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Development and Validation of New Approaches for the Detection of Sight-Threatening Diabetic Macular Edema

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Development and Validation of New Approaches for the Detection of Sight-Threatening Diabetic Macular Edema

By

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A dissertation submitted in partial satisfaction of the requirements for the degree of

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Abstract

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Clinically significant macular edema (CSME) results from the microvascular complications of diabetes in the central retina of the eye. Diabetes-induced disregulation of the intra- and extra-vascular fluid homeostasis leads to the accumulation of fluid in the retinal tissue. When left untreated, CSME can result in vision loss by disrupting normal metabolic processes, ischemia and by mechanical disruption of the intricate microstructure of the retina. CSME can be succesfully treated when detected in time. Vision loss caused by CSME is largely irreversible and, therefore, the emphasis is on early detection. Challenges in CSME detection result in a significant number of untreated patients with devastating visual consequences. Tele-medicine screening which relies on either monoscopic or stereoscopic fundus photographs is widely implemented and has good sensitivity and specificity for the detection of retinopathy levels. Contrary to that, CSME detection in fundus photographs faces substantial challenges. Stereoscopic photographs require dilation which increases the risk of vision loss due to an elevated intraocular pressure which may lead to angle closre glaucoma and is generally time consuming and incovenient for patients which further decreases compliance with screening. Non-dilated stereophotography suffers from unacceptably high number of stereophotographs of poor quality. Monoscopic images have low sensitivity for CSME detection because of the lack of stereopsis. Programs which use monoscopic images must rely on surrogate markers of CSME. There is currently no consensus on how to detect CSME in monoscopic images. Different programs use different surrogate markers primarily because there is lack of evidence in scientific literature regarding the unequivocal validity of a particular CSME detection method.

This dissertation includes three studies that share a common goal of improving the accuracy of CSME detection. The anticipated impact of this research is that we will be able to improve the efficiency with which we identify patients requiring treatment, thereby increasing their chance for reducing the risk of vision loss due to CSME.

The first study presented in Chapter 3 of this dissertation evaluates the ability of hard exudates, a surrogate marker of DME, located within one-disc diameter from the center of the macula to detect CSME. This study tests the “real world” implementation of this approach in a diabetic retinopathy

screening program and attempts to resolve the discrepancies regarding the accuracy of this method that exists in the literature. The second study presented in Chapter 4 of this dissertation tests whether the proximity of hard exudates to the fovea is associated with more severe cases of CSME and thus warrants a more expedited referral and intervention. In addition, we proposed an OCT-based adaptation of CSME severity scale based on the DME severity scale derived from the Early Treatment Diabetic Retinopathy Study (ETDRS) data. This is a step forward towards developing a more repeatable and objective structural measure of sight-threatening diabetic macular edema which correlates with functional measures of retinal health, such as visual acuity and electroretinographic recordings. Finally, the third study presented in Chapter 5 builds on the findings of the first two studies and utilizes a novel approach for the detection of CSME. This approach is based on the combined measure of the proximity of hard exudates to the fovea and the areal extent of exudation in the central macula. We have also evaluated the association of the photopic 30 Hz flicker ERG measurements with CSME. Finally, the model of CSME detection which combines relevant clinical variables was evaluated as the CSME detection tool.

The results of the studies presented in this dissertation support the use of a new approach – radially arranged sectors in the central macula - for the detection of CSME. This approach was shown to accurately classify patients with and without CSME. Moreover, the increase in the number of sectors affected by hard exudates is also associated with the increase in probability of having severe CSME, justifying a more expedited referral for treatment. Finally, while significantly associated with CSME, the latency of the 30 Hz photopic flicker ERG response does not seem to offer additional power to discriminate between patients with and without CSME.

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Chapter 1: Introduction and Background

1.1 Diabetes Mellitus

1.1.1 Definition and Classification

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia or elevated levels of plasma glucose. The mechanisms leading to hyperglycemia may involve dysregulation in insulin secretion, insulin action or a combination of both[1]. Generally, diabetes mellitus can be classified as type 1, type 2, gestational diabetes, and other specific types[1]. Type 1 DM is an autoimmune condition which is typically diagnosed earlier in life. Type 2 DM is thought to be related to insulin resistance and relative insulin insufficiency and may be undiagnosed for many years. Gestational diabetes refers to glucose intolerance with the onset during pregnancy[1]. Other specific types of diabetes is a category that combines those cases of diabetes that are associated with genetic defects, are chemically-induced or caused by any type of injury to the pancreas[2]. Such secondary causes of diabetes are rare and, ultimately, lead to hyperglycemia, a common causative factor in microvascular complications of all types of diabetes. Therefore, other specific types of diabetes are mentioned but are not discussed in detail.

1.1.2 Epidemiology

Diabetes mellitus is becoming one of the most prevalent diseases worldwide, with well over 300 million people projected to be affected by the year 2030[3]. According to different estimates, between 9% and 14% of adults in the United States have diabetes and almost a third of those are undiagnosed[4,5]. Racial and ethnic differences in diabetes prevalence exist in the U.S[6]. It is estimated that 15.9% of American Indians/Alaska Natives have diabetes. 13.2% of Non-Hispanic Blacks and 12.8% of Hispanics are affected by diabetes. Non-Hispanic Whites and Asian Americans have the lowest prevalence of diabetes - 7.6% and 9%, respectively[7]. Slightly higher prevalence of diabetes is reported in men than women, however, this relationship may not be true in different sub-populations[7,8]. The incidence of diabetes increases with age[7].

Type 1 DM (DM1) accounts for 5% - 15% of all cases of diabetes worldwide and for over 85% of cases in people under 20 years of age[7,9]. It may occur in children as well as adults[10]. The incidence of DM1 varies geographically with the highest incidence in North America and Europe. US has one of the highest rates of new cases of DM1 in the world, with a range of 10-20 cases per 100,000 individuals per year[9,11]. It has been noted that the incidence of DM1 is highest in the 10 - 14 year old age group[9,11]. In contrast with the type 2 DM, prevalence of DM1 is greater among White individuals – 2.55 cases per 1000 – when compared with Black (1.62/10000), Hispanic (1.29/1000), Asian (0.6/1000), and American Indian (0.35/1000) [12].

Type 2 DM (DM2) accounts for 85%-95% of diabetes cases[1,7]. Prevalence of DM2 varies geographically and across the ethnic groups as well as income levels[11]. In general prevalence is lower in the developing countries and in the rural areas. It has been noted that individuals moving from the areas of low prevalence to the areas of high DM2 prevalence are at high risk for developing diabetes. Gene-environment interaction has been suggested to play a role in this

trend[11]. In the United States, low-income urban Hispanics and Pima Indians have the highest prevalence of DM2 of 20% and 50 %, respectively[11]. In addition, DM2, although uncommon in children, is being diagnosed more frequently among African Americans, American Indians, and Hispanics/Latinos[7].

Gestational diabetes complicates 1% - 14% of all pregnancies, depending on the population studied[1,13]. In the U.S., African-American, Hispanic, Asian, and Native American women are at higher risk or GDM than non-Hispanic white women[14].

The economic burden of diabetes in the United States is difficult to overstate with an estimated $245 billion spent in 2012. This includes direct medical costs as well as costs related to disability and inability to work[4].

1.1.3 Pathophysiology and Risk Factors

A brief discussion of the normal glucose metabolism will aid in understanding the pathophysiology of diabetes. Under normal physiologic conditions, the majority of glucose disposal occurs in an insulin-independent tissue, such as brain, liver, and gastrointestinal tract. About 25% of glucose disposal occurs in insulin-dependent tissue, such as muscle and adipose tissue[15]. Post-prandial elevation of plasma glucose stimulates pancreatic beta-cells to release insulin. Elevated levels of insulin cause the suppression of basal endogenous glucose production by liver and kidneys and lead to glucose uptake by the peripheral tissue (muscle and adipose) as well as by liver and gut[15,16]. While the majority on insulin-dependent uptake occurs in the muscle tissue, adipose tissue contributes greatly to the glucose homeostasis by the release of the free fatty acids (FFA)[15,16].

DM1 is caused by the cell-mediated autoimmune destruction of the beta-cells in the pancreas which leads to the functional absence of insulin[2,17]. There is a strong genetic component to DM1 pathogenesis. Polymorphisms of the human leukocyte antigen (HLA) II genes, coding for the major histocompatibility complex (MHC), are the major genetic determinants of the DM1[17]. In addition to the genetic determinants of the disease, environmental factors play a role which is not well defined[9,18]. One of the major risk factors for the development of DM1 is the presence of more than two autoantibodies specific to the molecules required for the normal insulin production and function, such as the islet cells or insulin[18]. Children who test positive for such antibodies have 70% chance of developing DM1 in 10 years and 84% chance of developing DM1 in 15 years[18]. Other risk factors include age, sex, race, geographic location, as well as seasonality. The incidence of diabetes increases from birth through puberty with a peak between the ages of 10-14 years[19,20]. In populations of European origin males are reported to be at higher risk for DM1, while in populations of non-European origin, females are at higher risk[21]. With respect to race and ethnicity as a risk factor, non-Hispanic Whites are at highest risk for the development of DM1[19]. The combination of seasonal and geographic effects has been noted in the literature as a risk factor for the development of DM1[9]. In the United States, children born in the northern latitudes during April-July months were at higher risk for the development of DM1[22]. Similar trend was reported in Ukraine, New Zealand, and Israel[23,24,25]. Such trend was not observed among children born in more southern latitudes in the US, as well as in East Asia and Cuba[9,22].

The cause of DM2 is multifactorial and is characterized by the abnormalities of carbohydrate and fat metaboism[26]. Individuals with DM2 develop peripheral insulin resistance and relative insulin deficiency over time[2,27]. The interplay between the genetic and environmental factors is likely the underlying cause but the exact mechanism remains uclear[26].

In the early stages of the disease, insulin resistance develops but glucose tolerance remains normal due to the compensatory increase in insulin secretion[15]. When plasma glucose reaches a certain level, beta-cells are unable to keep up with the compensatory insulin secretion and the blood glucose levels rise further exacerbated by the fact that the basal hepatic glucose production is no longer suppressed. This is followed by the impaired glucose tolerance and, eventually, overt diabetes[15]. Maturity onset diabetes of youth (MODY) is a subtype of DM2 which is characterized by absolute insulin deficiency with or without decrease in insulin sensitivity. MODY may result from a single gene mutation in one of at least six different genes[27].

The most common cause of insulin resistance and the major risk-factor for the development of DM2 is obesity[27]. Physical inactivity, independent of obesity, has been shown to be a risk factor for the development of DM2[28]. Compared to moderate alcohol intake, high alcohol intake as well as no alcohol intake were shown to be associated with an increased incidence of type 2 diabetes[29]. Other risk factors reported in the analysis of the data gathered during the Nurses' Health Study are smoking or history of smoking and poor diet high in saturated fat, low in fiber with high glycemic index[30]. In addition, positive family history of diabetes was shown to be a strong risk factor for the development of DM2[31].

Women diagnosed with gestational diabetes mellitus (GDM) appear to develop beta-cell dysfunction with underlying chronic insulin resistance being present before the pregnancy. The insulin resistance is likely not due to the defects in the insulin binding receptors on the skeletal muscles and may involve post-receptor signaling pathways[32]. In addition to insulin resistance, fetus-related antigenic load has been hypothesized to play a role in the development of GDM, although this theory has not been completely developed or illucidated[33]. Among the risk factors for the development of GDM are maternal obesity (body mass index or BMI of 25 and above), age older than 25 years, previous history of abnormal glucose metabolism or poor obstetric outcome, family history of diabetes, or being a member of ethnic groups with high prevalence of diabetes[34,35,36].

1.1.4 Diagnostic Criteria

Diagnosis of diabetes is typically established based on hemoglobin A1C criteria or plasma glucose criteria[10]. Type 1 diabetes has a characteristic combination of symptoms and relatively acute onset of blood glucose elevation. In such cases a specific diagnostic threshold of blood glucose is not required[37]. On the other hand, Type 2 diabetes is characterized by insidious onset with blood glucose levels rising gradually. Specific diagnostic cut points are required in such cases to differentiate pathologic hyperglycemia from normal distribution of glucose concentration in nondiabetic population[37]. Historically, metabolic stress tests, such as the 2-hour 75-g oral glucose tolerance test (OGTT) were used for diagnosis of diabetes. The support for the use of blood glucose levels at two hours after the ingestion of glucose for the diagnosis of diabetes was offered by the study of blood glucose levels in Pima Indians[38]. In this study of a high-risk population a bimodal distribution of blood glucose levels was found at 2 hours after the oral ingestion of glucose making

it possible to separate, with reasonable accuracy, patients with and without diabetes[38]. Fasting plasma glucose (FPG) and OGTT are both used for the diagnosis of diabetes. The measure of hemoglobin A1C, in contrast to FPG and OGTT, captures the long-term glycemic exposure and shows better inter-test variability[37]. Presently, hemoglobin A1C, FBG, and OGTT may be used for the diagnosis of diabetes. The following diagnostic criteria are established for diabetes based on these three tests[11,18]:

1) A1C $\geq$ 6.5%. This should be confirmed with a repeat A1C test. The confirmation is not required for symptomatic patients with plasma glucose > 200 mg/dL.
2) 2-h PG $\geq$ 200 mg/dL during an OGTT.
3) FBG $\geq$ 126 mg/dL.

1.1.5 Diabetes Management

The overall treatment goal in patients with diabetes is to prevent the acute complications and reduce the risk of long-term complications of hyperglycemia[39]. Diabetes is a complex, multifactorial disease with a potential for a number of serious complications. A multidisciplinary and holistic approach is required for a successful management of patients with diabetes. Such efforts should include self-management education, addressing modifiable risk factors for the disease progression and complications, screening for complications and comorbidities, as well as the pharmacologic approach for controlling hyperglycemia[39].

Self-management education has been shown to be effective in improving glycemic control and quality of life[40,41]. Modifiable risk factors include smoking, dietary, obesity, lack of exercise. Numerous studies have demonstrated detrimental health effects of smoking in the general population. It has also been reported in epidemiologic literature that estimated 65% of cardiovascular disease death rates were attributable to the interaction between diabetes and smoking[42]. American Diabetes Association (ADA), therefore, recommends not to use cigarettes or other tobacco products, including e-cigarettes[39]. With respect to the dietary modifications, ADA recommends an individualized approach to the medical nutrition therapy to meet individual needs of the patient and improve compliance. General recommendations include portion control, healthy food choices (may be better suited for individuals with DM2), consistent carbohydrate intake for those on a fixed insulin therapy to reduce risk for hypoglycemia, modest weight loss, incorporating cultural and religious beliefs in meal planning, carbohydrate-counting and focus on low-glycemic load foods, individualized recommendations for alcohol, sodium, and fat intake by an expert in medical nutritional therapy[43]. A meta-analysis of 11 randomized and 3 nonrandomized studies evaluating the effect of exercise intervention of 8 or more weeks on the glycemic control and body mass revealed a reduction in hemoglobin A1C of 0.66% in the exercise group but no effect on body mass[44]. Specific recommendations for physical activity include at least 60 min of physical activity each day for children with diabetes and at least 150 min/week of moderate-intensity aerobic physical activity for adults. Adults with DM2 should be encouraged to perform resistance training at least twice per week[39].

At the time of diabetes diagnosis patients should undergo a comprehensive medical evaluation to assess for the presence of comorbidities and complications of diabetes. Patients should be evaluated for the presence of a fatty liver disease which was found to be significantly associated with diabetes in nonalcoholic individuals[45]. Sleep apnea is frequently found in patients with DM2

because of its association with obesity[46,47]. Diabetes, possibly only DM2, is associated with increased risk of liver, pancreas, and endometrial cancer. This association has been attributed to the common risk factors in diabetes and cancer such as older age, obesity, and physical inactivity as well as hyperinsulinemia and hyperglycemia[48]. Patients should also be assessed for the history and risk of fractures since the relative risk of fractures is 6.3 for patients with type 1 diabetes and 1.7 for patients with type 2 diabetes[49]. The rate of cognitive decline and an increased risk of dementia have been reported in patients with diabetes. Age- and sex-adjusted incidence of all-cause dementia was higher in patients with diabetes[50,51,52]. Attention should be given to the cognitive status of patients with diabetes. Complications of diabetes will be discussed in detail later in this chapter.

The standard pharmacologic treatment for individuals with type 1 diabetes is insulin therapy. Diabetes Control and Complications Trial (DCCT) has demonstrated that intensive blood glucose control with three or more daily insulin injections or an external pump delays the onset and slows the progression of vision-threatening retinopathy, neuropathy, and nephropathy[53]. In this study, the median hemoglobin A1C in the intensive control group was maintained at about 7.4% in contrast to 9.1% maintained in conventional control group[53,54]. The ADA recommends a glycemic target for non-pregnant adults of hemoglobin A1C less than 7%[39]. In addition to insulin therapy, other treatment options are available. Pramlintide, an amylin analog, induces weight loss and lowers insulin dose, presumably by delaying gastric emptying, decreasing pancreatic secretion of glucagon, and enhancing satiety[39]. Pancreas and islet cell transplantation is available for a narrow range of clinical scenarios whenever patients are undergoing simultaneous renal transplantation or deal with recurrent ketoacidosis in spite of the best glycemic management efforts[55].

Oral hypoglycemic therapy is recommended for patients with DM2. Metformin, if not contraindicated, is the preferred initial agent[39]. The most compelling evidence in favor of the intensive glycemic control and the use of metformin specifically comes from the United Kingdom Prospective Diabetes Study (UKPDS)[56,57]. If the A1C target is not achieved after approximately three months, a clinician may consider a combination therapy of metformin and either sulfonylurea, thiazolidinedione, DPP-4 inhibitors, SGLT2 inhibitors, GLP-1 receptor agonists, or basal insulin[39]. The glycemic goal for the treatment of DM2 is recommended to be as close to normal blood glucose levels as possible (upper limit of normal A1Cis 6.1%) without causing hypoglycemia[58,59]. It is well recognized that medical treatment of other comorbid conditions such as dyslipidemia, hypertension, hypercoagulability, improves outcomes[58,60].

With respect to the gestational diabetes, the risk of diabetic fetopathy, a result of maternal postprandial hyperglycemia, can be reduced by lowering postprandial response. This is optimally achieved by carbohydrate restriction[36]. When dietary restrictions are insufficient to control blood glucose level, insulin therapy may be of benefit in reducing the risk of fetal overgrowth, shoulder dystocia, cesarean delivery, and hypertensive disorders[61].

While gestational diabetes presents risk for the fetus and for the mother, the occurrence of gestational diabetes in the absence of preexisting diabetes does not increase the risk of diabetic retinopathy, which will be the focus of the work presented in this dissertation[62]. Therefore, the following sections will be most relevant to the issues related to DM1 and DM2.

1.2 Complications of Diabetes Mellitus

Animal model studies as well as the epidemiologic studies implicate hyperglycemia in the pathogenesis of long-term complications of diabetes[63,64,65,66,67,68]. Long-standing hyperglycemia has detrimental effect on almost every part of the body. The focus of this work is on the retinal complications of diabetes but an overview of other ocular and systemic complications of diabetes will be presented to provide a comprehensive picture of the potential complications that threaten the quality of patient's life.

1.2.1 Systemic complications

The chronic hyperglycemia seen in diabetes mellitus is associated with the damage and disruption in normal function of various organs, including kidneys, nerves, eyes, heart, blood vessels[1]. Complications of diabetes can be classified as acute metabolic complications or chronic micro- and macro-vascular complications. The acute metabolic complications include diabetic ketoacidosis (DKA), hyperosmolar non-ketotic coma (HNC), lactic acidosis (LA), and hypoglycemia. DKA and HNC are caused by insulin deficiency while hypoglycemia is a side-effect of treatment for diabetes (mostly related to insulin therapy). LA is frequently associated with cardiovascular disease associated with hypoxia and lactic acid buildup[69]. DKA is defined in clinical terms as absolute insulin deficiency with hyperglycemia (>200 mg/dl), increased ketone production, increased lipolysis, hyperketonemia, and acidosis[69]. DKA is more likely to occur in patients under 30 years of age with incidence estimates ranging from 4.6/1000 person-years to 13.4/1000 person-years[69]. While mild forms of DKA can be managed in the ambulatory care setting, more advanced cases require hospitalization for the initiation of intravenous insulin therapy and correction of the acid-base and electrolyte disturbances[69]. HNC is characterized by the presence of relative insulin deficiency and hyperglycemia (>1000 mg/dl) which is accompanied by elevated serum osmolality, dehydration, and stupor progressing to coma if uncorrected, but without the presence of ketosis or acidosis. This is a rare complication of diabetes and occurs most commonly in patients over the age of 65. Dehydration, steroid medications, acute illness, cerebral vascular disease, may play a role in precipitating HNC. Treatment may involve short hospitalization, hydration, and small doses of insulin[69]. LA is defined by elevated lactic acid with acidosis and without ketoacidosis. LA may be precipitated by hypoxia and phenformin, a biguanide agent. Treatment involves hydration, restoration of electrolyte balance, correction of acidosis and hyperglycemia[69]. Hypoglycemia is a complication of glucose-lowering therapy in patients with diabetes which is more common in patients with more aggressive glycemic control target (A1C of 6.5%)[70,59]. Hypoglycemia may be categorized as mild (glucose levels of 60-70 mg/dl) or severe (glucose levels <40 mg/dl)[69,70]. Hypoglycemia may be associated with a range of symptoms such as sweating, palpitations, cognitive dysfunction, and seizures and can lead to coma and death[70]. In most cases, oral carbohydrates can be used to treat episodes of hypoglycemia but temporary hospitalization may be required in severe cases.

