. Several theories by which high glucose levels causes dysfunction of these organs have been proposed, including the aldose reductase, advanced glycation endproduct, reactive oxygen intermediates, and protein kinase C theory¹. Multiple studies have shown a relationship between the degree of glucose levels and the development of complications, including diabetic eye disease. The Diabetes Control and Complication Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have provided evidence that intensive glucose control reduces the risk of visual loss in patients with type 1 and type 2 diabetes, respectively^{2,3}. Despite the growing understanding of the complex processes involved in diabetic complications, diabetic retinopathy (DR) remains a leading cause of vision loss in working-aged people⁴. DR is characterized by capillary closure and retinal vascular hyperpermeability. Early histological signs of DR include the loss of pericytes and capillary basement membrane thickening. These processes may lead to outpouchings of capillaries and the formation of microaneurysms⁵. Micro-aneurysms are frequently the first clinically visible hint of DR during eye examination. The number of micro-aneurysms is associated with DR progression and breakdown of the inner endothelial blood-retinal barrier (BRB), characterized by hard exudates and localized areas of retinal vascular hyperpermeability^{6,7}. The leakage of fluid into the retinal tissue may exceed clearance, leading to a major cause of visual loss in diabetic patients: diabetic macular edema (DME).

DIABETIC MACULAR EDEMA

The pathogenesis of DME is not fully understood. Although the breakdown of the BRB is considered to be a major mechanism in the development of DME, other mechanisms may include capillary non-perfusion, neuronal damage, and alterations of the vitreous gel⁹. Vascular Endothelial Growth Factor (VEGF) is a major mediator of proangiogenic factors which may produce DME by altering endothelial tight junctions and transcellular flow⁵. Intraocular delivery of anti-VEGF therapies are now used widely in the treatment of DME. However, it is estimated that about 50% of DME patients are refractory to anti-VEGF therapies^{10,11}. At present, robust biomarkers to identify which DME patients are potential good and non-responders to anti-VEGF therapies have not been established.

NECESSITY OF BIOMARKERS FOR DR

The global rise in diabetes will result in 552 million individuals being affected by this chronic sight-threatening disease by the year 2030¹². By the year 2050, 1 in 3 persons in the United States are projected to have diabetes, with approximately half having some degree of DR

at any point in time¹³. Thus, ocular complications from diabetes are a major and expanding public health concern. There is a critical need to identify biomarkers for DR¹⁴. Although anti-VEGF agents are effective, there is a need for the substantial proportion of patients who do not respond completely to anti-VEGF therapy¹¹. One difficulty that prevents the development of new treatments is the identification of robust biomarkers of DR that could help define targets for new therapies or provide more effective management strategies¹⁵. Another is the prolonged period needed to assess changes in vision and progression of disease by fundus photography. Associations found to date are generally not sensitive or specific enough to be reliable biomarkers for DR¹⁶.

IMAGING BIOMARKERS FOR DR

Imaging techniques in DR include fundus photography, fluorescein angiography (FA) and optical coherence tomography (OCT). FA visualizes areas of retinal hyperpermeability, but these areas are difficult to quantitate and often show a low correlation with retinal thickening on OCT¹⁷. Retinal features visualized by noninvasive imaging techniques such as OCT may be potential candidates for biomarkers of DME. The definition of a biomarker is ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention’¹⁸. They may be part of the pathophysiological pathway of the endpoint of interest¹⁹. OCT generates high-resolution cross-sectional images by measuring the echo time delay and magnitude of back-reflected light. It is a noninvasive imaging modality that allows quantitative measurements of retinal thickness, as well as evaluation of anatomic lesions different from retinal thickening²⁰. Specific OCT patterns have been reported to be predictive of outcome after anti-VEGF therapy. Patients with cystoid macular edema (CME) pattern have been found to achieve better visual acuity and greater changes in retinal thickness after anti-VEGF therapy, while patients with diffuse pattern have been reported to exhibit a less favorable outcome²¹⁻²³. In addition to specific OCT patterns, anatomic characteristics such as the presence of highly reflective spots, disruption of inner/outer retinal layers and intraretinal cysts have been suggested to be relevant features in predicting the response of DME patients to anti-VEGF therapies²⁴⁻²⁸.