Diabetes-related microvascular disease is the leading cause of blindness, renal failure and nerve damage. Macrovascular complications of diabetes result from atherosclerosis and increase the risk of cardiovascular disease (CVD)[71,72]. A number of mechanisms of hyperglycemia-induced tissue damage have been described in the literature and include an increase in polyol pathway flux,

increase in advanced glycation end products (AGEs), activation of protein kinase C (PKC), accumulation of the reactive oxygen species, and increase in hexosamine pathway flux[71,73,74]. The integrating paradigm of hyperglycemia-induced overproduction of superoxide by the mitochondrial electron-transport chain has been proposed by Brownlee[71], and provides a conceptual framework for understanding the pathophysiologic changes that lead to the micro- and macro-vascular disease in diabetes.

1.2.1.1 Macrovascular complications

Large epidemiologic studies have shown that CVD is the primary cause of death in people with DM1 and DM2[75,76]. Patients with DM2 carry up to 400% increase in the risk of stroke[77]. It is evident that DM1 and DM2 accelerate atherosclerotic changes[78]. Atherosclerosis likely results from chronic diabetes-exacerbated inflammation and injury to the walls of the arteries in the periphery or in the coronary vascular system[78]. The initial step in the formation of atherosclerotic lesions is deposition of the "fatty streak" which are the regions of increased low-density lipoprotein (LDL) concentration within the arterial intima[79,80]. It appears that these lipoproteins accumulate in the intima of the arteries during the ambient state of hypercholesterolemia, a common comorbidity in patients with diabetes, as they bind to the components of extracellular matrix after leaking through the endothelium, thus increasing the residence time of the lipid particles within the extracellular space of the intima. Further binding of LDL to proteoglycan molecules of the subendothelial extracellular matrix makes them particularly susceptible to such chemical modifications as oxidation and nonenzymatic glycation[79,80]. Formation of nonenzymatic glycation products appears to be the common biochemical link between chronic elevated blood glucose levels and long-term diabetic complications[81]. The result of such modifications is the formation of hydroperoxides, lysophospholipids, oxysterols, and aldehydic breakdown products of fatty acids and marks the initiation of inflammatory process. Arterial endothelial cells begin to secrete vascular cell adhesion molecules (VCAM-1), intercellular adhesion molecules (ICAM-1) as well as chemokines, such as CCL2. Various cytokines such as cytokines interleukin (IL-1) and tumor necrosis factor (TNF-alpha) can upregulate the expression of VCAM-1. This promotes the adhesion of leukocytes and activated platelets to the endothelium resulting in the release of even more chemokines to increase immune cell infiltration. Recruitment of monocytes, T-cells and dendritic cells occurs. Once inside the intima layer of the artery, monocytes undergo differentiation into macrophages and engage in receptor-mediated endocytosis of lipoprotein particles which results in transformation of macrophages into lipid-filled foam cells[78,80,82]. Foam cell formation occurs when lipid droplet formation in the macrophage cytoplasm exceed the efflux which suggests that this process is concentration dependent. Retention of foam cells leads to the accumulation of smooth muscle cells and extracellular matrix within the intima with the formation of a fibrous cap which overlies a necrotic lipid core – a result of apoptotic foam cells. These lesions can become unstable and rupture, resulting in clinical manifestations of CVD[78]. In addition to atherosclerotic changes, there is evidence of hypercoagulability and impaired fibrinolysis in DM2. This combination likely further increases the risk of cardiovascular events in DM2[83].

1.2.1.2 Diabetic nephropathy

Diabetic nephropathy is defined by proteinuria > 500 mg in 24 hours in the presence of diabetes and is preceded by microalbuminuria[72]. Diabetic nephropathy can be staged based on the values of urinary albumin excretion set forth by the ADA[84]. Cumulative incidence of microalbuminuria

in patients with DM1 was reported to be 12.6% over 7.3 years by the European Diabetes Prospective Complications Sutdy[85]. UKPDS reported the incidence of microalbuminuria to be 2% per year and the prevalence at 10 years of 25%[86]. Albuminuria is caused by the changes in the kidney structure induced by chronic hyperglycemia with underlying mechanism of injury similar to that seen in diabetic retinopathy[72]. Structural changes include increase in glomerular basement membrane width, diffuse mesangial sclerosis, hyalinosis, microaneurysms, and hyaline arteriosclerosis[87]. Diabetic nephropathy is the leading cause of renal failure in the United States. It increases the risk of death from cardiovascular disease and, therefore, prevention and treatment efforts are needed to decrease the morbidity[72,87]. The best strategy for prevention or controlling nephropathy is to maintain lowest safe glucose levels[72]. In addition to the glucose lowering therapy, aggressive blood pressure control is recommended. Renin-angiotensin system blockade has been shown to have nephroprotective effects beyond the effect expected from blood pressure lowering alone. The reduction in risk of progression to microalbuminuria attributable to the effects of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) may be as much as 60-70%[72].

1.2.1.3 Diabetic neuropathy

The diabetic neuropathies are heterogeneous and present with diverse manifestations. They may be classified as focal or diffuse. One of the most common types is diabetic peripheral neuropathy (DPN)[77]. It is defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes[72]. Symptoms of DPN may be rather unpleasant: pain, tingling, burning, and numbness, although up to 50% of patients may be asymptomatic, increasing the risk of injury to the feet which may lead to the amputation[72,77]. Once again, the mechanism of neural injury is likely related to accumulation of AGEs, oxidative stress, and polyol accumulation[72]. Another type of diabetic neuropathy is autonomic neuropathy which can manifest as cardiovascular autonomic neuropathy (CAN), gastroparesis, constipation, diarrhea, anhidrosis, bladder dysfunction, erectile dysfunction. CAN is the most clinically important form of diabetic autonomic neuropathy because of the life-threatening consequences of this condition[88]. Dysregulation of blood pressure and heart rate responses either after exercise or when resting, difficulty in thermoregulation, sudden death and silent myocardial ischemia have been attributed to CAN and have a direct negative effect on patient's daily life[88]. There is currently no specific treatment for diabetic neuropathy. Glycemic control was shown to effectively prevent DPN and CAN in DM1 as well as slow progression in DM2[77].

1.2.2 Ocular complications

Diabetes is blamed for 12,000 to 24,000 new cases of blindness each year in the United States making it the leading cause of blindness among working-age adults[89,90]. The most well-recognized complications of diabetes are diabetic retinopathy and maculopathy. However, other ocular changes related to diabetes which may threaten vision do occur. These changes will be discussed in the following sections.

1.2.2.1 Glaucoma

Glaucoma refers to a progressive optic neuropathy accompanied by the visual field changes and likely captures a number of different mechanisms of optic nerve damage. In the absence of a

proliferative diabetic retinopathy which is characterized by retinal ischemia, patients with diabetes are at risk for a primary open angle glaucoma. The Rotterdam Study found a threefold increase in glaucoma prevalence among patients with newly diagnosed diabetes[91]. The Blue Mountain eye study has reported the significant association between diabetes and glaucoma and ocular hypertension, although in 67% of cases glaucoma was diagnosed before the diabetes[92]. Not all the studies have found association between diabetes and glaucoma, however. The Baltimore Eye Survey found no additional risk for open angle glaucoma among patients with diabetes[93]. The lack of clear trend may be related to the difference in the definition of what constitutes glaucoma for each of the studies. Patients with proliferative retinopathy are at risk for a neovascular glaucoma, characterized by the growth of new blood vessels on the iris and in the iridocorneal angle, blocking the outflow of aqueous humor. Rapid rise in the intraocular pressure in this type of glaucoma puts patient's vision at immediate risk. Treatment with anti- vascular endothelial growth factor (VEGF) injections or pan-retinal photocoagulation results in regression of the neovascularization.

1.2.2.2 Ocular movement disorders

Patients with diabetes are at risk for cranial nerve palsies which frequently result in diplopia. Diplopia may be rather disrupting to patients but usually resolves within three months. Sixth and third cranial nerve palsies are most common in diabetes. The mechanism is thought to be related to ischemia. Diabetic third nerve palsies present a diagnostic and management challenge because of the need to rule out life-threatening causes such as intracranial aneurysm or tumor. In such cases pupillary sparring and completeness of the palsy are important clinical features that help differentiate diabetic vs. compressive lesions[94].

1.2.2.3 Lenticular changes

Numerous epidemiologic studies have found the association between diabetes and cataracts. Cataract is a major cause of vision impairment in people with diabetes[94]. The progression of cataract formation depends on diabetes duration and blood glucose control. Accumulation of sorbitol and the advanced glycosylated end products have been implicated in the progression of cataract formation. Aldose reductase turns excess glucose into sorbitol which cannot cross cell membranes and creates osmotic stress in the lens. These changes are thought to result in the refractive changes and cataract formation[95,71]. When visual impairment due to cataract becomes significant, cataract surgery is the standard treatment. In general, cataract surgery outcomes are good in general population, presence of proliferative diabetic retinopathy or macular edema may complicate the outcome[94].

1.2.2.4 Diabetic retinopathy

Diabetic retinopathy is characterized by the presence of microaneurysms and hemorrhages, in its earliest clinically-detectable stages. Further progression is accompanied by the increase in the number of retinal hemorrhages, appearance of intraretinal microvascular abnormalities (IRMA), venous abnormalities, areas of retinal non-perfusion and ischemia, exudates. Eventually, retinopathy can progress to the proliferative stage which involves the formation of new blood vessels and vitreous hemorrhages. The breakdown in the blood-retina barrier may lead to the appearance of macular edema. Diabetic retinopathy is a leading cause of new cases of legal blindness among working-age Americans[96]. The main causes of vision loss in patients with

diabetes are macular edema, vitreous hemorrhages, tractional retinal detachment, and macular capillary nonperfusion[97]. The overall prevalence of diabetic retinopathy for adults aged 40 years and older in the United States is 3.4%, however, age, racial and ethnic differences exist. According to the National Eye Institute (NEI), the highest prevalence is in the older Hispanic group, surpassing 15% after the age of 65[98]. Increased duration of diabetes and poor glycemic control are important risk factors for retinopathy progression. The Los Angeles Latino Eye Study reported about 18% prevalence of proliferative diabetic retinopathy, which carries the highest risk for vision loss, among participants who had diabetes for more than 15 years[99]. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) reported that the proliferative diabetic retinopathy was present in 50% of participants diagnosed with DM1 with 20 years' duration of the disease[66]. In patients with type 2 DM, proliferative diabetic retinopathy develops in 2% of patients who have diabetes for less than 5 years, this number increases to 25% for patients who have diabetes for 25 years or longer[100]. The degree of hyperglycemia is the main modifiable risk factor in progression of diabetic retinopathy[56,59,101,60,102].

The key aspect in the management of diabetic retinopathy is staging its severity. The original classification of diabetic retinopathy was proposed at the Airlie House symposium in 1968[103,104]. This grading scheme has been modified a number of times. The classification established by the Early Treatment Diabetic Retinopathy Study (ETDRS), which associated the risk of vision loss with various levels of retinopathy and demonstrated an effective treatment for more severe stages of retinopathy and macular edema, has become the gold standard for staging of diabetic retinopathy in research[105,106]. This grading scheme is impractical for use in clinical practice because it utilizes thirteen complex severity stages that are often unnecessary in clinical care[104,105]. The International Clinical Diabetic Retinopathy and Diabetic Macular Edema Severity Scale was developed based on the ETDRS and WESDR data to standardize and simplify communication among clinicians[107]. According to this retinopathy severity scale, five levels are identified: 1) No apparent retinopathy, 2) Mild nonproliferative diabetic retinopathy, 3) Moderate nonproliferative diabetic retinopathy, 4) Severe nonproliferative diabetic retinopathy, 5) Proliferative diabetic retinopathy. The following clinical findings define the five stages, 1) No abnormalities, 2) Microaneurysms only, 3) More than just microaneurysms but less than severe nonproliferative diabetic retinopathy, 4) Any of the following: more than 20 intraretinal hemorrhages in each of 4 quadrants; definite venous beading in 2+ quadrants; prominent IRMA in 1+ quadrants and no signs of proliferative retinopathy, 5) One or more of the following: neovascularization, vitreous/preretinal hemorrhage[107]. The international classification scale is useful for population screening and incorporates evidence on disease progression form the ETDRS[103].

The Diabetic Retinopathy Study (DRS) and ETDRS demonstrated that laser photocoagulation treatment for patients with proliferative diabetic retinopathy reduces the risk of vision loss[106,108]. A recent study has shown that intravitreal anti-VEGF injections for the treatment of proliferative diabetic retinopathy may be a reasonable alternative to the panretinal photocoagulation[109].

1.3 Diabetic macular edema (DME)

Diabetic macular edema is a leading cause of vision loss in patients with diabetes affecting an estimated 2.7% of adults with diabetes[96,110,111]. The focus of this dissertation is on developing and

evaluating the new strategies of diabetic macular edema detection in a screening setting. This is especially relevant now that many large healthcare delivery systems have implemented screening programs for the detection of treatable diabetic retinopathy and macular edema. Such institutions include the Veterans Health Administration, Kaiser Permanente and The UK National Health Service (NHS), to name a few. The detection of sight-threatening diabetic macular edema is not standardized and lacks the desired accuracy. The rest of the discussion in Chapter 1 will be dedicated to the issues related to the diagnosis, pathophysiology, detection and treatment of DME.

1.3.1 Definition and Pathophysiology

Diabetic macular edema refers to retinal thickening in the macular region of the eye that is the result of the microvascular complications of diabetes. Diabetes-induced dysregulation of the intra- and extra-vascular fluid homeostasis leads to the accumulation of fluid in the retinal tissue. The ETDRS Fundus Photograph Reading Center determined the presence of macular edema in the eye if there was a retinal thickening at or within 1 disc diameter (diameter of the optic nerve head ~ 1800 microns) of the center of the macula or definite hard exudates in this region[112]. Based on the clinical features associated with the increased risk for vision loss, ETDRS[112] has defined Clinically Significant Macular Edema (CSME) as any of the following characteristics:

1) Thickening of the retina at or within 500 microns of the center of the macula.
2) Hard exudates at or within 500 microns of the center of the macula, if associated with thickening of adjacent retina (not residual hard exudates remaining after disappearance of retinal thickening).
3) A zone or zones of retinal thickening 1disc area or larger, any part of which is within 1-disc diameter of the center of the macula.

In general, edema is defined as an excessive volume of fluid accumulated in the tissue. This accumulation can happen intracellularly or within the collagen-mucopolysaccharide matrix distributed in the interstitial spaces[113]. Intracellular edema occurs due to the dysregulation of the cellular ion channel function due to a metabolic insult caused by chronic hyperglcemia[114]. Extracellular or interstitial macular edema is a result of retinal vascular leakage caused by the loss of the blood-retinal barrier (BRB) function[73]. The retina has a dual blood supply with the outer retina being supplied by the choriocapillaris and the inner retina – by the branches of the central retinal artery. The endothelium of choroidal capillaries is fenestrated and permeable to various molecules in the vascular circulation. The retinal pigment epithelium forms the barrier between the choroidal circulation and the photoreceptors and is the basis of the outer BRB. The capillaries in the inner retina have a continuous endothelium with tight junctions and are the basis of the inner BRB. According to Starling's equation, net water transport over the endothelium is determined by the sum of hydrostatic pressure and osmotic and oncotic pressure exerted within and outside of the blood vessel walls[73,113]. Hyperglycemia-induced loss of BRB leads to the leakage of plasma solutes into the interstitial compartment increasing the oncotic and osmotic pressure in the interstitial compartment. This pressure gradient favors movement of fluid into the extravascular tissue. The accumulation of fluid in the tissue, however, is counteracted by a number of safety factors that act to limit edema formation[113]. These counter-measures include active and passive movement of fluid and leaked protein away from the retina as well as myogenic factors[114]. Under the normal conditions osmotic pressure within the choroidal tissue draws water from the retina to the choroid. Intraocular pressure plays a role in normal relative dehydration of the interstitial retinal

compartment[114]. Water is also removed from the retina by active transport by the RPE cells[114]. Chronic hyperglycemia leads to a diminished capacity of these compensatory mechanisms which promotes the accumulation of fluid in the retina. Historically, DME has been classified as focal and diffuse[115]. As the name implies, focal edema refers to localized areas of retinal thickening and is caused by the leaking microaneurysms or IRMA. Diffuse leakage from the dilated retinal capillaries in the general macular area leads to the formation of the diffuse edema[114].

1.3.2 Epidemiology

The overall prevalence of DME among patients with diabetes in the U.S. is 3.8%, in those over 40 years of age[116]. The prevalence of DME is highest among the non-Hispanic blacks and is 3-fold higher than in non-Hispanic whites[116]. Factors associated with DME presence are longer duration of diabetes, higher mean A1C, and insulin use[116]. The four-year incidence of either CSME or any DME is 12% for patients with mild NPDR. The incidence increases to 23% for patients with moderate NPDR[96]. Up to 30% of patients with CSME develop moderate vision loss which is defined as doubling of the visual angle[115]. WESDR study of the long-term incidence of macular edema reported that a 10-year incidence of DME in the younger-onset group of participants was 20.1% and 25.4% in the older-onset group among those taking insulin[117]. The Los Angeles Latino Eye Study (LALES) found the prevalence of DME to be 10.4% and CSME – 6.2% in a cohort of patients of primarily Mexican ancestry[99]. CSME is a leading cause of vision loss in patients with diabetes affecting an estimated 2.7% of adults with diabetes[96,110,111].

1.3.3 Detection

The definition of CSME was set forth by the ETDRS and its presence was assessed by stereoscopic contact lens biomicroscopy or stereoscopic photography[112]. Regardless of the method, stereoscopic view of the macula is required for the definitive diagnosis of CSME.

1.3.3.1 Binocular biomicroscopy

Binocular biomicroscopy is usually performed by an ophthalmologist or an optometrist after the pharmacologic dilation of the pupil. It can be performed using a contact or non-contact condensing lens.

1.3.3.2 Stereoscopic macular photography

Stereoscopic macular photography is the standard way of detecting CSME in a research setting[105,118,119]. It correlates well with the detection of CSME based on the contact lens biomicroscopy[120]. The specifics of this technique will be discussed in Chapter 2, General Methods section.

1.3.3.3 Fluorescein Angiogram (FA)

Fluorescein angiography is performed by the intravenous injection of fluorescein dye. Once in the systemic circulation, the dye travels to the eye via the central retinal artery and posterior ciliary arteries. The process of fluorescein diffusion through the choroidal and retinal capillary networks

can be observed by sequential fundus photography using specialized fundus camera. Such camera detects the fluorescence emitted after the excitation of the dye by the blue light. As discussed in Section 1.2.1, the retinal vasculature has tight junctions which prevent the diffusion of the dye into the retina, under the normal conditions. Any abnormalities in the retinal vasculature, such as microaneurysms, increased permeability, neovascularization, as well as areas of non-perfusion, will become apparent during this procedure. FA is not used for the diagnosis of CSME, rather it is used identify the lesions requiring treatment once the decision for treatment has been made on clinical grounds[112,115].

1.3.3.4 Optical coherence tomography (OCT)

Introduced in 1991, OCT has become an invaluable tool for evaluation of retinal disease including DME[121]. This technique uses low-coherence interferometry to determine the relative depth of the tissue layers and is analogous to ultra-sonic pulse echo imaging[121]. The light reflected from the tissue creates interference patterns with the light reflected from the reference mirror and processed into an A-scan. Multiple A-scans are combined into a two-dimensional B-scan, a cross-sectional map of the retina[121,122]. The advantages of this technique are its non-invasive approach, high axial and lateral resolution, and speed. Current commercially available spectral domain (SD) OCTs have axial resolution of 4-5 microns.

OCT has been shown to provide accurate and reproducible measurements of the retinal structure[123,124,125]. This makes it possible to quantitatively evaluate retinal thickness in vivo and follow its changes over time. This has not been possible prior to the implementation of OCT in clinical practice. Since CSME has been defined prior to the introduction of OCT technology based on the subjective evaluation of retinal thickness, OCT measurements cannot be directly applied for the diagnosis of CSME. Nevertheless, center-involved DME can be defined in terms of OCT measurements and is used as an outcome measure in clinical trials evaluating the effectiveness of various treatment modalities for diabetic macular edema[126]. In addition, OCT scans of the macula offer an opportunity to detect retinal thickening at the early stages which may not be detectable on a clinical examination and study patterns of DME beyond focal and diffuse classifications[127,128].

1.3.4 Treatment options

The initial conclusive evidence of the treatment benefit for CSME was provided by the ETDRS study which demonstrated a 50% reduction in risk (24% vs. 12%) of moderate vision loss in a group of patients with CSME who received laser photocoagulation treatment when compared to those who did not[112].

1.3.4.1 Laser photocoagulation

Photocoagulation treatment for macular edema is indicated when CSME is diagnosed based on the stereoscopic examination of the macula. FA is performed to guide the selection of the treatable lesions within 2 disc diameters of the center of the macula which include microaneurysms, IRMA, diffusely leaking capillary bed, retinal avascular zones[112]. Focal lesions are treated with focal laser burns at the site of the lesion with the goal of closure of leaking areas by thermal photocoagulation[115]. Areas of diffuse leakage or nonperfusion are treated in a grid pattern[112]. The therapeutic mechanism of grid laser application is less clear but may be related to the decrease in

oxygen demand by destruction of photoreceptors. Alternatively, application of laser burns to the RPE cells may promote its function of eliminating fluid from the retina[115].

1.3.4.2 Steroids

Several case reports have shown that intravitreal steroid injections had positive short-term effect on the resolution of cystoid diabetic macular edema non-responsive to grid laser photocoagulation[129,130]. The mechanism of this effect is thought to be the reduction in inflammatory mediators and stabilization of the vascular endothelium[131]. A randomized clinical trial comparing the effectiveness of intravitreal triamcinolone treatment to focal/grid laser treatment for DME showed that laser photocoagulation treatment was superior to steroid injections in the long-term with respect to the visual acuity and macular thickness. In addition, the rate of cataract surgery and intraocular pressure elevation was higher in the groups of subjects receiving various doses of triamcinolone injections[131,132].

1.3.4.3 Anti-VEGF (vascular endothelial growth factor)

The rationale for using intravitreal anti-VEGF injections for the treatment of DME comes from the recognition that BRB breakdown in diabetes is mediated in part by VEGF, a 45 kD glycoprotein[133]. One of the proposed mechanisms by which VEGF increases vascular permeability is through the phosphorylation of the protein occludin, a key component of the tight junctions of the endothelial cells[134]. VEGF is released in response to retinal ischemia brought on by diabetes.

Several clinical trials have demonstrated the effectiveness of bevacizumab and other anti-VEGF agents in the treatment of center-involved CSME[135,136,137,110]. The improvement in acuity appears to be greater in patients treated with intravitreal anti-VEGF agents as compared to those treated with focal/grid laser. While all three currently available anti-VEGF agents were shown to have similar effect on DME, Aflibercept appears to be more effective in patients with poor (20/50 or worse) visual acuity at baseline[110].

1.3.5 Summary and Rationale for Current Work

Diabetes affects a significant and growing part of the population in the U.S. and worldwide. Clinically significant macular edema, an ocular manifestation of the microvascular complications of diabetes is the leading cause of visual impairment in the working age adults in the U.S. Timely and appropriate treatment for CSME has been shown to reduce the risk of future vision loss and improve visual acuity. The diagnosis of CSME requires a stereoscopic evaluation of the macula by an ophthalmologist or an optometrist. It has been estimated that up to 40% of patients with diabetes do not comply with the recommended schedule of eye exams[138]. This number is likely higher in safety-net populations with greater number of barriers to healthcare. A telemedicine approach for the detection of vision-threatening complications of diabetes has been implemented by a number of private and government-run programs with the hope of eliminating some of the barriers to eye care access and identifying those individuals who are at highest risk of vision loss. Such diabetic retinopathy screening (DRS) programs rely on acquisition of monoscopic digital fundus images for the detection of vision-threatening retinopathy. As detailed in Section 1.2.3, the definitive detection of CSME requires a stereoscopic view of the macula which is impossible in monoscopic images. Attempts to use stereoscopic images have been made but require a more

complex procedure and result in a higher number of ungradable images than desired[139]. The detection of CSME in monoscopic images requires identification of a reliable surrogate marker of CSME and implementation of an accurate screening approach. This dissertation includes three observational clinical studies conducted over a span of several years with an overarching goal of developing and validating an accurate method for the detection of CSME in the DRS setting. This work is clinically important because there is no standard approach for the detection of CSME by various DRS programs. This lack of standard reflects the lack of data on the validity of most of the methods used. The data that is available provides conflicting information and is difficult to interpret because the diagnostic standard against which various detection methods were tested vary from study to study[140,139,141,118,142,119,120]. In general, diagnostic methods of CSME detection include digital or film stereoscopic photography as well as the dilated fundus exam with stereoscopic biomicroscopy. In recent years, however, the importance of including objective measures of retinal thickening, like the ones obtained using optical coherence tomography, became increasingly clear. Most of the recent clinical trials evaluating the effectiveness of treatment for CSME, rely on OCT information to define the inclusion criteria. In addition, a recent study has demonstrated that some of the CSME detection approaches used by DRS programs and epidemiologic studies fail to detect up to 40% of patients with center-involving DME as assessed by OCT. We have attempted to address these shortcomings in Chapter 4 and Chapter 5 by using both diagnostic modalities for CSME detection and including OCT as an integral part of the diagnostic testing against which the DRS detection approaches are evaluated.

The initial motivation for the work detailed in this dissertation came from the studies by Bresnick, et al. and Rudnisky, et al. where the presence of hard exudates (HE) at various locations in the macula was shown to provide reasonable sensitivity but sub-optimal and varying specificity for the detection of CSME. The basis for using HE as a surrogate marker for CSME detection comes from the mechanism of HE formation in the retina. As discussed in Section 1.2.1, chronic hyperglycemia-induced BRB breakdown leads to the leakage of plasma components into the retinal tissue. The leaked lipoproteins are precipitated in the retina and form bright yellow deposits – hard exudates. HE are readily visible on fundus images because of their color and sharply-defined borders. While some DRS programs use the presence of microaneurysms (MAs) in addition to HE for the detection of CSME, this combination was shown to have no advantage over using HE alone[141].

As a first step, Chapter 3 presents a study which tested the effectiveness of using HE within 1800 microns from the center of the macula for the detection of CSME under a "real world" clinical scenario. The reasoning for this study was to test the implementation of this method in clinical setting and to resolve the discrepancy in diagnostic accuracy of this detection method evident from the work by Rudnisky and Bresnick. In Chapter 4 we develop and propose OCT-based adaptation of the CSME severity scale based on the ETDRS criteria and evaluate whether the distance of HE from the center of the macula affects the severity of CSME. The underlying clinical question answered in this chapter is whether a more urgent intervention is required for the patients who present with HE within 500 microns from the center of the macula when compared to those with HE outside of 500 microns but within 1800 microns from the center of the macula. In addition, we proposed a new CSME detection method that captures the combined measure of the proximity of HE to the fovea and the areal extent of exudation in the central macula. Finally, in Chapter 5 we have tested this new detection approach in a sample of new study participants. Previous studies have reported that the latency and the amplitude of the electroretinogram (ERG) responses are

affected in patients with DME[143,144]. We have evaluated the association between the ERG measurements and the diagnosis of CSME. We have combined those and other relevant clinical variables in a model of CSME detection and evaluated its performance.

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Chapter 2: General Methods

2.1 Introduction to design and methodology

This chapter introduces the general methods of study design, data collection, and analysis that is common to all of the studies included in this dissertation. Each of the subsequent chapters will detail the specific methods pertinent to each of the studies. Some of the information will be repeated to maintain clarity and logical continuity of the study reports. All three studies share observational cross-sectional design. This is appropriate since we were mainly interested in the measurements of association of clinical variables and CSME as well as the estimates of sensitivity, specificity, positive and negative predictive values of the screening methods. In the first study, Chapter 3, study participants were invited to attend two study visits within the recommended timeframe but without the strict oversight, beyond the usual clinical oversight. This was performed to approximate the "real world" implementation of the DRS program and to evaluate the effectiveness of the proposed CSME screening approach. The average time between the visits was slightly over one month. In several cases that time exceeded 100 days. Since the presented studies investigated patients with CSME and other types of sight-threatening diabetic retinopathy - severe non-proliferative (sNPDR) and proliferative (PDR) – care was taken to provide the study participants with the appropriate clinical care. All of the data collection occurred within the Alameda Health System (AHS). Highland hospital is a flagship hospital within the AHS. All of the study participants who were diagnosed with CSME, sNPDR, and PDR were referred to a retina specialist for further management at Highland hospital.

2.2 Recruitment of participants

Study participants for all of the clinical studies were recruited at the AHS. Each participant was paid $10 for a study visit. The purpose of the study, data collection procedures, potential adverse events, and participant rights were discussed at the time of the recruitment. The procedures followed the tenets of the Declaration of Helsinki and the study protocols were approved by the Institutional Review Boards at UC Berkeley and AHS. For the study included in Chapter 4, the study sites included the following locations in Northern California: Eastmont Wellness Center in Oakland, Winton Wellness Center in Hayward, Newark Wellness Center in Newark, and Highland hospital in Oakland. For studies covered in Chapter 3 and 5, Eastmont Wellness Center was the data collection site.

The following inclusion criteria were common to all of the studies: all consecutive adult (18 years old or over), non-pregnant patients with diabetes mellitus type 1 or type 2 presenting for the DRS or an eye exam at a study site. Exclusion criteria were the presence of macular pathology other than DME, retinal vascular occlusions, glaucoma, evidence of laser or anti-VEGF treatment for DME (and PDR for ERG data analysis), significant media opacity rendering the digital photographs or OCT scans ungradable. There was no exclusion or attempt to balance the participant groups with and without CSME based on ethnicity, sex, duration of diabetes or any other factors. In the study covered in Chapter 3 the sample was enriched with patients who were more likely to have CSME. Based on the evidence presented by large epidemiologic studies, the following factors were considered: age 40 years and older, Latino/Hispanic ethnic group, duration of diabetes greater than 5 years, and A1C of 7% and above[1,2]. We recruited one hundred and forty-three participants for the first study, Chapter 3. A retrospective analysis of the cross-sectional data

from one thousand eight hundred and fourteen participants was included in the analysis for the second study, Chapter 4. Finally, two hundred and twenty-five participants were recruited for the third study, Chapter 5. I was responsible for the study design, data analysis, as well as participant recruitment and data collection in the three studies covered in this dissertation.

2.3 Procedures

All of the study procedures followed the signing of the required consent forms by the study participants. Study participants were free to withdraw their participation in the data collection procedures at any time.

2.3.1 Visual acuity testing

For the study covered in Chapter 3, visual acuity was collected as part of an eye exam which itself was part of the study procedures. In this study monocular best-corrected Snellen acuity was measured at a distance of 20 feet after the refraction. Visual acuity measurements were not required for the study covered in Chapter 4. For the study covered in Chapter 5, monocular, best-corrected for near work visual acuity was measured at 40 cm using Bailey-Lovie LogMAR near card. This was performed after the refraction.

2.3.2 Fundus photography

2.3.2.1 Three-field monoscopic photography

Forty-five-degree digital color fundus photographs were obtained using Canon CR6-NM, for the first and the second study, and Canon CR-DGI for the third study. Pupillary dilation was not necessary for the first and the second study prior to fundus photography. Fundus photography in the third study was carried out after the pharmacologic dilation of the pupil which was achieved during an ocular examination by the study optometrist using Tropicamide 1% and Phenylephrine 2.5%. In all cases three fields in the posterior pole were captured according to the EyePACS DRS protocol[3], Figure 2.1. This imaging strategy was selected because it had been validated for the detection of referable retinopathy against the ETDRS standard photographs and the dilated fundus exam[4,5] and is much easier for the study participants to handle than the stereoscopic 7-field 35-mm film photography. This is an important consideration when compliance with study procedures has the potential to affect the quality of data and differential attrition of the study participants.

All the photographs were de-identified and uploaded for storage and grading on to the EyePACS web server (www.eyepacs.org). For the second and third study, relevant images were transferred from EyePACS server to the computer located in the laboratory at UC Berkeley for customized marking and grading to ensure accuracy and standardization of the grading procedures.

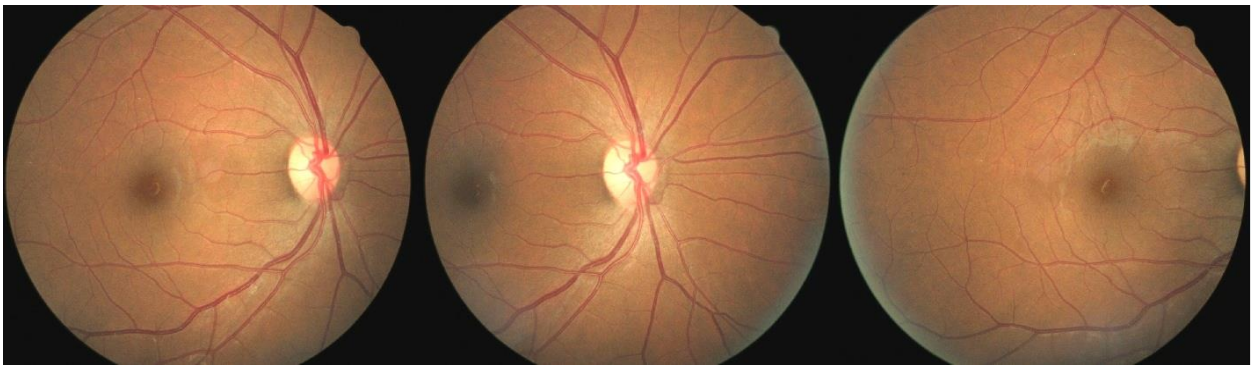

Figure 2.1. Three-field fundus photographs. Primary field (left image) captures macula and the optic nerve head, roughly equidistant from the center of the image. Secondary field (middle image) is centered on the optic nerve head. Tertiary field (right image) captures area temporal to the fovea with the edge of the optic nerve head disc in the view.

2.3.2.2 Stereoscopic macular photography

Stereoscopic macular photography was performed only in the study reported in Chapter 5. This was an important step for achieving the diagnostic standard of CSME detection against which the proposed screening methods were tested. Stereoscopic digital fundus photography is a validated diagnostic standard used in research[6,7,8,9].

We used the Canon CR-DGI digital fundus camera to obtain non-simultaneous 45^0 stereoscopic images of the macula using a modified technique first described by Lee Allen in 1964 and detailed by Tyler[10,11]. Stereoscopic images were obtained after the pharmacologic pupillary dilation. Under this technique, the illuminating ring of the fundus camera is displaced laterally from the center of the pupil to the left and then to the right edge of the dilated pupil with the goal of achieving the widest possible stereoscopic base separation between the two rings that allow for the clear view of the macula, Figure 2.2. Figure 2.3 shows an example of the left and the right stereoscopic pair of the same fundus obtained using the technique described above. Notice the presence and the position of the left and the right edge artifacts indicating that the wide-base stereoscopic separation was achieved. Stereoscopic images were de-identified and uploaded to the EyePACS web server as described above.

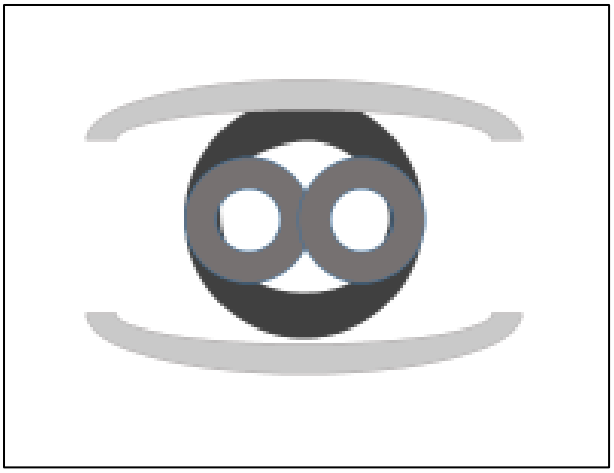

Figure 2.2. The schematic drawing of a dilated pupil with the two illuminating rings of the fundus camera positioned within the dilated pupil, each at the farthest opposite edge of the pupil.

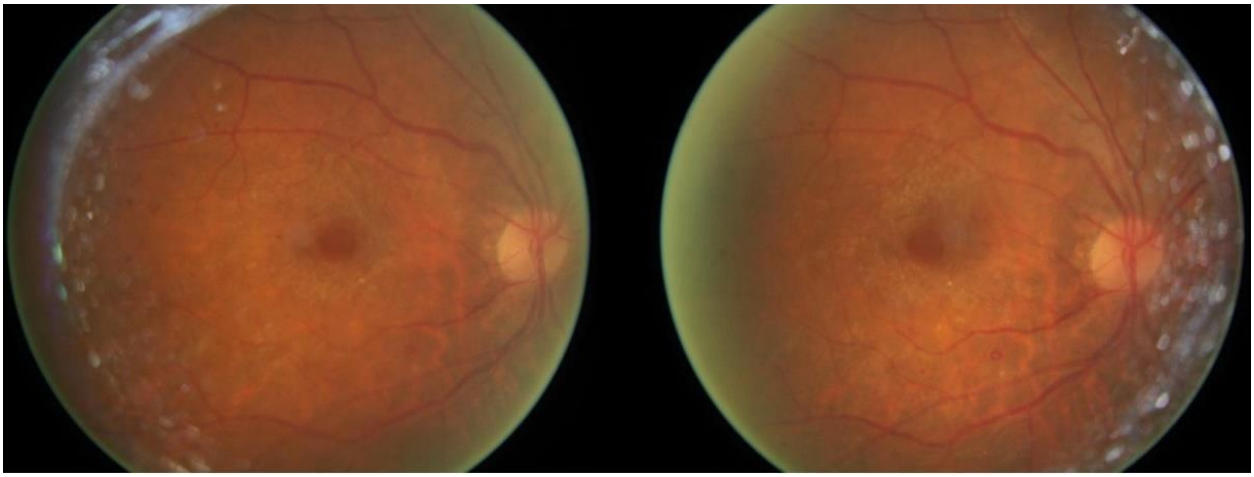

Figure 2.3. An example of the stereoscopic pair of images of the same fundus obtained using the CR-DGI fundus camera. A prominent epiretinal membrane is visible in the central macula. This subject would be excluded from analysis because of the macular pathology other than diabetic macular edema

2.3.3 Optical coherence tomography

As discussed in Chapter 1, optical coherence tomography (OCT) has been used in ophthalmology for over 20 years and allows for quantitative assessment of retinal thickening and a more detailed qualitative characterization of the retinal changes in patients with DME. We performed OCT scans of the macula using the iVUE SD-OCT device, Optovue, Inc. using Retina Map protocol, Figure 2.4.

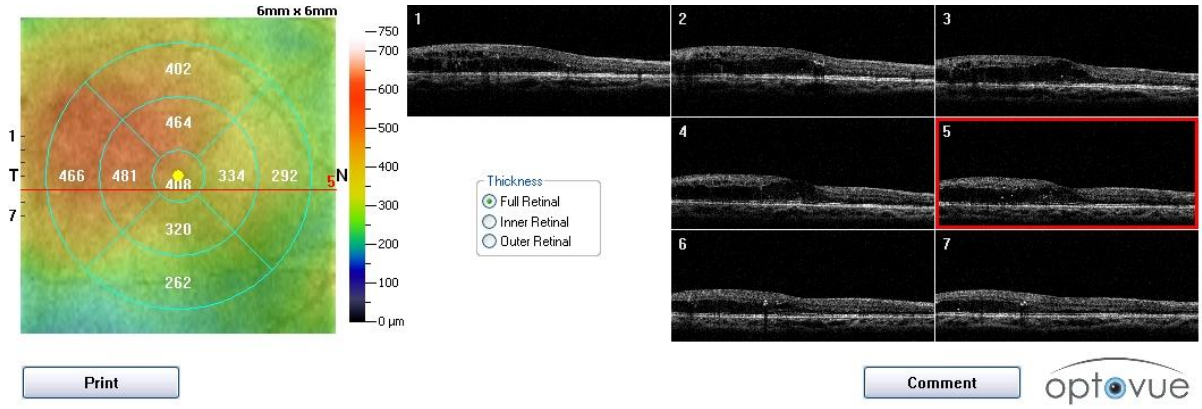

Figure 2.4. An example of the Retina Map OCT scan of the macula. The left panel shows a topographic map with thickness values averaged within each sector. The right panel shows seven b-scans obtained from the central macula (slices 1-7 marked on the left side of the left panel).

iVue OCT provides a 32^0 x 32^0 field of view and uses an 840nm light source. It captures 26,000 A-scans/second and allows for 5-micron axial resolution and 15-micron lateral resolution. OCT scans of the macula were obtained for the studies reported in Chapter 4 and Chapter 5 through undilated and dilated pupils, respectively. In Chapter 4, OCT information was used to develop and propose an OCT-based adaptation of the DME severity scale derived from the ETDRS data[12]. In Chapter 5, OCT data was used in conjunction with the dilated biomicroscopic evaluation of the retina to establish the diagnosis of CSME as a clinical standard of CSME detection[13,14,15].

2.3.4 Electroretinogram

We performed undilated, photopic 30 Hz flicker electroretinography (ERG) using the RETeval hand-held device, Figure 2.5A. ERG data was collected for the study reported in Chapter 5. ERG provides the electrophysiologic evaluation of retinal function by measuring the electrical activity in the retina in response to a flash of light[16]. Light-adapted 30 Hz flicker ERG is one of the six full-field ERG protocols recognized and specified by the International Society of Clinical Electrophysiology of Vision (ISCEV). RETeval is a hand-held ERG device that complies with ISCEV standards for the photopic 30 Hz ERG by delivering a 28.3 Hz flickering white-light stimulus (chromaticity coordinates, x = 0.33, y = 0.33) that is produced by brief (<5 ms) flashes from blue (470 nm), green (530 nm), and red (622 nm) LEDs in a ganzfeld[17,16]. The constant retinal illuminance for a reasonable range of pupil sizes (pupil diameter < 6.5 mm) in the undilated eye is delivered by RETeval by adjusting the white light stimulus intensity in response to real-time pupillary measurements according the following formula, T = L x p, where T is retinal illuminance, L is the photopic luminance in cd m^{-2}, and p is pupil area in mm^2 [18]. The device automatically measures the implicit time (time between the onset of a flash of light and the peak of the wave), and amplitude (the peak-trough distance of a sinusoid fit to the fundamental component[19] (Figure 2.5B). This is achieved by using a special algorithm using discrete Fourier transformation and

cross-correlation analysis[18]. The electrical response from the retina is measured using skin electrodes instead of the corneal electrodes.