NOVEL BIOMARKERS FOR DR

Aiello, King, and colleagues have identified VEGF as an important mediator in the development of DME by proteomic analysis of the vitreous fluid of patients with DR³³. Other potential mechanisms and biomarkers of DR may be found in other and more accessible fluids, such as the peripheral blood of patients with DR. The increased understanding of the

processes involved in DR has offered approaches to select candidate circulating biomarkers based on pathogenic mechanism and structural damage. Recently, a number of circulating biomarkers associated with pathogenic mechanisms and structural retinal tissue damage of DR have been independently and significantly associated with DR³⁴⁻³⁹. Endothelial progenitor cells are circulating cells that have been suggested to play roles in the regeneration of vessel damage and may be involved in the pathogenesis of DR^{35,36,40,41}. Other candidate biomarkers are related to structural damage of the retina in DR^{34,38,39,42}. However, little is known about the potential value of these biomarkers in patients with DME. In addition to further research investigating the potential of these biomarkers, it is essential to investigate potential VEGF-independent mechanisms of DME, such as the plasma kallikrein kinin system (KKS).

KALLIKREIN KININ SYSTEM

The KKS has been discovered by Feener and colleagues as a potential mediator of BRB loss and VEGF-independent mechanism of DME⁴³⁻⁴⁹. Plasma kallikrein is activated by the coagulation factor XIIa, which is released by the contact system during inflammation and bleeding⁴⁷. Once generated, plasma kallikrein divides high molecular weight kininogen to produce bradykinin, which can stimulate vasoactive hormones (e.g. nitric oxide) that influence the BRB⁴⁸. Components of the KKS, including plasma kallikrein and factor XII, have been identified in the vitreous fluid of patients with DME⁴⁴. These findings suggest that components of the KKS in plasma may be potential biomarkers in patients with DME.

AIM OF THE THESIS

The aim of the thesis is to identify potential novel biomarkers for DR. Identification of potential novel biomarkers for DR could contribute to the definition of targets for new therapies and provide more effective management strategies for DR.

OUTLINE OF THE THESIS

Components of the KKS may be potential biomarkers for DR. In Chapter 1a, we review the role of the KKS in diabetes and the mechanisms that contribute to KKS activation. The physiological actions of components of the KKS are described. The review provides an overview of the factors that have been associated with activation of the KKS and the functions that have been supported by clinical data. In addition, genetic studies of the KKS gene and the biochemical functions are summarized. Future directions and new strategies to monitor KKS activity are also discussed. As discussed above, the biological effects of the KKS are primarily

mediated by bradykinin. Chapter 1b characterizes the effects of bradykinin on retinal structure and proteome of the rat retina. This chapter concludes with Chapter 1c, which examines the associations between DR, kidney disease, and cardiovascular disease by characterizing individuals with type 1 diabetes in the United States and Finland.

The aim of study in Chapter 2 was to examine potential imaging biomarkers for DR. In chapter 2a, we examine the predictive value of OCT patterns and retinal features on visual outcomes and retinal thickness of patients with DME receiving anti-VEGF treatment. This is important, since there are no methods available in clinical practice to assess which patients with DME are good-responders and non-responders to anti-VEGF therapy. Within the prospective, multicenter BRDME (comparing the effectiveness and costs of Bevacizumab to Ranibizumab in patients with Diabetic Macular Edema) study, we evaluated the potential of retinal features visualized by OCT as biomarkers in patients with DME that receive anti-VEGF therapies. Although diabetic neuroretinal abnormalities have been previously described, such as thinning of inner retinal layers⁵⁰, changes in individual retinal layers have not been fully explored across multiple decades of diabetes duration. In chapter 2b, we assessed retinal layer thicknesses on OCT on 1413 eyes of 776 patients across a wide range of age, duration of diabetes, and DR severity.

In Chapter 3a, we determined whether specific circulating biomarkers could be used as potential markers for patients with DME that receive anti-VEGF therapy. The candidate biomarkers were selected based on literature on structural damage and pathogenic mechanism of DR and included mRNA levels of CD34+, CD133+, rhodopsin, RPE65, and retinoschisin. Blood samples were collected at baseline in a cohort of patients from the BRDME study and OCT images and visual acuity were analyzed during anti-VEGF treatment to assess the associations between candidate markers and changes in retinal thickness and visual acuity. Chapter 3b focuses on the retinal and vitreous protein profiles from participants of the Joslin 50-Year Medalist Study. The Joslin Medalist Study characterized over 1000 people with type 1 diabetes duration of 50 years or more. Over 35% of these individuals exhibit only no-mild diabetic retinopathy (DR), independent of glycemic control, suggesting the presence of endogenous protective factors against DR. The goal of the study was to identify these protective factors with a high therapeutic potential to prevent or stop the progression of DR.

1. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414(6865):813-820.
2. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK prospective diabetes study (UKPDS) group. *Lancet*. 1998;352(9131):837-853.
3. Progression of retinopathy with intensive versus conventional treatment in the diabetes control and complications trial. diabetes control and complications trial research group. *Ophthalmology*. 1995;102(4):647-661.
4. Tamayo T, Rosenbauer J, Wild SH, et al. Diabetes in europe: An update. *Diabetes Res Clin Pract*. 2014;103(2):206-217.
5. Klaassen I, Van Noorden CJ, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog Retin Eye Res*. 2013;34:19-48.
6. Ehrlich R, Harris A, Ciulla TA, Kheradiya N, Winston DM, Wirostko B. Diabetic macular oedema: Physical, physiological and molecular factors contribute to this pathological process. *Acta Ophthalmol*. 2010;88(3):279-291.
7. Antcliff RJ, Marshall J. The pathogenesis of edema in diabetic maculopathy. *Semin Ophthalmol*. 1999;14(4):223-232.
8. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet*. 2010;376(9735):124-136.
9. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. *N Engl J Med*. 2012;366(13):1227-1239.
10. Mitchell P, Bandello F, Schmidt-Erfurth U, et al. The RESTORE study: Ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology*. 2011;118(4):615-625.
11. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for diabetic macular edema: Results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology*. 2012;119(4):789-801.
12. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract*. 2011;94(3):311-321.
13. Saaddine JB, Honeycutt AA, Narayan KM, Zhang X, Klein R, Boyle JP. Projection of diabetic retinopathy and other major eye diseases among people with diabetes mellitus: United states, 2005-2050. *Arch Ophthalmol*. 2008;126(12):1740-1747.
14. Simo-Servat O, Simo R, Hernandez C. Circulating biomarkers of diabetic retinopathy: An overview based on physiopathology. *J Diabetes Res*. 2016;2016:5263798.
15. Cunha-Vaz J, Ribeiro L, Lobo C. Phenotypes and biomarkers of diabetic retinopathy. *Prog Retin Eye Res*. 2014;41:90-111.
16. Ting DS, Tan KA, Phua V, Tan GS, Wong CW, Wong TY. Biomarkers of diabetic retinopathy. *Curr Diab Rep*. 2016;16(12):125-016-0812-9.
17. Danis RP, Scott IU, Qin H, et al. Association of fluorescein angiographic features with visual acuity and with optical coherence tomographic and stereoscopic color fundus photographic features of diabetic macular edema in a randomized clinical trial. *Retina*. 2010;30(10):1627-1637.
18. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95.
19. Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS*. 2010;5(6):463-466.
20. Panozzo G, Gusson E, Parolini B, Mercanti A. Role of OCT in the diagnosis and follow up of diabetic macular edema. *Semin Ophthalmol*. 2003;18(2):74-81.
21. Kim BY, Smith SD, Kaiser PK. Optical coherence tomographic patterns of diabetic macular edema. *Am J Ophthalmol*. 2006;142(3):405-412.