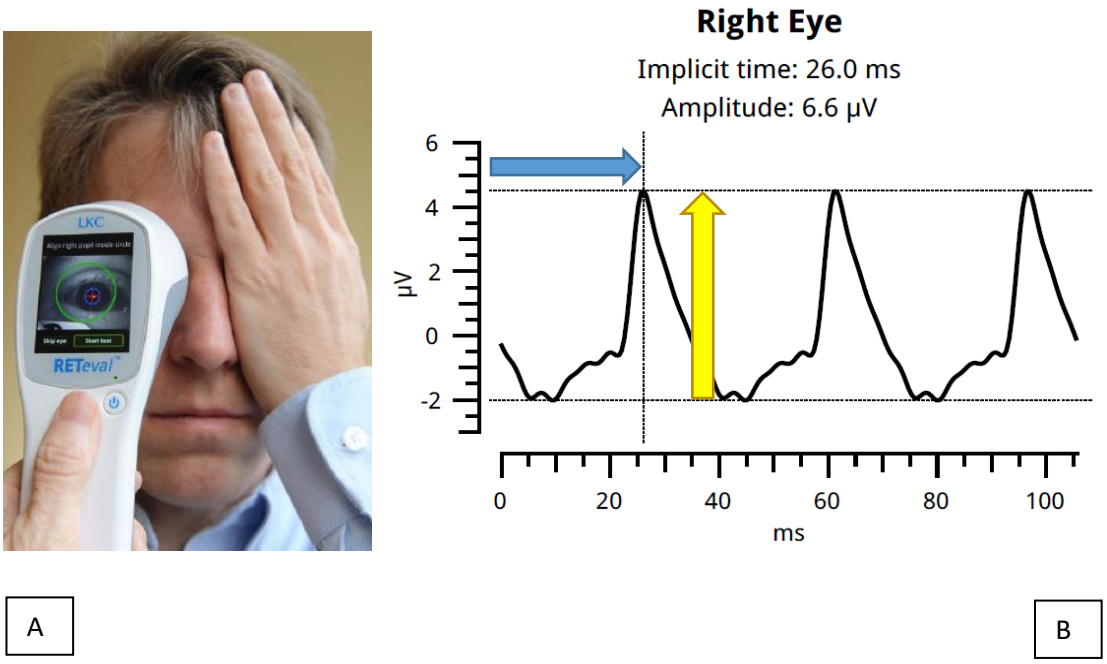

Figure 2.5. A) Hand-held ERG device, RETeval, LKC Technologies, Inc., being used to obtain ERG recording. B) ERG waveform generated by RETeval. Blue arrow indicates the measure of implicit time. Yellow arrow indicates the measure of amplitude.

The cone system is the main physiologic generator of the light-adapted 30 Hz flicker ERG[16]. Existing literature suggests that abnormalities in the implicit time and the amplitude of the 30Hz photopic ERG may be significantly associated with the presence of CSME[20,21].

2.4 Retinopathy and CSME grading

The level of diabetic retinopathy was graded by two EyePACS certified graders with a third grader adjudicating the discrepancies in grading. Grading was performed using the EyePACS diabetic retinopathy grading tool[22] following the International Clinical Diabetic Retinopathy Severity Scale[23].

CSME grading of the stereoscopic photographs was performed by two masked graders on a 27-inch color-calibrated monitor with 1920x1080 resolution. Stereoscopic pairs were presented on the monitor using custom program (Matlab, Mathworks, Inc.) and viewed through the Screen-Vu stereoscope. The grading program allowed the grader to enlarge the macular area of each of the stereo pairs in a standardized fashion to allow for a more detailed inspection of the questionable areas. The diagnosis of CSME was established based on the ETDRS criteria (see Chapter 1,

Section 1.2.1). The size and the location of the retinal thickening were measured using a measuring grid which was calibrated on a subset of photographs captured by each of the fundus cameras used in the three clinical studies, Figure 2.6. Cases with inter-grader disagreement were adjudicated by a third expert grader.

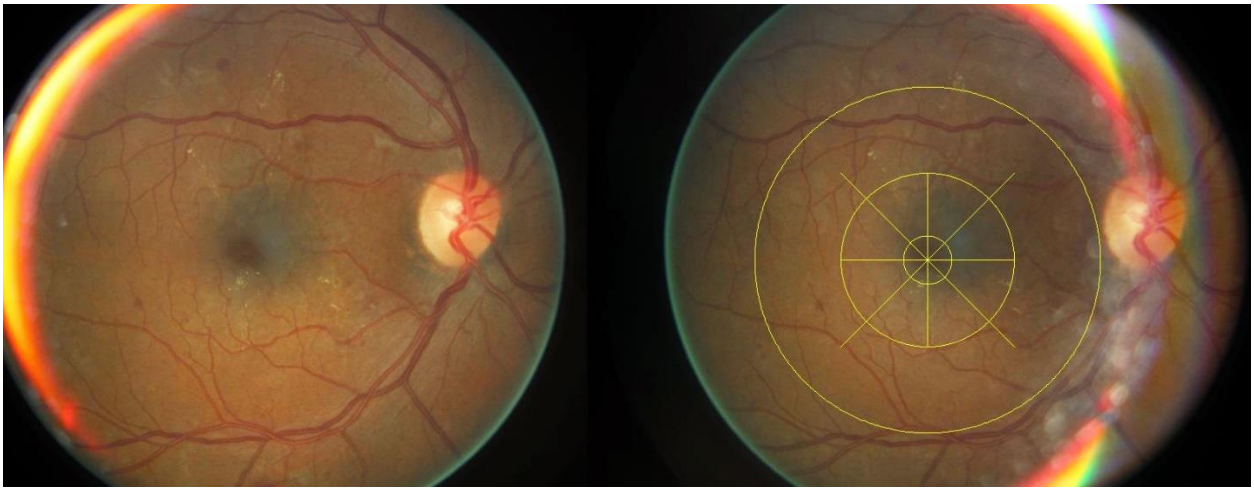

Figure 2.6. An example of the measuring grid (right image) applied during grading of the stereoscopic photographs for the presence of CSME. The radii of the inner, middle and outer circles of the grid are 500 um, 1800 um or 1-disc diameter, and 3600 um, respectively.

2.5 Hard Exudate detection and grading

One of the monoscopic images captured during the non-simultaneous stereophotography was used to determine a number of sectors affected by hard exudates. A custom Matlab program was used to place the eight-sector grid centered on the fovea to assure the consistency in the orientation of the grid placement throughout the study participants and the graders (Figure 2.7). The number of sectors within a circle with the radius equal to one-disc diameter that were affected by hard exudates (Sectors) were established by the two graders masked to each other's grading. This procedure was followed by the studies reported in Chapter 4 and 5. The study reported in Chapter 3 used the standard EyePACS grading approach to estimate the presence of HE within 1-disc diameter of the center of the macula.

2.6 Macular edema grading

The details of macular edema grading are provided in Chapters 4 and 5 and describe the specific grading procedures suited for the goals specific to each of the studies.

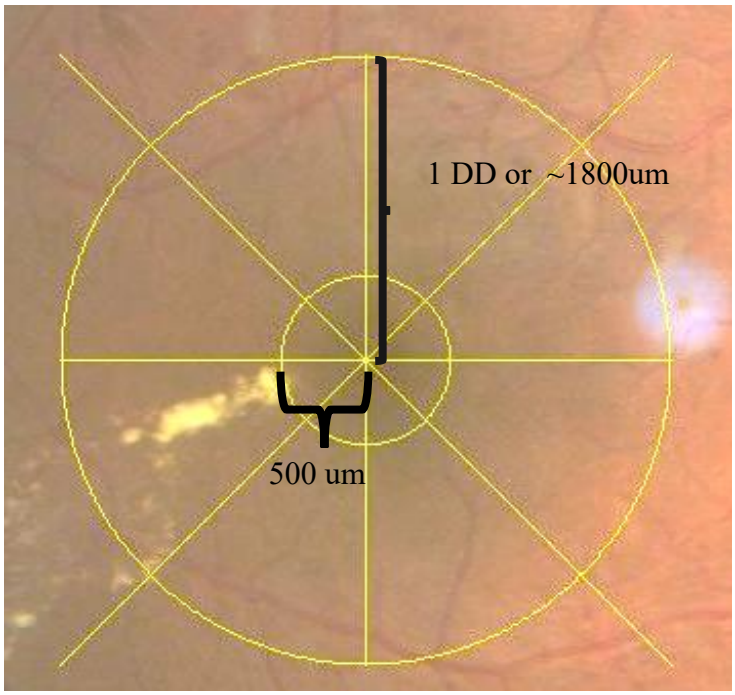

Figure 2.7. Macular grid with eight radially arranged sectors centered on the fovea. Only one sector is affected by HE in this case.

2.7 Other clinical and demographic data

Each participants' blood pressure was measured prior to dilation using a Welch Allyn Connen ProBP 3400 unit. In addition, the last two most recent blood pressure measurements were collected from the patient's medical record. This was done to obtain an idea regarding the patient's average systolic and diastolic blood pressure, since it would likely capture more meaningful information regarding the patient's average blood pressure control. In addition, age, sex, ethnicity, duration of diabetes and type, insulin dependence were recorded based on the patient's medical record review. We also accessed each patient's medical records for the following information: HbA1c, fasting blood glucose, serum cholesterol, creatinine, and blood urea nitrogen or BUN, all collected within three months of the patient's visit. All of the information was de-identified and stored in a secure location according to the requirements of the Committee for the Protection of Human Subjects at UC Berkeley.

2.8 Statistical treatment of the data

The statistical analysis was different in each of the reported studies but some approaches were common for all three. We relied on the receiver operating characteristic (ROC) curves to evaluate the accuracy of models proposed for the detection of CSME. This approach is commonly used in clinical studies. The derived sensitivity, specificity, negative and positive predictive values (NPV and PPV) have been reported for the detection of CSME in the past. Utilizing this approach in the current work facilitates the comparison of the results of our studies with those reported elsewhere

in the literature. The ROC graph plots sensitivity values on the y-axis and the false positive rate or 1-specificity on the x-axis, Figure 2.8. The cutoff values on the plot are chosen to maximize the sensitivity and specificity values.

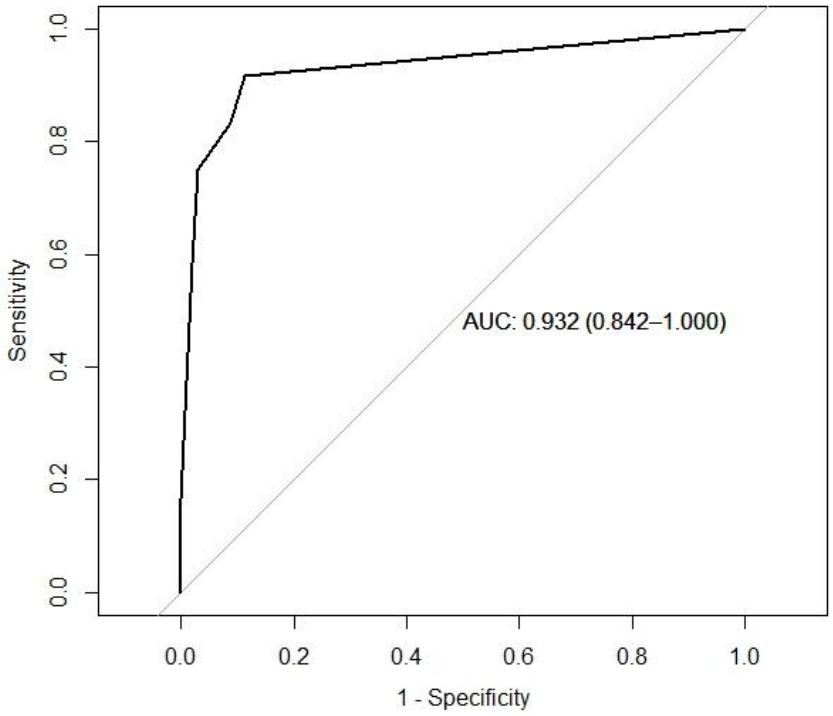

Figure 2.8. An example of an ROC curve. Area under the curve (AUC) and the confidence intervals are shown below the diagonal line which reflects chance performance.

The overall accuracy of the test in its ability to separate patients with CSME from those without CSME is measured by the area under the ROC curve (AUC). The closer this number is to 1, the closer the proposed test is to perfect performance. AUC of 0.5 represents a purely guessing rate. Sensitivity, specificity, NPV, and PPV are calculated for the selected cutoff on the ROC curve. Sensitivity reflects the proportion of participants who were correctly identified by the test as having the disease or a/(a+c), Table 2.1.

	True CSME+	True CSME-
HE+	a	b
HE-	c	d

Table 2.1. An example of a 2x2 table summarizing the performance of hard exudates (HE) in detection of CSME.

Specificity is the proportion of participants who were correctly identified by the test as not having CSME or d/(b+d) (Table 1). Positive predictive value reflects the probability of having a disease for a patient who tested positive for HEs or a/(a+b) (Table 1). Using similar logic NPV reflects the probability of not having a disease for a patient who tested negative for HEs. PPV and NPV should be interpreted with caution as they are directly affected by the prevalence of the disease in the sample and should be discussed in such context[24].

Another common statistical approach used for the evaluation of the data reported in this dissertation is the use of a multivariable logistic regression to evaluate the association and estimate the odds of CSME, an outcome variable, with several independent or "predictor" variables. This approach is well-suited for our work because CSME is a binary variable (present or absent) and it may be influenced by a number of factors such as presence of hard exudates, abnormalities in the ERG waveform as well as other clinical variables discussed in greater detail in Chapter 1. Those include duration of diabetes, blood glucose control, etc.

Logistic regression may be generally formulated as,

$$\text{Log}(P/1\text{-}P) = b_0 + b_1 x$$

Where "P" is the probability of the outcome, in this case CSME; "b_0" is the intercept coefficient which is a theoretical value in our case as it represents the baseline probability of CSME. b_1 is log odds ratio associated with one-unit increase in variable "x". Log odds ratio can be easily converted to the odds ratio (OR) by exponentiating the coefficient beta. This equation can be expanded to include multiple variables. Each exponentiated coefficient beta may be interpreted as the OR of having CSME holding all other variables constant. In essence, this task is equivalent to testing a series of hypotheses that each proposed "predictor" variable is associated with the outcome – CSME. This technique is appropriate for several reasons: our outcome is a binary variable, "predictor" variables are a combination of continuous and categorical variables, and the model allows to evaluate the magnitude and significance of each variable holding other variables constant.

2.9 References

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Chapter 3: The Utility of Hard Exudates for the Referral of Clinically Significant Macular Edema

3.1 Prelude

Hard exudates (HE) near the center of the macula had been shown to have reasonable sensitivity of 94% but varying specificity (54% - 81.6%) in detection of CSME, as noted in Chapter 1, Section 1.2.9. It was important to rigorously test this approach to resolve the reported discrepancy in the specificity in order to aid the diabetic retinopathy screening (DRS) programs in making a decision on the implementation of this approach in detection of CSME. Additionally, comparison of the new approaches for the detection of CSME (Chapter 5) to this existing approach are simplified if the uncertainty regarding its accuracy is minimized. The results of study were published in Optometry and Vision Science in 2014.

In retrospect, the implemented study design, Section 3.4, offered us an opportunity to evaluate the utility of this screening approach in a "real world" setting. Evaluation of HE presence in the macula as well as diagnosis of CSME on the same day is important in understanding the relationship between these two variables but ignores the fact that there is usually a delay between the detection of HE at the time of the DRS visit and the visit of the same patient to the eye care provider either for treatment or evaluation. We have gained an understanding of what that time delay may be and whether it influences the effectiveness of the proposed screening approach.

3.2 Abstract

Purpose. The purpose of this study was to determine whether hard exudates (HEs) within one-disc diameter of the foveola is an acceptable criterion for the referral of diabetic patients suspected of clinically significant macular edema (CSME) in a screening setting.

Methods. One hundred and forty-three adults diagnosed as having diabetes mellitus were imaged using a nonmydriatic digital fundus camera at the Alameda County Medical Center in Oakland, CA. Nonstereo fundus images were graded independently for the presence of HE near the center of the macula by two graders according to the EyePACS grading protocol. The patients also received a dilated fundus examination on a separate visit. Clinically significant macular edema was determined during the dilated fundus examination using the criteria set forth by the Early Treatment Diabetic Retinopathy Study. Subsequently, the sensitivity and specificity of HEs within one-disc diameter of the foveola in nonstereo digital images used as a surrogate for the detection of CSME diagnosed by live fundus examination were calculated.

Results. The mean age of 103 patients included in the analysis was 56 +/- 17 years. Clinically significant macular edema was diagnosed in 15.5% of eyes during the dilated examination. For the right eyes, the sensitivity of HEs within one-disc diameter from the foveola as a surrogate for detecting CSME was 93.8% (95% CI: 81.3% - 100%) for each of the graders; the specificity values were 85.1% (95% CI: 77.0% - 91.9% and 88.4% (95% CI: 81.4% - 95.4%). For the left eyes, the sensitivity values were 93.8 (95% CI: 81.3% - 100%) and 75% (95% CI: 50.0% - 93.8%) for each of the two graders, respectively; the specificity was 87.4% (95% CI: 80.4% - 94.3%) for both graders.

Conclusions. This study supports the use of HE within a disc diameter of the center of the macula in nonstereo digital images for CSME detection in a screening setting.

3.3 Introduction

The International Diabetes Federation recommends that patients with type 2 diabetes mellitus receive a dilated fundus exam by a qualified provider at the time of diagnosis of diabetes and every 1-2 years thereafter if no retinopathy is present[1]. More frequent retinal examinations are indicated if any retinopathy is present. For patients with type 1 diabetes mellitus a dilated fundus exam is recommended 2 years after the diagnosis and annually thereafter[2]. The current data indicate that, on average, only 60% of patients with diagnosed diabetes comply with these recommendations[3]. Even poorer compliance is reported among patients of lower socioeconomic status[4]. This underscores the need for a simple and effective screening tool for the detection of sight threatening retinopathy, since early detection and prompt treatment of retinal disease among diabetic patients can prevent vision loss[5,6].

Tele-ophthalmology screening for diabetic retinopathy has been shown to be effective in detecting diabetic retinopathy in a primary care setting[7]. Stereoscopic digital retinal photography with pupil dilation has been validated as an acceptable method for detecting and grading the severity of diabetic retinopathy (DR) and diabetic macular edema (DME)[8,9]. The international classification of diabetic retinopathy (ICDR) developed by the International Council of Ophthalmology and adopted by the American Academy of Ophthalmology uses the presence and severity of retinal lesion types to stratify the risk of progression to sight-threatening complications from DR[10,11]. Several organizations throughout the world, including the Canadian Teleophthalmology Network in Alberta and Inoveon diabetic retinopathy screening program in Oklahoma, have implemented diabetic retinopathy detection programs using stereoscopic retinal photography and the ICDR[12,13]. Non-mydriatic retinal cameras have been developed to reduce the discomfort and potential hazards of pupil dilation. However, stereoscopic photography without pharmacological pupil dilation is difficult to perform, and it results in images ungradable for retinal thickening in up to 20% of eyes[14,15]. On the other hand, determining retinal thickening in nonstereoscopic images is not possible. Therefore, a number of diabetic retinopathy detection programs, such as the Scottish Diabetic Retinopathy Screening Program[16] and the Veterans Administration Diabetic Retinopathy Screening Program[17], that have adopted nonmydriatic photography, use the presence and location of hard exudates close to the center of the macula, as a surrogate to detect and stage DME. There has been only limited validation of hard exudates as a surrogate for DME. Bresnick et al.[18] performed a retrospective analysis of the photographic database of the Early Treatment of Diabetic Retinopathy Study (ETDRS) using the criterion of hard exudates within one-disc diameter of the foveola, and identified CSME with a sensitivity of 94% and a specificity of 54%. Rudnisky et al.[19] reported the sensitivity of HE within two disc diameters of the foveola to be 93.9% in detecting ophthalmoscopically confirmed CSME; the specificity was reported to be 81.6%. Retinal images, in both of these studies, were obtained after pupillary dilation.
The purpose of the present study was to test in a prospective clinical design the validity of using hard exudates within one-disc diameter of the foveola in non-mydriatic, non-stereo digital retinal images as a criterion for referring diabetic patients suspected of having CSME, compared to the standard clinical technique of stereo biomicroscopy with a condensing lens or a contact fundus lens.

3.4 Methods

This study was conducted at the Alameda County Medical Center in Oakland, CA, a diabetic retinopathy screening site within the EyePACS telemedicine network[20]. Adult patients with known diabetes were recruited for the study. The recruitment process was purposely enriched by patients who were deemed likely to have diabetic retinopathy based on their medical records.

The study protocol required two patient visits to the clinic, one for retinal photography and the other for a live retinal exam. If the interval between the first and the second clinic visit exceeded 100 days, the patient was excluded from the study because of uncertainty regarding the stability of their retinopathy. Retinal imaging was performed during the first clinic visit using a non-mydriatic fundus camera, the CR6-45NM (Canon Inc., Tokyo, Japan) without pupillary dilation. Non-stereoscopic, forty-five degree images of three retinal fields per eye were obtained in accordance with the EyePACS imaging protocol[21]. The primary field included the macula and the optic nerve, the centers of which were located equidistant from the center of the image (default position of the camera. The second field was obtained with the optic nerve at the center of the image. The third field was captured with the optic nerve to the far nasal side of the field with the macula below and nasal to the center of the picture (Figure 3.1).

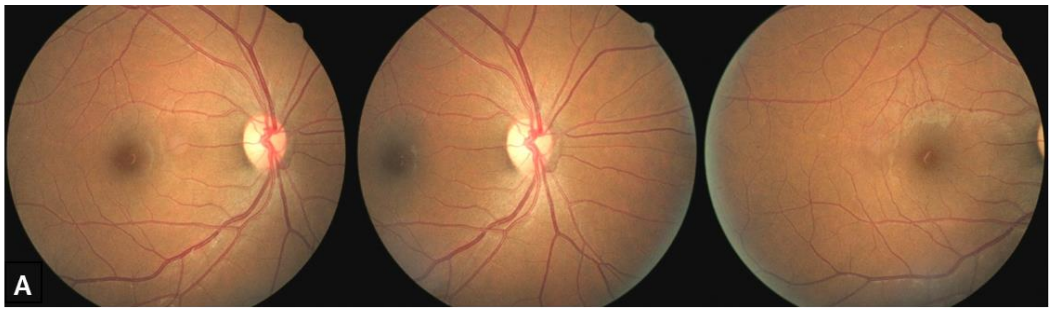

Figure 3.1. Images of the right eye obtained according to the EyePACS imaging protocol.

The captured images were uploaded to the EyePACS website[20] and graded independently for macular edema by two graders according to the EyePACS grading protocol[22]. A presumptive diagnosis of clinically significant macular edema (CSME) was made when hard exudates were noted at or within one-disc diameter of the foveola. During the second clinic visit, patients received a dilated fundus exam of the macular region. The dilated fundus exams were performed by using a non-contact 90D condensing lens and a biomicroscope. The examiner was masked to the retinal imaging findings. The presence, extent, and location of retinal thickening were noted, as well as the presence and location of hard exudates. In those cases in which the presence of retinal thickening could not be determined with certainty, a Goldmann macula contact lens was also used. The diagnosis of CSME on the dilated fundus exam was made according to the criteria set forth by the Early Treatment Diabetic Retinopathy Study[23]: 1) retinal thickening within 500 microns of the center of the macula; or 2) hard exudates within 500 microns of the center of the

macula with adjacent retinal thickening; or 3) retinal thickening of one-disc area in size or greater, any part of which is located at or within one-disc diameter from the center of the macula.

The sensitivity and specificity values for CSME detection using hard exudates at or within one-disc diameter of the foveola graded in the retinal images were calculated and compared to CSME identified during the dilated fundus exam as the "gold standard." The statistical analysis was performed separately for the right (OD) and the left (OS) eyes.

3.5 Results

One hundred forty-three adult diabetic patients were recruited for the study. Forty of these patients were excluded from the study because the time between the first and the second clinic visits exceeded 100 days. The length of this interval was considered as sufficient to cast doubt on the stability of retinal thickening. The mean time interval between the first and the second clinic visits of the remaining 103 patients was 33±31 days. Forty-nine percent were females. The mean age of the included patients was 56±17 years. Ethnic composition of the study population is presented in Figure 3.2.

For the right eyes, CSME was diagnosed in 16 (15.5%) eyes by biomicroscopy during the dilated exam. Based on retinal images, a presumptive diagnosis of CSME was made independently by the two graders in 28 (26.4%) and in 25 (24.2%) cases, respectively (Table 3.1). The agreement between the graders for the detection of HE within 1-disc diameter from the center of the macula in the right eyes was substantial, Cohen's kappa equal to 0.67, percent agreement was 87.3%. The agreement was similar when grading the left eyes, Cohen's kappa – 0.71, percent agreement was 89.3%.

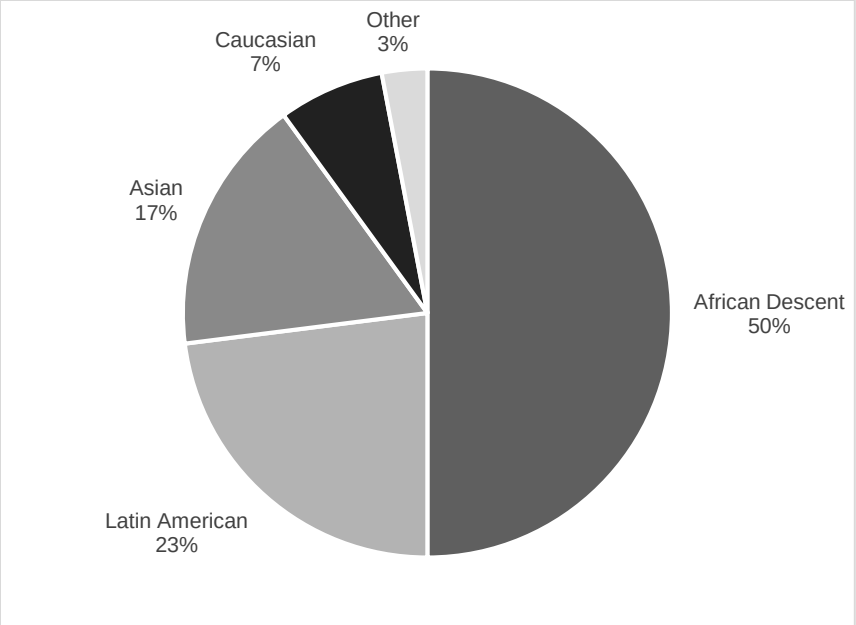

Figure 3.2. Ethnic makeup of the sample.

	CSME On Fundus Exam		**CSME Grader 1 On Image Grading**		**CSME Grader 2 On Image Grading**	
	OD	OS	OD	OS	OD	OS
Number of eyes	16	16	28	26	25	23
Percent Value	15.5%	15.5%	26.4%	25.2%	24.2%	22.3%

Table 3.1. CSME: Dilated biomicroscopic exam vs. Image grading

The ROC curve analysis based on the data from the right eyes for Grader 1 is summarized in Figure 3.3. The area under the ROC curve (AUC) is 89.4% (95% CI: 82.2% - 96.6%). The ROC curve analysis of the left eye data from Grader 1 is shown in Figure 3.4. The AUC is 90.6% (95% CI: 83.5% - 97.6%). The sensitivity, specificity, positive and negative predictive values based on the data from Grader 1, for both right and left eyes, are summarized in Table 3.2.

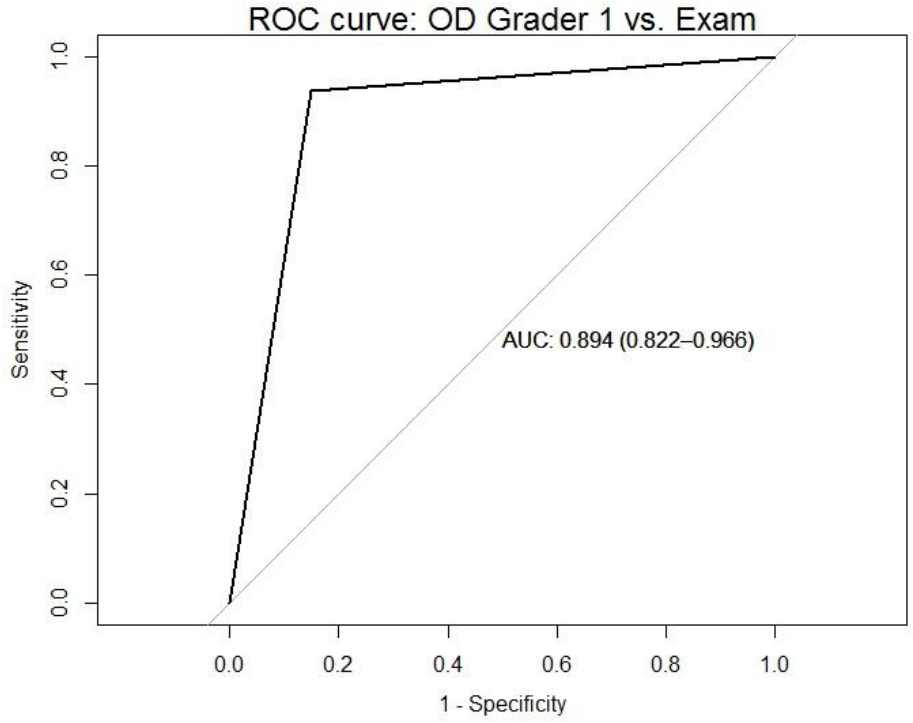

Figure 3.3. Graphical summary of the results based on the analysis of the Grader 1, right eye data.

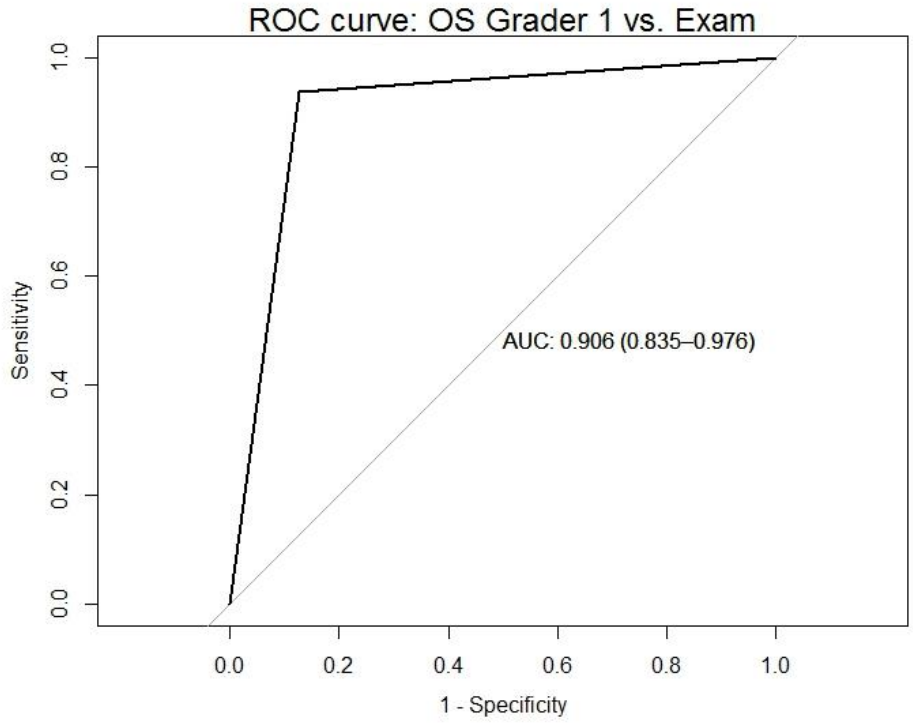

Figure 3.4. Graphical summary of the results based on the analysis of the Grader 1, left eye data.

	Grader 1	
	OD (95% CI)	**OS (95% CI)**
Sensitivity	93.8% (81.3% - 100%)	93.8% (81.3% - 100%)
Specificity	85.1% (77.0% - 91.9%)	87.4% (80.5% - 94.3%)
PPV	53.6% (42.1% - 68.2%)	57.7% (45.5% - 75.0%)
NPV	98.7% (95.9% - 100%)	98.7% (96.1% - 100%)

Table 3.2. Predictive power of the surrogate method of CSME detection based on the data from Grader 1.

The ROC curve analysis based on the data from the right eyes for Grader 2 is summarized in Figure 3.5. The area under the ROC curve (AUC) is 91.1% (95% CI: 84.1% - 98.1%). The ROC curve analysis of the left eye data from Grader 2 is shown in Figure 3.6. The AUC is 81.2% (95% CI: 69.7% - 92.7%). The sensitivity, specificity, positive and negative predictive values based on the data from Grader 2, for both right and left eyes, are summarized in Table 3.3.

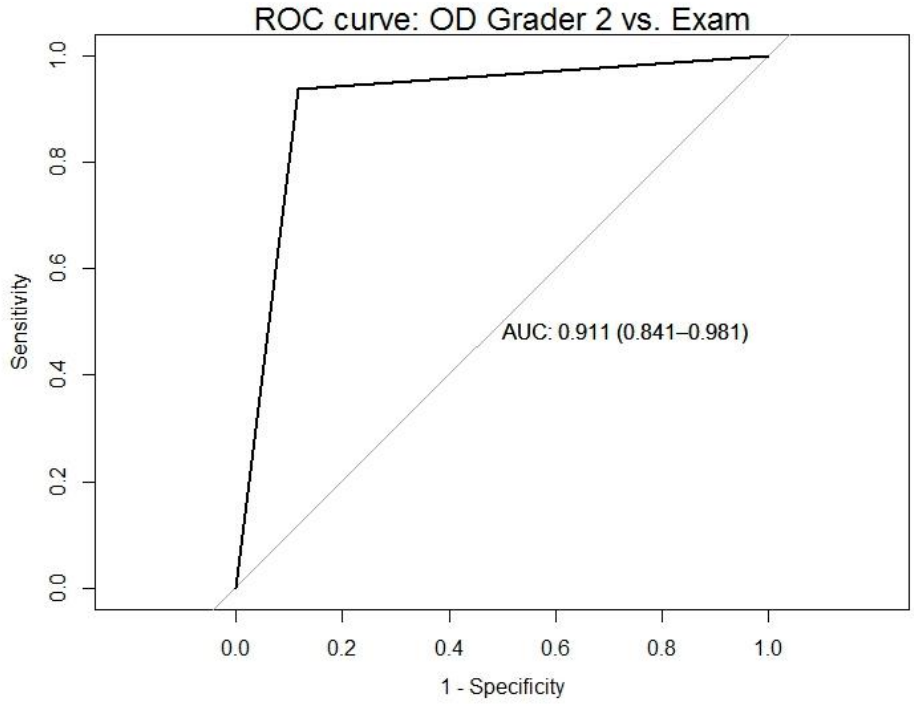

Figure 3.5. Graphical summary of the results based on the analysis of the Grader 2, right eye data.

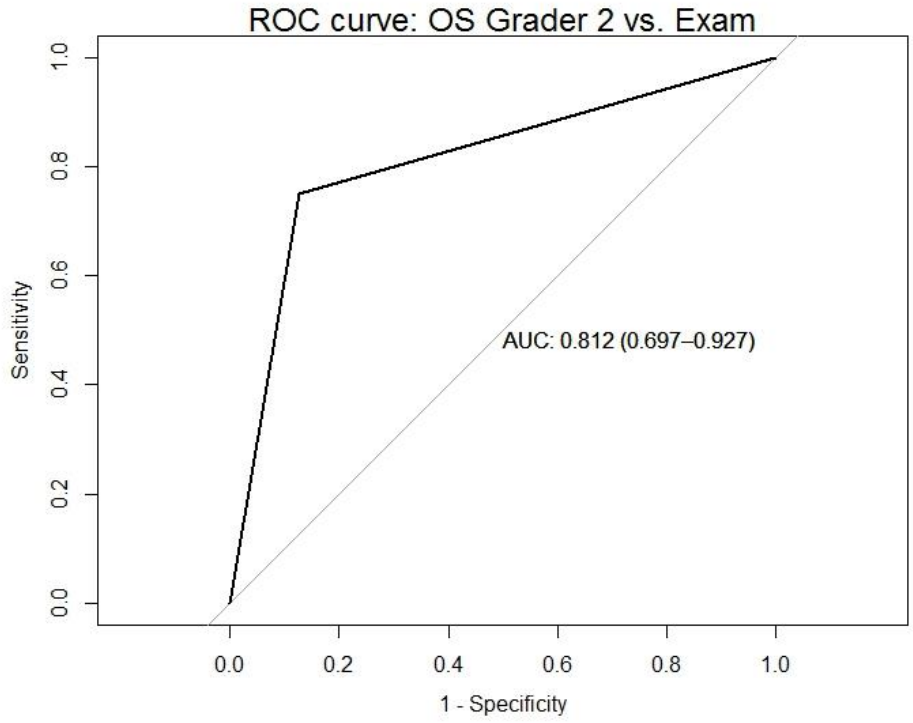

Figure 3.6. Graphical summary of the results based on the analysis of the Grader 2, left eye data.

	Grader 2	
	OD (95% CI)	**OS (95% CI)**
Sensitivity	93.8% (81.3% - 100%)	75.0% (50.0% - 93.8%)
Specificity	88.4% (81.4% - 95.4%)	87.4% (80.4% - 94.3%)
PPV	60.0% (47.1% - 76.5%)	52.2% (37.5% - 70.6%)
NPV	98.7% (96.0% - 100%)	95.0% (90.8% - 98.7%)

Table 3.3. Predictive power of the surrogate method of CSME detection based on the data from Grader 2.

3.6 Discussion

When a hard exudate was present at or within one-disc diameter from the foveola in undilated non-stereoscopic fundus photographs, CSME (determined by a dilated biomicroscopic fundus examination) was detected with good sensitivity and specificity. These results indicate that HE is a valid surrogate marker for the detection of CSME when stereophotography is inadequate, unavailable, or infeasible.

Having a valid alternative for the detection of CSME is important for several reasons. It assures that diabetic retinopathy screenings can successfully detect sight-threatening macular edema without dilation and/or stereoscopic photographs of the macula. In addition, diabetic retinopathy screenings that rely on non-mydriatic stereo-imaging can result in a high proportion of ungradable images for the detection of retinal thickening[14,15]. When one image of a stereo-pair for the macula is unusable, a valid surrogate marker for CSME is very useful. The results of this study also suggest that HE near the center of the macula can be used by primary care providers and emergency room physicians to screen for CSME using a direct ophthalmoscope.

Our sensitivities correspond to results published by Bresnick[18] comparing hard exudates within one-disc diameter of the center of the macula versus "gold standard CSME" graded in ETDRS stereoscopic photographs. Our specificities are somewhat higher than those reported by Bresnick, although both sensitivities and specificities are similar to data published by Rudnisky in 2006[19] reporting the ability of hard exudates within two disc diameters of the fovea to detect CSME that was confirmed by a dilated fundus examination using a retinal contact lens. However, our study is different from these other two studies because we obtained our retinal images undilated, more closely matching the common screening condition of nonmydriatic, nonstereo retinal imaging.

The lower sensitivity demonstrated by Grader 2 in detection of CSME in the left eyes prompted further investigation. Of the three CSME cases that Grader 2 missed, one case showed unmistakable exudates well within one-disc diameter of the foveola, and therefore may have been a case of data-entry error. The other two cases showed a single small exudate at the border of one-disc diameter from the foveola. The difference in grading in those cases may be attributed to variability in judgment between graders for the threshold of hard exudates detection by each grader. This highlights the importance of testing grading systems for intra- and inter-grader repeatability, and of performing quality control of image grading in DR screening programs.

Low positive predictive values point to a relatively high "over-referral," although it may be acceptable in a screening setting, particularly since this sample only included diabetic patients. In this case it is more advantageous to over-refer than under-refer since the consequence of under-referral is the significant degradation of patient's vision. In addition, it has been shown that eyes with hard exudates at or within 1-disc diameter of the center of the macula, but without CSME, have about a 50% risk of developing CSME in one year (Bresnick, GH, unpublished data). Therefore, one could argue that these high-risk eyes should be followed more closely even in the absence of CSME. This study further validates the use of the hard exudates surrogate by screening programs that utilize non-stereoscopic images for the detection of diabetic retinopathy.

This study has limitations. First, the time interval between imaging and dilated eye exams was 33±31 days. It is possible that existing edema may have resolved during this period, causing the specificities found in this study to be underestimated. It is also possible that new edema developed, artificially increasing our sensitivities. However, both possibilities are unlikely since our findings are similar to those reported by Rudnisky[19]. In their study, fundus photographs were obtained on the same day as a dilated fundus exam and the presence of HE within 2DD of the fovea was used as a surrogate for CSME detection. They report that HE within 2DD of the foveola has a sensitivity of 93.9% and a specificity of 81.6% for CSME detection. Thus, it may be inferred that any significant changes in retinal thickening occur slowly, and that the elapsed time in our study likely did not have a major impact on our results.

Finally, the most widely accepted gold standard for detecting CSME is the use of 30-degree film-based stereo macular photographs performed and graded according to the ETDRS protocol[24]. However, our use of the dilated biomicroscopic exam as the gold standard in the present study is supported by the high correlation reported between CSME detected by contact lens biomicroscopy compared with CSME detection by the ETDRS protocol[25].

Further validation studies to compare the hard exudates surrogate for macular thickening with more objective means such as optical coherence tomography (OCT) will be reported in Chapter 5. While OCT is widely accepted as an objective method for detecting diabetic maculopathy, it is currently too costly and technically challenging to integrate into existing retinopathy detection programs in primary care settings. Low-cost and reliable methods of detecting CSME, such as the use of a hard exudate surrogate marker described here, are needed to meet the challenge of widespread screening for this vision threatening condition.