22. Shimura M, Yasuda K, Yasuda M, Nakazawa T. Visual outcome after intravitreal bevacizumab depends on the optical coherence tomographic patterns of patients with diffuse diabetic macular edema. *Retina*. 2013;33(4):740-747.
23. Kim NR, Kim YJ, Chin HS, Moon YS. Optical coherence tomographic patterns in diabetic macular oedema: Prediction of visual outcome after focal laser photocoagulation. *Br J Ophthalmol*. 2009;93(7):901-905.
24. Sun JK, Lin MM, Lammer J, et al. Disorganization of the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. *JAMA Ophthalmol*. 2014;132(11):1309-1316.
25. Seo KH, Yu SY, Kim M, Kwak HW. Visual and morphologic outcomes of intravitreal ranibizumab for diabetic macular edema based on optical coherence tomography patterns. *Retina*. 2015.
26. Sun JK, Radwan SH, Soliman AZ, et al. Neural retinal disorganization as a robust marker of visual acuity in current and resolved diabetic macular edema. *Diabetes*. 2015;64(7):2560-2570.
27. Otani T, Kishi S. Tomographic findings of foveal hard exudates in diabetic macular edema. *Am J Ophthalmol*. 2001;131(1):50-54.
28. Nishijima K, Murakami T, Hirashima T, et al. Hyperreflective foci in outer retina predictive of photoreceptor damage and poor vision after vitrectomy for diabetic macular edema. *Retina*. 2014;34(4):732-740.
29. Yanyali A, Bozkurt KT, Macin A, Horozoglu F, Nohutcu AF. Quantitative assessment of photoreceptor layer in eyes with resolved edema after pars plana vitrectomy with internal limiting membrane removal for diabetic macular edema. *Ophthalmologica*. 2011;226(2):57-63.
30. Shin HJ, Lee SH, Chung H, Kim HC. Association between photoreceptor integrity and visual outcome in diabetic macular edema. *Graefes Arch Clin Exp Ophthalmol*. 2012;250(1):61-70.
31. Wilkins JR, Puliafito CA, Hee MR, et al. Characterization of epiretinal membranes using optical coherence tomography. *Ophthalmology*. 1996;103(12):2142-2151.
32. Montero JA, Ruiz-Moreno JM, De La Vega C. Incomplete posterior hyaloid detachment after intravitreal pegaptanib injection in diabetic macular edema. *Eur J Ophthalmol*. 2008;18(3):469-472.
33. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med*. 1994;331(22):1480-1487.
34. Shalchi Z, Sandhu HS, Butt AN, Smith S, Powrie J, Swaminathan R. Retina-specific mRNA in the assessment of diabetic retinopathy. *Ann N Y Acad Sci*. 2008;1137:253-257.
35. Rigato M, Bittante C, Albiero M, Avogaro A, Fadini GP. Circulating progenitor cell count predicts microvascular outcomes in type 2 diabetic patients. *J Clin Endocrinol Metab*. 2015;100(7):2666-2672.
36. Brunner S, Scherthaner GH, Satler M, et al. Correlation of different circulating endothelial progenitor cells to stages of diabetic retinopathy: First in vivo data. *Invest Ophthalmol Vis Sci*. 2009;50(1):392-398.
37. Hu LM, Lei X, Ma B, et al. Erythropoietin receptor positive circulating progenitor cells and endothelial progenitor cells in patients with different stages of diabetic retinopathy. *Chin Med Sci J*. 2011;26(2):69-76.
38. Butt A, Ahmad MS, Powrie J, Swaminathan R. Assessment of diabetic retinopathy by measuring retina-specific mRNA in blood. *Expert Opin Biol Ther*. 2012;12 Suppl 1:S79-84.
39. Hamaoui K, Butt A, Powrie J, Swaminathan R. Concentration of circulating rhodopsin mRNA in diabetic retinopathy. *Clin Chem*. 2004;50(11):2152-2155.

40. Brunner S, Hoellerl F, Schmid-Kubista KE, et al. Circulating angiopoietic cells and diabetic retinopathy in type 2 diabetes mellitus, with or without macrovascular disease. *Invest Ophthalmol Vis Sci.* 2011;52(7):4655-4662.
41. Zerbini G, Maestroni A, Palini A, et al. Endothelial progenitor cells carrying monocyte markers are selectively abnormal in type 1 diabetic patients with early retinopathy. *Diabetes.* 2012;61(4):908-914.
42. Wong A, Merritt S, Butt AN, Williams A, Swaminathan R. Effect of hypoxia on circulating levels of retina-specific messenger RNA in type 2 diabetes mellitus. *Ann N Y Acad Sci.* 2008;1137:243-252.
43. Kita T, Clermont AC, Murugesan N, et al. Plasma kallikrein-kinin system as a VEGF-independent mediator of diabetic macular edema. *Diabetes.* 2015;64(10):3588-3599.
44. 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;7(6):2516-2525.
45. Gao B, Clermont A, Rook S, et al. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. *Nat Med.* 2007;13(2):181-188.
46. Phipps JA, Clermont AC, Sinha S, Chilcote TJ, Bursell SE, Feener EP. Plasma kallikrein mediates angiotensin II type 1 receptor-stimulated retinal vascular permeability. *Hypertension.* 2009;53(2):175-181.
47. Liu J, Clermont AC, Gao B, Feener EP. Intraocular hemorrhage causes retinal vascular dysfunction via plasma kallikrein. *Invest Ophthalmol Vis Sci.* 2013;54(2):1086-1094.
48. Clermont A, Chilcote TJ, Kita T, et al. Plasma kallikrein mediates retinal vascular dysfunction and induces retinal thickening in diabetic rats. *Diabetes.* 2011;60(5):1590-1598.
49. Feener EP, Zhou Q, Fickweiler W. Role of plasma kallikrein in diabetes and metabolism. *Thromb Haemost.* 2013;110(9):434-441.
50. van Dijk HW, Verbraak FD, Kok PH, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci.* 2010;51(7):3660-3665.

Chapter 2

Role of Kallikrein Kinin System and Common Factors in Diabetes Complications