3.7 References

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Chapter 4: Proximity of Hard Exudates to the Foveal as a Marker of Severe Clinically Significant Macular Edema

4.1 Prelude

Results of the study covered in Chapter 3 support the use of the CSME detection method based on the presence of HE within one-disc diameter from the center of the macula. These results are not substantially different than those reported when a two-disc diameter cutoff is used for the detection of CSME. Several screening programs rely on the detection of HE within 500 microns from the center of the macula. We have speculated that the proximity of HE to the fovea may be associated with more severe cases of DME, requiring an expedited referral. To our knowledge, there was no attempt to test this hypothesis prior to this study. In addition to providing evidence for the management of patients at highest risk for vision loss, this study proposed at OCT-based adaptation of the DME severity scale. While OCT is routinely used in clinical practice for the evaluation of patients with DME as well as used by clinical trials to define inclusion criteria, there is no information regarding the risk of vision loss associated with various features and extent of OCT-detected retinal edema. ETDRS has reported on the risk of vision loss based on certain retinal features but without the use of OCT because this technology was not available at that time. The semi-quantitative approach for classifying the severity of DME and correlating it with the extent of vision loss was developed based on ETDRS data. We have relied on this semi-quantitative approach to develop OCT-based classification of DME into severe and non-severe types. The results of this study were published in the Journal of Diabetes Science and Technology in 2015.

4.2 Abstract

Background: Hard exudates (HE) are used as a surrogate marker for sight-threatening diabetic macular edema (DME) in most telemedicine-based screening programs in the world. This study investigates whether proximity of HE to the center of the macula, and extent of HE are associated with greater clinically significant macular edema (CSME) severity. A novel method for associating optical coherence tomography (OCT) scans with CSME was developed.

Methods: Eligible subjects were recruited from a DRS program in a community clinic in Oakland, California. The ocular fundus of each subject was imaged using 3-field 45-degree digital retinal photography and scanned using central 7-line spectral domain OCT. Two certified graders separated subjects into 2 groups, those with and without HE within 500 microns from the center of the macula. A modified DME severity scale, developed from Early Treatment Diabetic Retinopathy Study data and adapted to OCT thickness measurements, was used to stratify CSME into severe and non-severe levels for all subjects.

Results: The probabilities of severe CSME in groups 1 and 2 were 14.4% (95% CI: 8.2%-23.8%) and 9% (95% CI: 2.4%-25.5%), respectively (P = .556). In a post hoc analysis, an increase in the number of sectors affected by HE within the central zone of the macula was associated with the increase in the probability of being diagnosed with severe CSME.

Conclusion: We have proposed OCT-based classification of DME into severe and nonsevere CSME. Based on this limited analysis, severity of CSME is related more to the extent of HE rather than proximity to the center of the macula.

4.3 Introduction

Diabetes mellitus is becoming one of the most prevalent diseases worldwide, with well over 300 million people projected to be affected by the year 2030.[2] According to different estimates, between 9% and 14% of adults in the United States have diabetes and almost a third of those are undiagnosed.[3,4] Microvascular complications of diabetes remain the leading cause of blindness among working-age adults in the US. [5] The economic burden of diabetes in the United States is difficult to overstate with an estimated $245 billion spent in 2012. This includes direct medical costs as well as costs related to disability and inability to work.[3] In spite of the scope of the problem, nationwide compliance with regular eye exams is only 60% in patients with diabetes.[6] In an effort to improve compliance with regular eye care among diabetic patients, diabetic retinopathy screening (DRS) programs in primary care and diabetes clinics have been implemented and shown to be effective.[7,8]

After proliferative diabetic retinopathy (PDR), the second most common cause of persistent severe vision loss in patients with diabetes is diabetic macular edema (DME).[9] The Early Treatment Diabetic Retinopathy Study (ETDRS) defined a subset of DME, clinically-significant macular edema (CSME), and demonstrated that prompt laser photocoagulation for CSME reduces the likelihood of moderate vision loss by 50%.[10] Since then, the use of anti-VEGF therapy that inhibits vascular endothelial growth factor was shown not only to reduce the risk of vision loss in patients with CSME but also to improve visual acuity and reduce central macular thickness.[11,12,13] Therefore, timely detection and prompt referral for suspected CSME by DRS programs is of critical importance. DRS programs generally use non-mydriatic, 2-dimensional monoscopic fundus photographs for the detection of sight-threatening retinopathy. Rather than directly observing increased retinal thickening from edema, they must rely on a surrogate marker of CSME. Presence and location of hard exudates (HE) are used to detect CSME, since the stereoscopic (3-dimensional) effect needed to detect retinal thickening (edema) is absent in such images. In patients with diabetes, eyes with HE within one optic nerve disc diameter (1DD – approximately 1800 microns) of the center of the macula manifest CSME more frequently than those without HE within 1DD. This surrogate marker has been shown to have good utility in referring patients with suspected CSME in a screening setting.[14,15] It is unknown whether eyes with HE within 500 microns of the fovea present with more severe sight-threatening diabetic macular edema than those with HE located greater than 500 microns but within 1DD from the fovea and, therefore, would require a more urgent referral. In an effort to improve the triaging ability of DRS programs, this study aims to answer this question.

4.4 Methods

Subject recruitment and data collection was carried out at four community health clinics, part of the Alameda Health System (AHS) in Northern California. Subjects were recruited from a consecutive stream of patients evaluated as part of an established DRS program. All patients with diabetes mellitus were eligible for recruitment if they were at least 18 years of age, not pregnant, and were able to understand and give informed consent to participate in the study. We obtained Retina Map OCT scans and three-field fundus photographs on both eyes of 1,814 adult patients with diabetes. The study protocol was approved by the Institutional Review Boards of the AHS and the University of California, Berkeley. Three-field non-mydriatic digital fundus photographs were obtained using a CR6-45NM fundus camera (Canon Inc., Tokyo, Japan) according to the

EyePACS protocol.[16] Macular thickness optical coherence tomography (OCT) scans were obtained using the iVUE OCT (Optovue, Inc., Fremont, CA) Retina Map scanning protocol. Only one eligible eye from each patient was used for analysis. 159 eyes with HE within 1DD from the center of the macula were initially selected. 36 eyes were excluded because of macular pathology other than diabetic macular edema or because of poor image quality. The level of retinopathy was established in each eye based on grading of the digital fundus photographs performed by an EyePACS-certified grader following the International Clinical Diabetic Retinopathy Severity Scale.[17]

Custom software, Matlab (MathWorks, Natick, MA) was used to apply a measuring grid to the photographs to judge the proximity of HE to the fovea and to determine the location in the central macula affected by hard exudates (Figure 4.1). We used a scaling factor to determine the distance from the fovea based on the measured pixels. Eyes were classified by two masked graders (TL and CW) as those having hard exudates within 500 microns from the center of the fovea (Group 1) and those with HE located at a distance greater than 500 microns from the center of the fovea but within 1DD (Group 2). In case of disagreement between the two graders, adjudication was performed by a third expert grader.

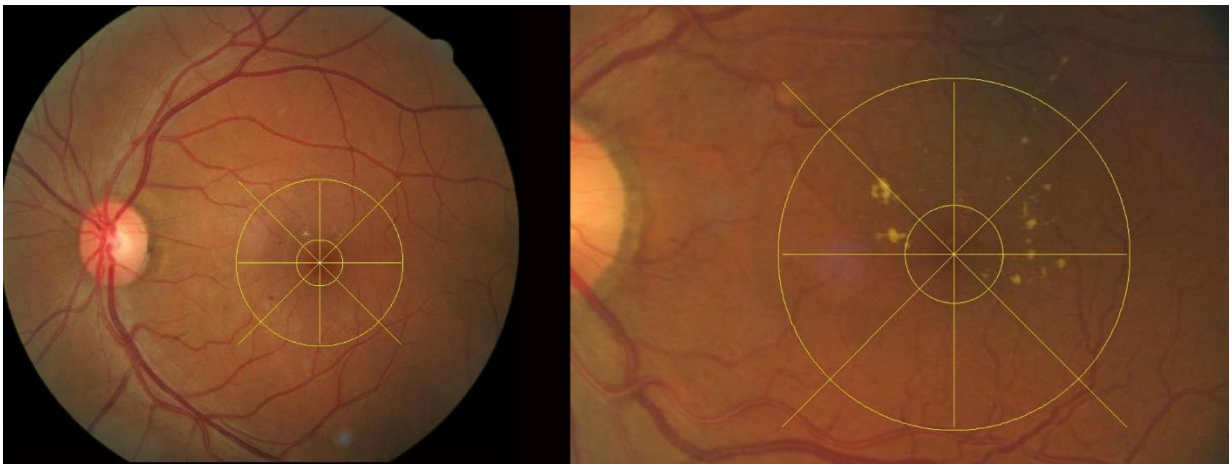

Figure 4.1. Digital fundus photographs. The radius of outer ring centered on the fovea is 1DD in size. The inner ring centered on the fovea is 500 microns in radius. A) 45^0 image showing hard exudates located further than 500 microns away from the center of the macula but within 1DD. HE are present in two out of eight sectors within the central zone (CZ – within 1DD). B) An enlarged image of the macula. HE are located within 500 microns from the center of the macula and in five out of eight sectors within the CZ.

Statistical analysis was performed in R (https://cran.r-project.org/). We have used a two-sided, two-sample t-test to compare groups with continuous variables. The Mann-Whitney test was performed when variables did not follow a normal distribution. A chi-square test was used to evaluate differences in proportions. The Fisher exact test was used when the cell count was small

(e.g. Table 4.1, Ethnicity). Inter-grader agreement was evaluated using Cohen's kappa. We also used logistic regression analysis to evaluate the relationship between the severity of CSME and the number of macular sectors affected by hard exudates, the proximity of HE to the center of the macula, the subject's age and sex, and the duration of diabetes. After selecting the most significantly associated independent variable we used ROC analysis to evaluate the characteristics of the screening test for the detection of severe CSME based on that variable. The ROC curve was built and analyzed using pROC package in R.[18]

4.4.1 Adaptation of the Clinically Significant Macular Edema Severity Scale to Optical Coherence Tomography

In order to stratify eyes by the severity of CSME and to determine the proportion of eyes with severe CSME in Groups 1 and 2, we adapted a modified diabetic macular edema severity scale developed from ETDRS data.[19] The ETDRS severity scale for diabetic macular edema was derived based on the extent of visual impairment as a function of the degree of retinal thickening in the macular center and the size and location of retinal thickening within the central zone (CZ, the area within 1DD from the center of the macula).[19] We adapted this scale to the OCT measurements in the following way. We defined severe CSME as the level of thickening in the central macula corresponding to Level 3B or worse on a 9-step ETDRS scale which was associated with the number of letters read at baseline equal to or less than 77[19]. This value approximates Snellen acuity of 20/30 or worse. We chose this level of visual impairment as a cutoff because we consider it to be clinically significant. Eyes were deemed to be in the severe CSME category if they met at least one of the following three criteria that match Level 3B or worse and are based on OCT scan analysis: 1) Retinal thickening at the macular center (central ring of 1mm in diameter centered on the fovea), defined as central macular thickness (CMT), greater than two standard deviations from the mean normal thickness derived from a normative database[20] but less than 1 x the reference thickness (RT-the 95^{th} percentile of normal retinal thickness in the region located 1 to 3 mm from the macular center) AND retinal thickening within the CZ that is at least one disc area in size; 2) CMT greater or equal to 1 x RT but less than 2 x RT AND area of retinal thickening within CZ that is equal to or greater than ½ disc area; 3) CMT equal to or greater than 2 x RT(Figure 4.2). It has been shown that age and sex have an effect on retinal thickness[21,22,23] so we corrected our cutoff RT values based on these parameters for each individual subject. These RT values were derived from a regression equation based on normative database.[20]

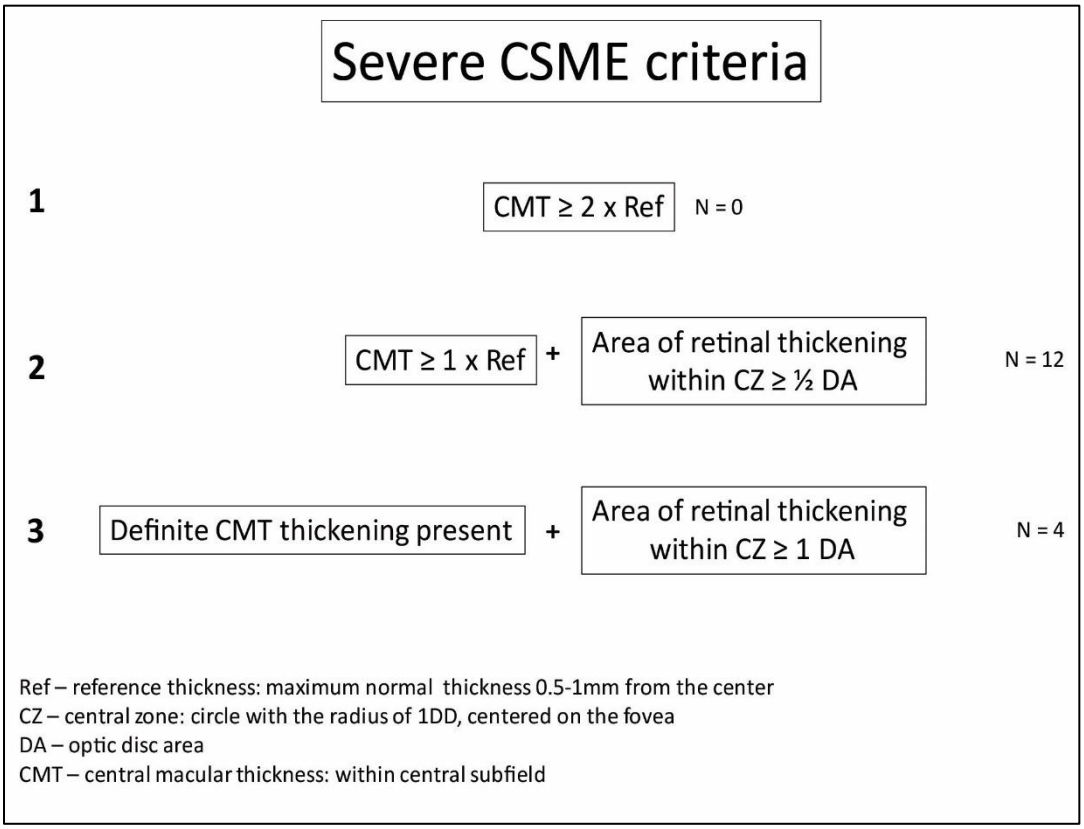

Figure 4.2. **S**evere CSME criteria based on the adaptation of diabetic macular edema severity scale. Ref was calculated as the 95th percentile of normal retinal thickness between 0.5 mm and 1mm from the fovea and adjusted based on subject's age and sex. The average Ref in this sample was 346.6 ± 4.6 microns. There were 16 cases of severe CSME out of 123.

The area of retinal thickening in the CZ was established by inspecting each topographic map and b-scans (Figure 4.3). We calculated and compared the proportion of eyes that met the criteria for severe CSME in Group 1 and Group 2.

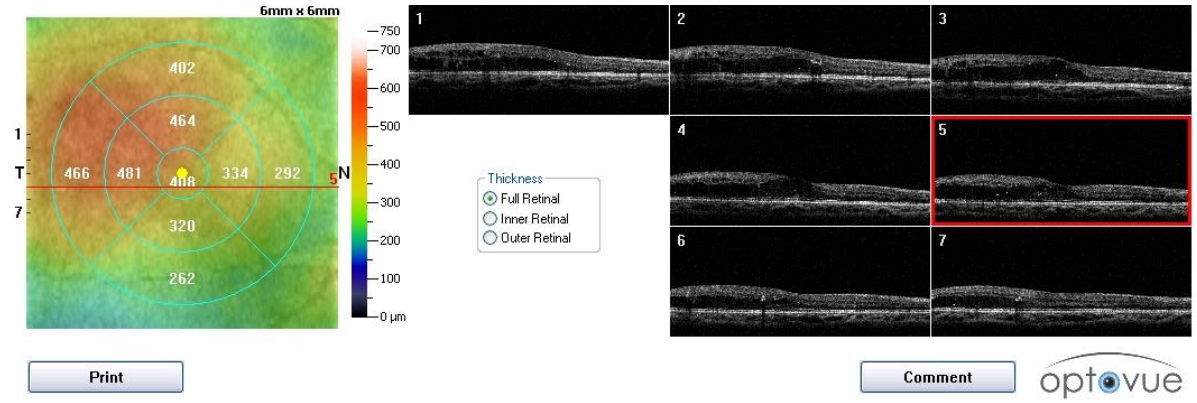

Figure 4.3. iVue OCT scan showing 6x6 mm topographic map on the left. The inner ring radius is 500 microns, the middle ring radius is one-disc diameter (CZ). Seven b-scans shown on the right are collected in the center of the macula with separation of 250 microns. The topographic map shows significant swelling within the CZ affecting the area that is at least 1DD in size. B-scans show accumulation of fluid in all seven scans.

4.5 Results

123 subjects with HE within 1DD from the center of the macula were included in the final analysis. The mean age of the sample was 54.9 ± 8.3 years. There were 54 (43.9%) females. HE within 500 microns of the center of the macula were observed in 90 subjects (73.2%) (Group 1). The inter-grader agreement for judging the location of HE from digital fundus photographs was good (k = 0.7). The distribution of retinopathy in our sample is shown in Figure 4.4.

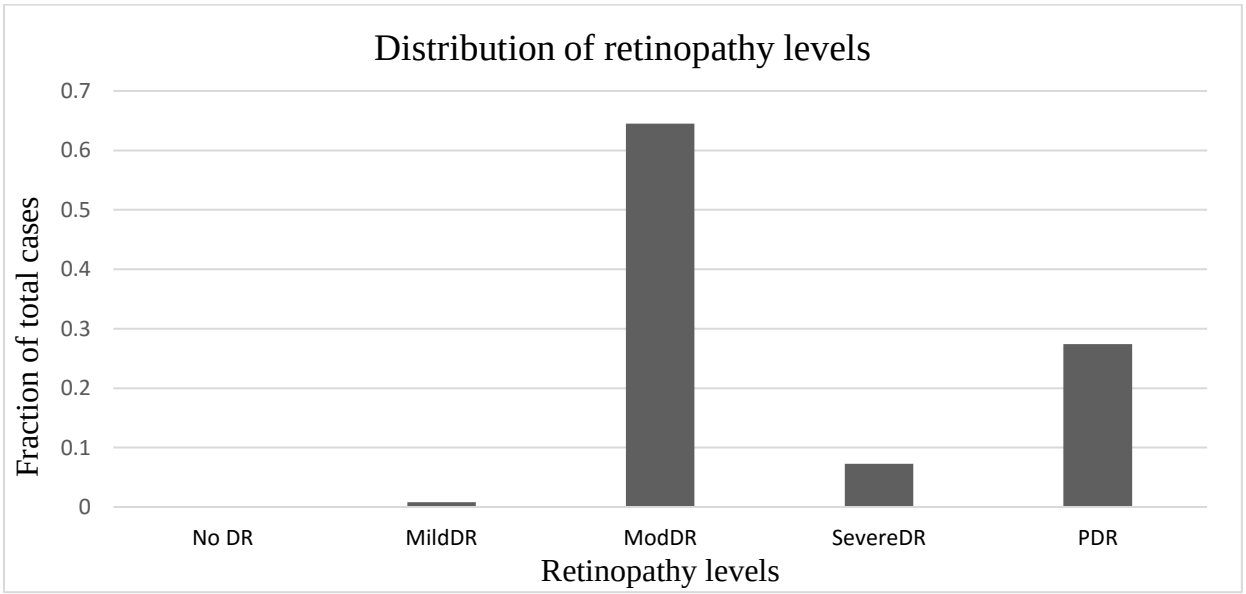

Figure 4.4. Distribution of retinopathy levels in the entire sample. Of note is the tendency towards more severe levels of retinopathy in this sample.

The overall prevalence estimate of severe CSME in our sample was 13.0% (95% CI: 7.8%-20.5%) (Figure 4.2). The demographic data is shown in Table 4.1.

	Sample Total	Group 1	Group 2	p-value
N (%)	123 (100)	90 (72.5)	33 (27.4)	
Females (%)	54 (43.9)	36 (40)	18 (54.5)	0.217
Mean age (sd)	54.9 (8.3)	54.7(8.4)	55.4(8.1)	0.684
Mean duration of DM (sd)	11.5 (5.9)	11.7(6.0)	10.7(5.6)	0.358
Ethnicity (%)				0.159
African Descent	26 (21.1)	23 (25.6)	3 (9.1)	
Hispanic	43 (35.0)	30 (33.3)	13 (39.4)	
Asian	45 (36.6)	29 (32.2)	16 (48.5)	
White	8 (6.5)	7 (7.8)	1 (3.0)	
Other	1 (0.8)	1 (1.1)	0	

Table 4.1. Descriptive statistics. P-values relate to the test statistic used to evaluate the difference in the corresponding variables between Group 1 and Group 2.

There was no difference in age, duration of diabetes, sex of the subject and ethnic composition between Groups 1 and 2. The prevalence estimate of severe CSME was 14.4% (95% CI: 8.2%-23.8%) in Group 1 and 9% (95% CI: 2.4%-25.5%) in Group 2. The difference in probabilities of severe CSME diagnosis was small, about 5%, between Group 1 and Group 2 and was not statistically significant (p-value = 0.556), as seen in Table 4.2. The associated odds ratio was 1.83 (95% CI: 0.46-10.66). However, it must be noted that our sample sizes were not large enough to confidently detect that small of a difference, if it was truly present. We have also compared the absolute value of CMT in Group 1 and Group 2. In Group 1, the median, 25^{th} and 75^{th} percentiles were 250.0 microns, 231.5 microns, and 271.8 microns, respectively. In Group 2 the corresponding values were 252.0 microns, 234.0 microns, and 267.0 microns (Figure 4.5). There was no difference in CMT between the two groups (p-value = 0.9). In post-hoc analysis, increase in the number of sectors affected by HE within the central zone of the macula was associated with the increase in the probability of being diagnosed with severe CSME (Figure 4.6). In the logistic regression analysis, patient's age, sex, and the number of macular sectors affected by HE were significantly associated with the presence of severe CSME (corresponding p-values: 0.011, 0.045, <0.001) when keeping other variables constant (Table 4.3). An increase in each year of subject's age was associated with the odds ratio of 1.14 (95% CI: 1.04-1.28) for having severe CSME. Females had significantly lower relative risk of having CSME compare to males with the odds ratio of 0.21 (95% CI: 0.036-0.871). Finally, a one-unit increase in the number of macular sectors

affected by HE was associated with the odds ratio of having severe CSME equal to 2.18 (95% CI: 1.45-3.55).

		Severe CSME	
		Present	Absent
HE within 500 microns from the center of the macula	Present	13	77
	Absent	3	30

Table 4.2. The group of subjects with the absence of HE within 500 microns from the center of the macula is equivalent to the group of subjects with HE located between 500 microns and 1DD from the center of the fovea. Fisher exact test was used to evaluate the association between the location of HE with respect to the center of the macula and the presence of severe CSME. Resulting p-value is 0.556 which means that subjects with HE within 500 microns of the center of the macula do not have higher proportion of severe CSME when compared to subjects with HE located between 500 microns and 1DD from the center of the macula.

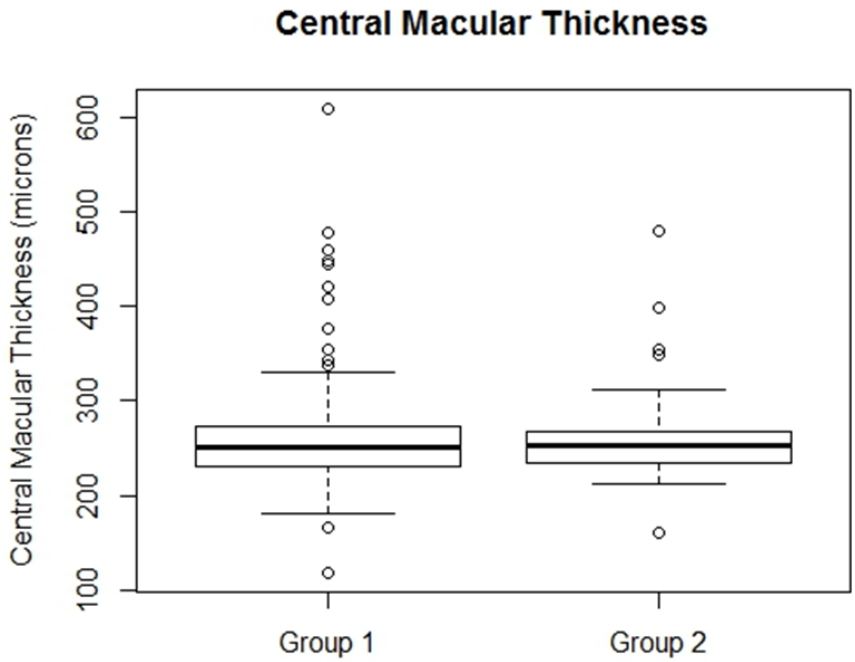

Figure 4.5. Box plot showing the distribution of CMT values in Group 1 and Group 2. The median value for each corresponding group is represented by the bold line inside each box. 75th percentile is represented by the upper boundary of the box plot. 25th percentile is represented by the bottom boundary of the box plot. Empty circles are outliers. A Mann-Whitney test was used to compare CMT distribution in Groups 1 and 2. The resulting p-value is 0.9. This means that the distribution in Group 1 is not different from the distribution in Group 2.

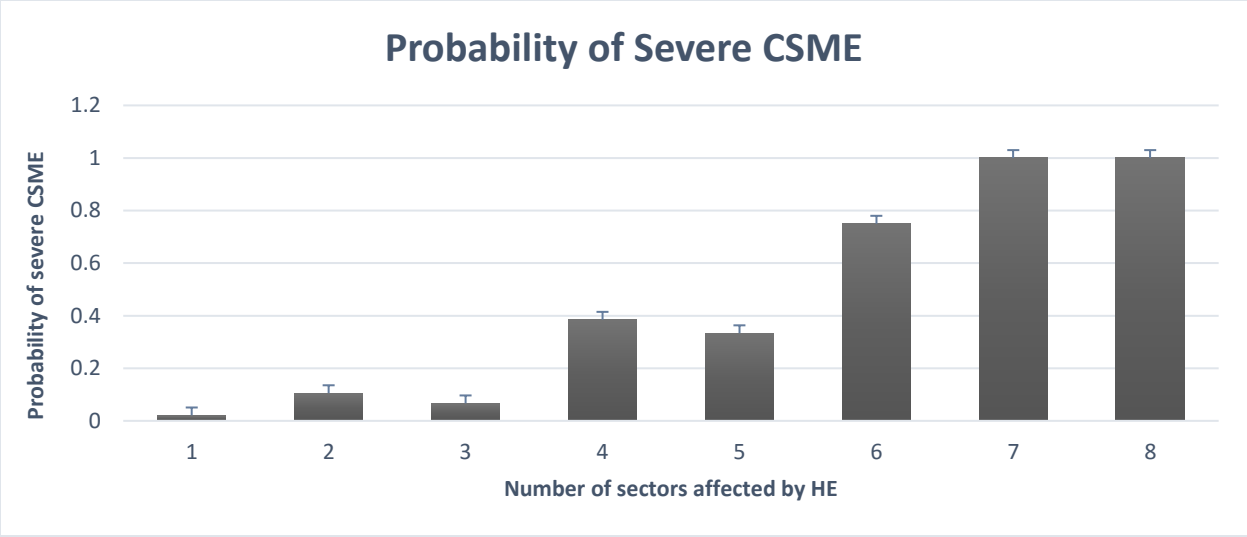

Figure 4.6. Probability of severe CSME as a function of the number of sectors within CZ that are affected by hard exudates.

Variable	Coefficient	p-value	Odds Ratio	95%CI
HE location	-0.257	0.755	0.773	0.158, 4.47
Subject's age	0.134	0.011*	1.14	1.04, 1.28
Duration of DM	-0.03	0.572	0.968	0.861, 1.08
Subject's sex	-1.58	0.045*	0.205	0.036, 0.871
Number sectors	0.779	<0.001*	2.18	1.45, 3.55

Table 4.3. Results of the logistic regression analysis. Severe CSME is the outcome variable. A one-year increase in subject's age was associated with increase in the odds ratio of 1.15. Female sex had a protective effect, with an odds ratio of 0.18. An increase by one additional macular sector affected by HE was associated with the odds ratio of 2.39.

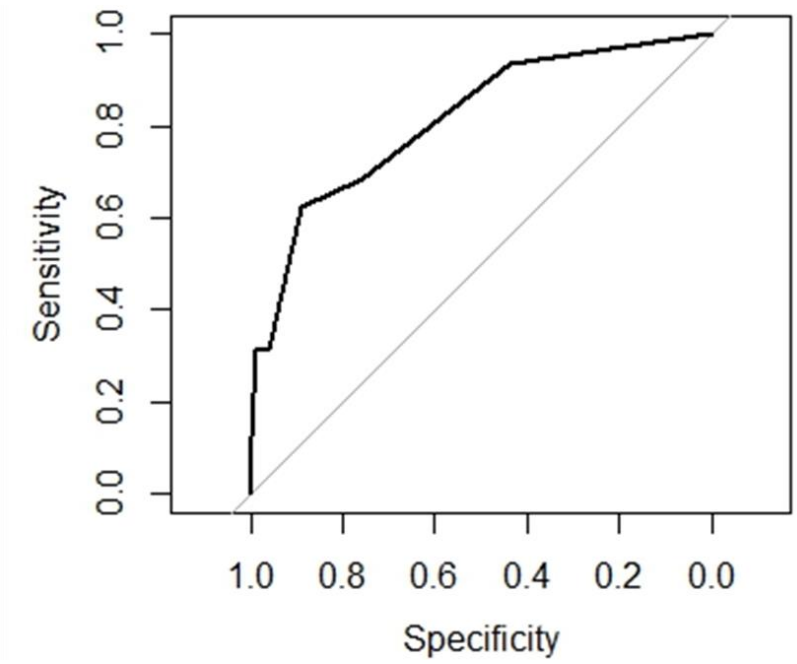

Figure 4.7. ROC curve illustrating test characteristics for the detection of severe CSME based on different number of macular sectors affected by HE. Area under the curve (AUC) = 81.54% (95% CI: 69.92-93.17).

An ROC curve was plotted using the number of sectors as a predictor of severe CSME (Figure 4.7). The AUC was 81.5% (95% CI: 69.9%-93.2%). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the local maxima on the ROC curve (thresholds) are shown in Table 4.4. The overall "best" cutoff value is 3.5 – that is, if more than three macular sectors are affected by HE, the sensitivity of this screening test is 62.5% and the specificity is 88.8%.

Threshold (# of sectors)	**1.5**	**2.5**	**3.5**	**5.5**	**6.5**
Sensitivity	93.75%	68.75%	62.50%	31.25%	12.50%
Specificity	43.93%	75.70%	88.79%	99.06%	100.00%
Positive Predictive Value	20.00%	29.73%	45.45%	83.33%	100.00%
Negative Predictive Value	97.92%	94.19%	94.06%	90.59%	88.43%

Table 4.4. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) for the different cutoff (threshold) values of the ROC curve based on the number of macular sectors affected by HE.

4.6 Discussion

We have proposed an OCT-based classification of diabetic macular edema (DME) into severe and non-severe CSME (Figure 4.2). The OCT-based approach to evaluating the severity of DME has the advantage of providing objectivity and, potentially, reducing the inter-observer variability as well as adding precision to the estimate of change over time. This classification is proposed based on our adaptation of the diabetic macular edema severity scale reported by Gangnon, et al.[19] We have adapted this severity scale because it offers two crucial components required for OCT-based classification of CSME into severe and non-severe subtypes. This scale defines the severity levels in semi-quantitative terms which can be replicated using OCT data and, importantly, it links each severity level to the degree of visual impairment which is an accepted criterion for defining the severity of DME. This adaptation is important because the information regarding the risk of vision loss in CSME was derived by the ETDRS study when OCT technology was not available.[10,24] In addition, the correlation between visual acuity and the absolute measurement of central retinal thickness in patients with DME is only modest which makes it difficult to establish an absolute cutoff value for CSME.[25]

We were able to use this classification to determine if an urgent referral for suspected sight-threatening diabetic macular edema is required by diabetic retinopathy screening programs based on the proximity of hard exudates to the center of the macula. Out of all the eyes with HE within 1DD of the center of the macula, nearly three quarters had HE located within 500 microns of the center of the macula. In our sample, the difference in the probability of being diagnosed with severe CSME in the group of eyes with HE within 500 microns of the center of the macula and those with HE between 500 microns and 1DD was small and not statistically significant. In addition, the central macular thickness was not different in those two groups. These results should be interpreted cautiously because of the relatively small sample sizes of the two groups compared and the risk of Type II error.

Additional analysis of our data revealed that the measure of extent to which HE affect the central macula improves the ability to discriminate between the eyes with severe CSME and those without it. We have used the number of sectors within the central zone as the measure of extent to which HE affect the central macula (Figure 4.1). The probability of being diagnosed with severe CSME increased as the number of sectors affected by HE within the central zone of the macula increased. A plausible explanation for this association between a larger number of sectors within the central zone and the increased likelihood of severe CSME presence is that this reflects the extent of blood-retina barrier permeability. The formation of HEs is associated with the breakdown of blood-retina barrier and increase in retinal vascular permeability.[26,27] It has been shown that the increase in blood-retina barrier permeability was significantly higher at baseline and at 18-month follow up visit in eyes that eventually developed CSME than in the eyes that did not.[28] Therefore, an increased number of sectors affected by HE within the central zone likely indicates a greater extent of increased blood-retina barrier permeability and may be useful in deciding on the urgency of referral once the patient is suspected of having CSME based on the presence of HE within 1DD from the center of the macula. We recognize that fundus photographs obtained during screening may vary slightly in orientation. We do not expect the orientation of the macular grid relative to the macular orientation to substantially change the utility of this test because the measure of interest is the number of sectors affected by hard exudates and not their specific segment location. Screening test characteristics for different cutoff values are provided in Table 4.4. A screening

program may adopt various cutoff values to implement a more urgent referral strategy reflecting the unique needs of the community it serves based on the evaluation of tradeoff between over-referral and under-referral with the ultimate goal of improving the cost-effectiveness of the screening program.

The limitation of this study is that the proposed OCT-adaptation of the diabetic macular edema severity scale has not been validated on an independent set of data. We intend to perform this validation in the future. This, however, does not limit the outcomes of the study because we have also compared the absolute central macular thickness in Group 1 and Group 2 and did not find it to be statistically different. In addition, the severe CSME group clearly included eyes with more advanced cases of CSME and therefore provides information regarding the distribution of more advanced cases of CSME in Groups 1 and 2. Another limitation of this study is a relatively small sample size. It is possible that in a larger sample size a small effect size could be evaluated more confidently.

It has been reported that racial minorities have a higher rate of sight-threatening complications from diabetes and lower compliance with eye exams than non-Hispanic Whites.[29] A strength of this study is that non-Hispanic Whites account for only 6.5% of our sample with the vast majority of our subjects representing Asian, Hispanic and African Descent ethnic groups. This ethnic distribution makes it easier to generalize the results of our study to the groups of people who stand to benefit most from the improvement in detection of sight-threatening diabetic retinopathy. Other programs with very different demographic characteristics should interpret our results with that in mind.

4.7 References

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Chapter 5: A New Approach for the Detection of Sight-Threatening Diabetic Macular Edema

5.1 Prelude

In the previous chapter we demonstrated the association between the number of radially arranged sectors affected by HE in the central macula and the severity of DME. This method may have further use as a detection approach for all types of CSME. In this chapter we report a study which was designed to evaluate the accuracy of this new approach in detecting CSME and suggest its implementation within the existing DRS model. In addition, we were interested in developing a model utilizing all available predictors of CSME which are feasible for use in a screening setting. In particular, previous studies have suggested that abnormalities in the amplitude and latency of the electrical response in the retina elicited using a 30 Hz photopic flicker may be associated with CSME presence. We have considered the level of overall retinopathy as one of the main confounding factors in this potential association. We have used statistical approach to control for this and other potential confounders in developing an overall model. We intend to publish the results of this study in 2016.

5.2 Abstract

Background: Clinically significant macular edema (CSME) is a leading cause of vison loss among patients with diabetes. The indicators of accuracy of the current approaches for the detection of CSME leave room for improvement. Here, we evaluate a new approach for the detection of CSME. We rely on a grid of radially arranged sectors to estimate the combined measure of the areal extent and proximity to the fovea of hard exudates (HE) as a surrogate for CSME.

Methods: 225 consecutive adult diabetic patients were enrolled. Presence of CSME was established by two methods: 1) dilated fundus exam (DFE) in conjunction with macular OCT and 2) stereoscopic macular photography. A macular grid composed of radially arranged sectors centered on the fovea was applied to the images of one eye of each patient. Two graders, masked to the results of the DFE and to the results of each other counted the number of Sectors affected by hard exudates (HE), and, during a separate grading session, established the presence of CSME from stereoscopic photographs. Discrepant cases were adjudicated by a third grader. ROC curve analysis was used to evaluate the accuracy of the Sectors in detecting CSME established by each method separately. A hand-held ERG device was used to measure the implicit time and amplitude of a photopic 30 Hz flicker ERG response, using skin electrodes.

Results: 207 eyes were analyzed. The inter-grader agreement for counting the number of Sectors was substantial (k = 0.67). The inter-method agreement for the diagnosis of CSME was also substantial (k = 0.75). Method 1 detected CSME in 9 eyes (4.3%). Method 2 - in 12 eyes (5.8%). When evaluating Sectors for their ability to detect CSME diagnosed on DFE, the area under the ROC curve was 97.6% (95% CI: 95.1% - 100%). When compared against the stereoscopic photographs, the area under the ROC curve was 93.2% (95% CI: 84.2% - 100%). The optimal sensitivity (91.7%, 95%CI: 75% - 100%) is achieved by using 1 Sector as a cutoff. The optimal specificity (96.9%, 95%CI: 94.4% - 98.9%) is achieved when using 3 Sectors as a cutoff. ERG implicit time was significantly (p=0.048) associated with the diagnosis of CSME but did not perform better than Sectors for the detection of CSME (area under the ROC curve – 80.9% (95%

CI: 68.1% - 93.6%)). The combined model which included Sectors and ERG implicit time did not perform better than Sectors alone.

Conclusion: The proposed detection method allows for an easy threshold optimization to aid in decision-making with respect to the timing as well as the type of service (general vs. specialty) to be specified in the referral. This grading approach will keep false positives and negatives to a desired minimum, while requiring no additional resources for Diabetic Retinopathy Screening programs.

5.3 Introduction

Diabetic retinopathy remains the leading cause of vision impairment in working-age adults in the US[1]. Clinically-significant macular edema (CSME) is a leading cause of vision loss in patients with diabetes affecting an estimated 2.7% of adults with diabetes[1,2,3]. The risk of vision loss associated with CSME can be significantly reduced and the chance of vision gain can be increased with appropriate treatment when detected in time[2,4]. CSME was originally defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) in terms of retinal thickening as detected by stereo retinal photography or clinical examination with stereo biomicroscopy[4]. In the absence of stereo photography or stereo biomicroscopy, the National Health and Nutrition Examination Survey (NHANES) and other epidemiologic studies, as well as various teleophthalmology programs rely on surrogate markers for the detection of CSME. Most, if not all, such programs rely on the detection of hard exudates (HE), bright-yellow lipoprotein deposits in the central macula, on two-dimensional digital fundus photographs as a surrogate for CSME detection[5,6,7,8,9]. There is, however, a lack of agreement on the best parameters for grading the presence and severity of CSME with respect to the location and the extent of HE in the macula[10,11]. In addition, a simple presence of HE in the macular region has been shown to have modest specificity[10,11,12]. A recent study revealed that close to 39% of patients with center-involved diabetic macular edema diagnosed by optical coherence tomography (OCT) which would be eligible for treatment were missed using NHANES screening criteria. Of those missed, over 25% had visual acuity of 20/40 or worse[13]. There is a clear need to improve the accuracy of CSME detection using new screening approaches which are validated against the accepted standards for determining the presence of CSME.

Our previous study showed that the proximity of HE to the fovea, 500 microns vs 1800 microns, was not associated with a higher proportion of subjects with more severe cases of CSME[14] (Chapter 4). We have proposed a new screening approach which relies on counting the number of radially arranged sectors (Sectors) centered on the fovea that are affected by HE. In the exploratory analysis (Chapter 4) we discovered that the proportion of subjects with more advanced degrees of CSME increased as the number of Sectors affected by HE increased[14].

The main objective of the current study was to investigate the screening test accuracy of Sectors for the detection of any CSME by comparing this screening method against: (1) digital stereo photography – a gold standard used in research[15,16,17,18,19] - and (2) a dilated fundus exam supplemented by the information from the OCT – a gold standard used in clinical practice[20,21,22]. The need for inclusion of OCT information for the detection of CSME has been recognized in a number of publications as it increases objectivity and diagnostic accuracy[11,13,23,22,24]. The inclusion of the two gold standard diagnostic modalities has allowed us to evaluate the internal validity of

the study by quantifying the inter-method agreement for CSME diagnosis. In addition, we can determine how robust our estimates of the screening test characteristics are by comparing the estimates calculated against the two different diagnostic modalities.

Our secondary aim was to evaluate whether the measurement of implicit time (IT) and amplitude (Amp) of the 30Hz photopic flicker electroretinogram (ERG) as well as patient's clinical and demographic characteristics may improve the detection of CSME in a screening setting. The inclusion of the ERG measurements was prompted by reports of abnormalities in the latency and amplitude of ERG waveform in patients with diabetic macular edema[25,26], and the development of the hand-held ERG technology specifically designed for the ease of implementation in a clinical setting[27,28].

5.4 Methods

Two hundred and twenty-five consecutive diabetic patients presenting for an eye exam at Eastmont Wellness Center, Alameda Health System in Oakland, CA were enrolled in this study. All non-pregnant adult patients with a diagnosis of diabetes mellitus type 1 or 2 and without prior history of glaucoma or non-diabetic macular pathology were eligible for enrollment. The study protocol was approved by the University of California Berkeley and the Alameda Health System Institutional Review Boards. Enrollment occurred between June of 2014 and December of 2015. All patients underwent refraction and a dilated fundus exam. 30Hz photopic flicker ERG of each eye was recorded prior to the dilation using RETeval device (LKC Technologies, Inc.). Blood pressure measurements were carried out using Connen ProBP 3400 unit (Welch Allyn, Inc.). Macular optical coherence tomography (OCT) scans of each eye were obtained after pharmacologic pupillary dilation using the Retina Map protocol on iVUE SD-OCT (Optovue, Inc.) (Figure 5.1).

Subsequently, three 45-degree digital photographs of the ocular fundi were obtained using Canon CR-DGI (Canon, Inc.) following EyePACS diabetic retinopathy screening (DRS) protocol[29]. In addition, non-simultaneous stereoscopic 45^0 digital photographs of the macula were obtained using a modified technique first described by Lee Allen in 1964 and detailed by Tyler.[30,31] The agreement for the diagnosis of CSME was shown to be good between the standard 30^0 stereoscopic film photographs and the 45^0 stereoscopic digital photographs[16]. All of the images were uploaded to a secure location in the EyePACS database. Patient's medical records were accessed to obtain clinical data measured within three months of imaging.

The level of retinopathy for each eye was established by evaluating digital fundus images by two experienced EyePACS-certified graders using the EyePACS grading system[32] following the International Clinical Diabetic Retinopathy Severity Scale.[33] Graders were blind to the findings of each other. Inter-grader agreement for the diagnosis of the level of retinopathy was evaluated using unweighted and weighted Cohen's kappa and percentage agreement value. When weighted kappa

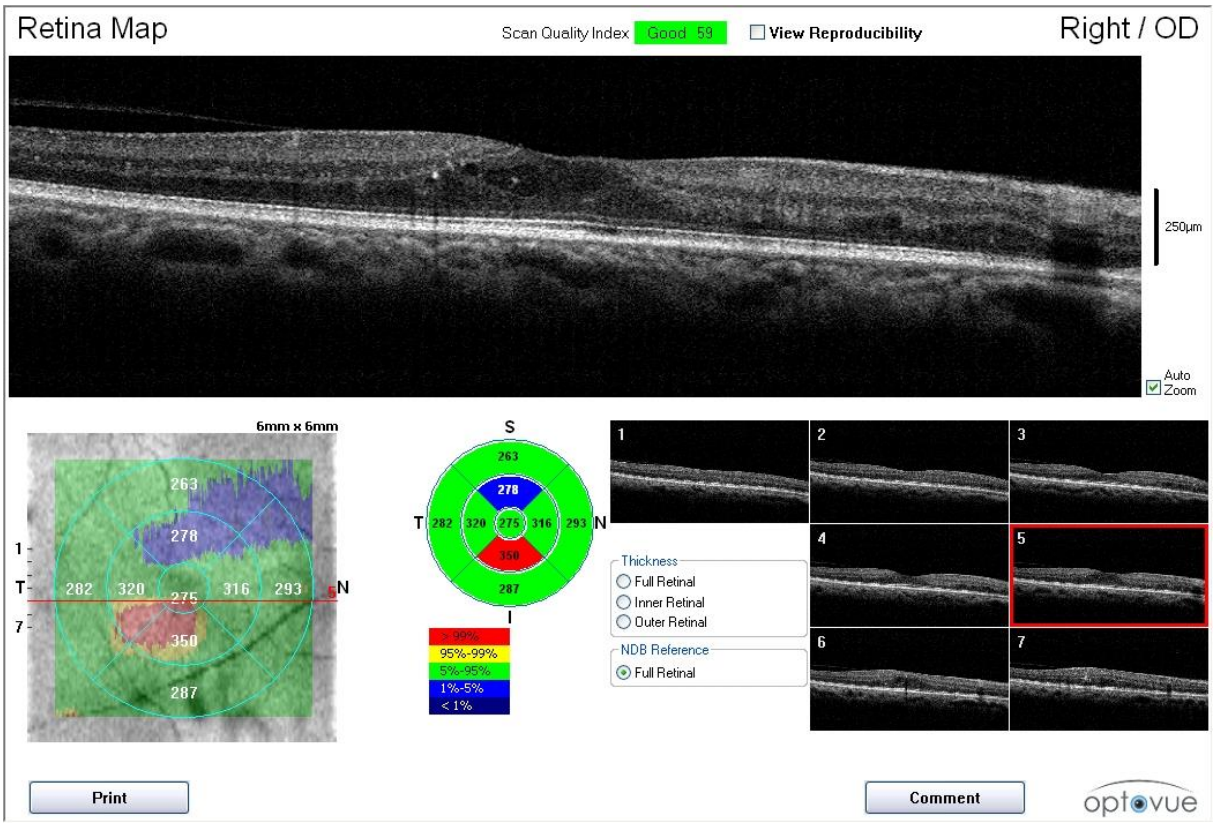

Figure 5.1. Illustration of the information from the OCT scan of the macula available to a clinician to aid in the diagnosis regarding the presence of CSME. In this case, an area of retinal thickening within 500 um from the center of the macula is apparent. The area of thickening extends into inferior and temporal quadrants.

was calculated, the weight of 1 was assigned to exact agreement and the weight of 0.75 was assigned for within one step agreement. The value of zero was assigned for the disagreement of more than one step.

Clinical diagnosis of CSME was established at the time of the exam by a clinician performing a dilated non-contact stereoscopic fundus biomicroscopy and after reviewing macular OCT scans. Clinicians were not required to use any specific cutoff value for the central subfield thickness for the diagnosis of CSME, although it is understood that retinal thickening less than 300 microns is difficult to detect reliably during stereoscopic biomicroscopy[34]. Rather, evaluation of OCT scans was performed in the context of the clinical exam with access to the iVUE OCT normative retinal thickness data and examination of the b-scans (Figure 5.1). Clinicians integrated information obtained from the dilated biomicroscopy and OCT scans to arrive at a clinical decision as would be done in practice. Stereoscopic photographs of the macula were evaluated by the two graders masked to the grading results of each other and to the results of the clinical diagnosis of CSME (Figure 5.2). One grader, was also involved in clinical evaluation of roughly half of the enrolled subjects; however, the grading of the stereoscopic photographs occurred several months after the clinical examination to reduce the possibility of recall. Stereoscopic photographs were graded on

a 27-inch color-calibrated monitor with 1920x1080 resolution. Stereoscopic pairs were presented on the monitor using custom program (Matlab, Mathworks, Inc.) and viewed through the Screen-Vu stereoscope. The diagnosis of CSME was established based on the ETDRS criteria[4]. Cases with intergrader disagreement were adjudicated by a third expert grader.

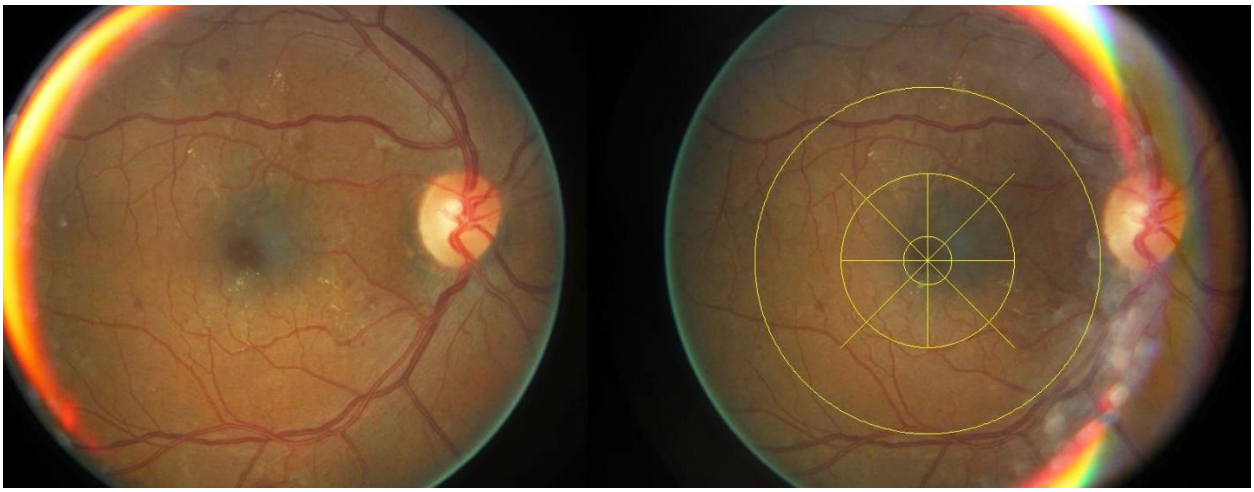

Figure 5.2. Stereoscopic pair of the right eye with a macular grid applied to the right image. The radii of the inner, middle and outer circles of the grid are 500 um, 1800 um and 3600 um, respectively.

One of the monoscopic images captured during the non-simultaneous stereophotography, equivalent to the standard EyePACS Field 3[29], was used to determine a number of sectors affected by hard exudates. A custom Matlab program was used to place the eight-sector grid centered on the fovea to assure the consistency in the orientation of the grid placement throughout the subjects and the graders (Figure 5.3). The number of sectors within a circle with the radius equal to one-disc diameter that were affected by hard exudates (Sectors) were established by the two graders blind to each other's grading (Figure 5.3). This grading was performed during sessions separate from the grading for the presence of CSME to minimize recall. The discrepancies in grading were adjudicated by a third grader. Weighted and unweighted Cohen's kappa and percent agreement was used to evaluate the inter-grader and inter-method agreement. The weight of 1 was assigned for the exact agreement and the weight of 0.75 was assigned for the agreement within one step. The weight of zero was assigned for disagreement of more than one step.

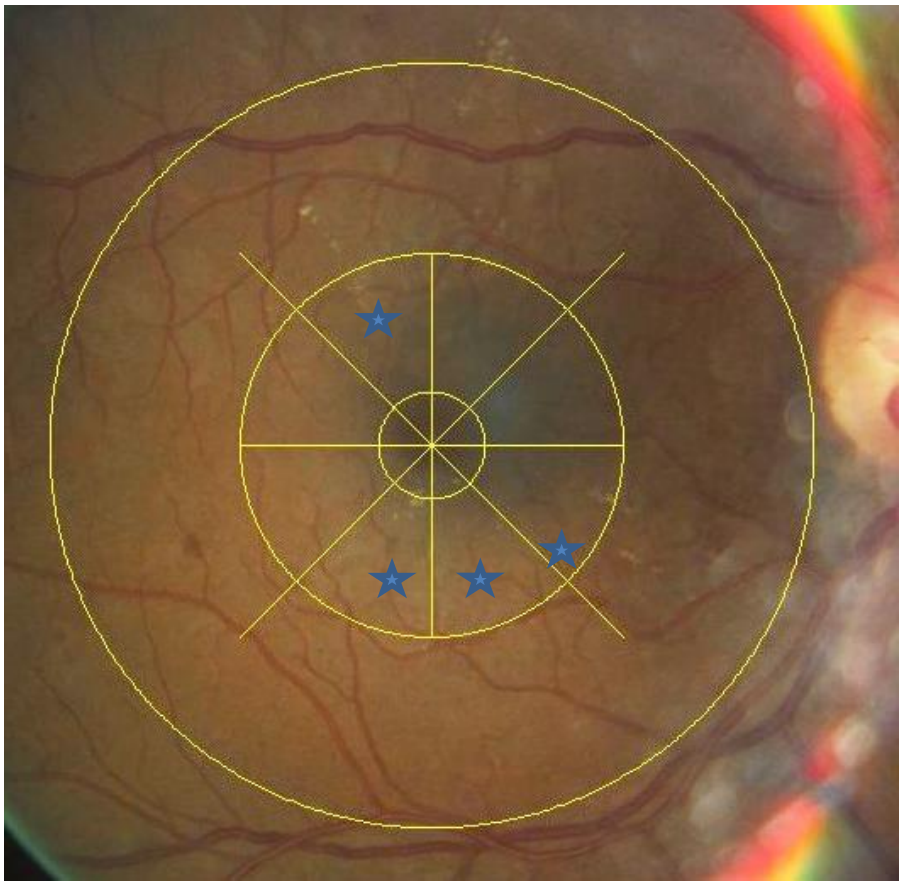

Figure 5.3. Macular grid with eight radially arranged sectors centered on the fovea. Only HE within one-disc diameter of the fovea (middle circle) were considered. A number of radial sectors affected by HE were counted. In this case, four sectors (starred) are affected by hard exudates.

A single eye per patient was used for the analysis to eliminate the need for correlation adjustment when both eyes of the same patient are included in the analysis. Exclusion criteria based on pre-existing conditions and poor image quality were applied on per-eye basis. The exclusion criteria were as follows, presence of any macular pathology other than diabetic macular edema, media opacity resulting in ungradable fundus images, retinal vascular occlusion, and intraocular pressure greater than 21mm Hg. Of the remaining eyes, those with CSME diagnosis based on either of the diagnostic modalities were eligible for inclusion. In cases where both eyes and neither of the eyes were diagnosed with CSME, the right eye was selected for the analysis.

All of the analysis was performed in R (p-cran.org). A two-sided t-test was used for comparison of continuous data. A Mann-Whitney test was used to compare continuous data that is not normally distributed. Fisher's exact test was used to assess categorical data. The significance level was set at p-value = 0.05. ROC curves and the indicators of diagnostic accuracy were calculated using pROC and Epi packages[35,36].

5.5 Results

Two hundred and seven eyes from 207 patients were selected for the final analysis. The demographic and clinical variables of the sample are summarized in Table 5.1. The CSME and non-CSME groups were balanced with respect to the clinical and demographic characteristics. Nine cases (4.3%) of CSME were identified during the OCT-assisted dilated fundus exam and twelve cases (5.8%) were identified during the grading of the stereoscopic photographs. The inter-grader agreement regarding the diagnosis of CSME based on stereoscopic photographs of the macula was substantial, based on the classification scale proposed by Landis and Koch[37], kappa = 0.66, percent agreement was 97.7%. The inter-method agreement for CSME diagnosis was substantial, kappa = 0.75, percent exact agreement was 97.6%. The inter-grader agreement in establishing the number of Sectors was substantial for the unweighted kappa – 0.67 and almost perfect for the weighted kappa – 0.81, percent exact agreement was 90.8% and percent within one sector agreement was 98.1%. The distribution of the diabetic retinopathy levels is shown in Figure 5.4. The inter-grader agreement for the retinopathy level diagnosis was substantial when agreement was calculated as unweighted kappa - 0.67 as well as weighted kappa - 0.80. The percent agreement between the two graders for the diagnosis of the level of diabetic retinopathy was 80.3% for the exact agreement and 97% for the agreement within one level of retinopathy. When evaluating the accuracy of Sectors in its ability to discriminate between patients with and without CSME, the presence of which was confirmed during the dilated fundus exam, the area under the curve (AUC) was 97.6% (95% CI: 95.1% - 100%) (Figure 5.5). The sensitivity, specificity, positive and negative predictive values (PPV, NPV) for the two optimal thresholds of 1 and 3 Sectors are summarized in Table 5.2.

The optimal thresholds were selected by evaluating the local maxima on the corresponding ROC curves and selecting those thresholds which resulted in the best sensitivity or specificity values while keeping the value of the other parameter in this pair at or above 75%. A cutoff value of 1 Sector has maximum sensitivity of 100%. A cutoff value of 3 Sectors has the best specificity value of 96.5% (95% CI: 93.4% - 98.9%). When evaluating the same Sector characteristics for the detection of CSME diagnosed by evaluating stereoscopic photographs, the AUC was 93.2% (95% CI: 84.2% - 100%) (Figure 5.6). The sensitivity, specificity, PPV, NPV for the three optimal thresholds of 1 and 3 Sectors are summarized in Table 5.3. Consistent with previous analysis, the best sensitivity is achieved by setting a cutoff value at 1 Sector – 91.7% (95% CI: 75% – 100%). Best specificity is achieved by setting the cutoff value at 3 Sectors – 96.9% (95% CI: 94.4% - 98.9%).

The ERG IT was significantly delayed in the CSME group when compared to those without the diagnosis of CSME but Amplitude was not significantly different (Figure 5.7). Initially, ERG IT was evaluated separately for its ability to detect CSME. This ROC curve is shown on Figure 5.8. The AUC is 80.9% (95%CI: 68.1% - 93.6%). This is well below the performance of Sectors summarized in Figures 5.5 and 5.6.

	Sample Total	(+) CSME	(-) CSME	P-value
Number of participants N (%)	207 (100)	12 (5.8)	195 (94.2)	
Females N (%)	122 (59.2)	6 (50.0)	116 (59.5)	0.55
Age in years (sd)	53.6 (10.8)	52.3 (8.3)	53.7 (10.9)	0.44
Duration of Diabetes, yrs (sd)	8.9 (7.4)	11.9 (7.4)	8.8 (7.4)	0.09
Ethnicity				0.06
Hispanic or Latino	125 (60.4%)	7 (58.3%)	118 (60.5%)	
Black or African American	39 (18.8%)	3 (25.0%)	36 (18.5%)	
Asian	21 (10.1%)	0	21 (10.8%)	
White	15 (7.2%)	0	15 (7.7%)	
American Indian	3 (1.5%)	0	3 (1.5%)	
Pacific Islander	4 (1.9%)	2 (16.7%)	2 (1.0%)	
Hemoglobin A1c % (sd)	8.4 (2.1)	8.8 (1.1)	8.3 (2.1)	0.17
Total Cholesterol, mg/dL (sd)	181.3 (67.3)	203 (48.1)	180 (68.3)	0.13
Systolic Blood Pressure, mmHg (sd)	129.1 (15.9)	130.5 (12.0)	128.9 (16.1)	0.62
Diastolic Blood Pressure, mmHg (sd)	76.5 (10.4)	78.6 (11.0)	76.4 (10.3)	0.43
Blood Urea Nitrogen, mg/dL (sd)	7.5 (7.6)	9.0 (7.7)	7.4 (7.6)	0.3
Creatinine, mg/dL (sd)	0.9 (0.6)	1.7 (2.0)	0.8 (0.3)	0.13

Table 5.1. Summary of clinical and demographic characteristics in the sample. Summary calculations are based on CSME cases diagnosed from stereoscopic photographs.

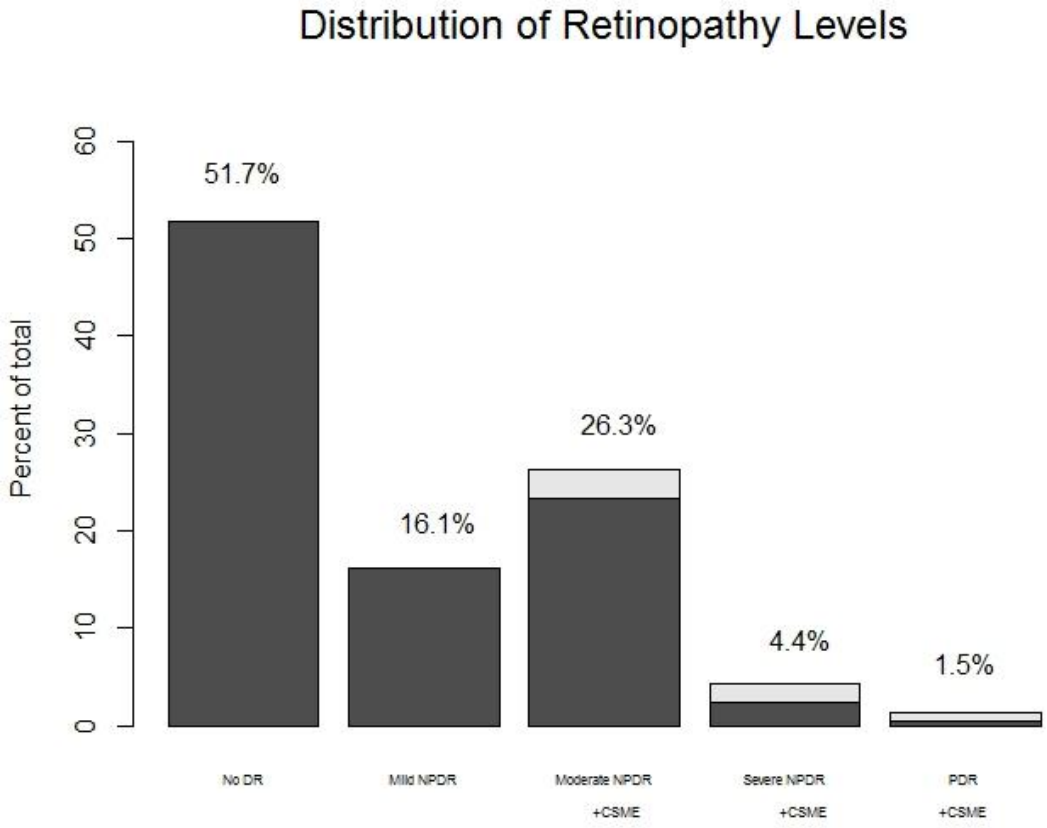

Figure 5.4. Distribution of retinopathy levels. Lighter shaded sub-bars represent the proportion of CSME cases within the corresponding level.

The multivariable logistic regression analysis was performed to assess the association between the measured clinical variables and the diagnosis of CSME. We have included those predictor variables that were significant in a bivariate analysis (p-value < 0.05). Those variables were Sectors, implicit time or the latency in the peak of the waveform (IT) of the 30Hz photopic flicker ERG, and the level of retinopathy. No interactions were found between the variables. Only Sectors (p-value = 0.039) and IT (p-value = 0.048) were found to be significantly associated with the diagnosis of CSME. The odds ratios derived from the regression model are summarized in Table 5.4. The goodness of fit of this model was assessed by plotting the probabilities predicted from the model against the probabilities calculated from the actual data(Figure 5.9). While it appears that the fit may be reasonably good for the lower range of probabilities, this plot is difficult to interpret because of a few probability intervals (only four data points on the plot). This is likely due to the relatively low number of CSME cases in the sample.

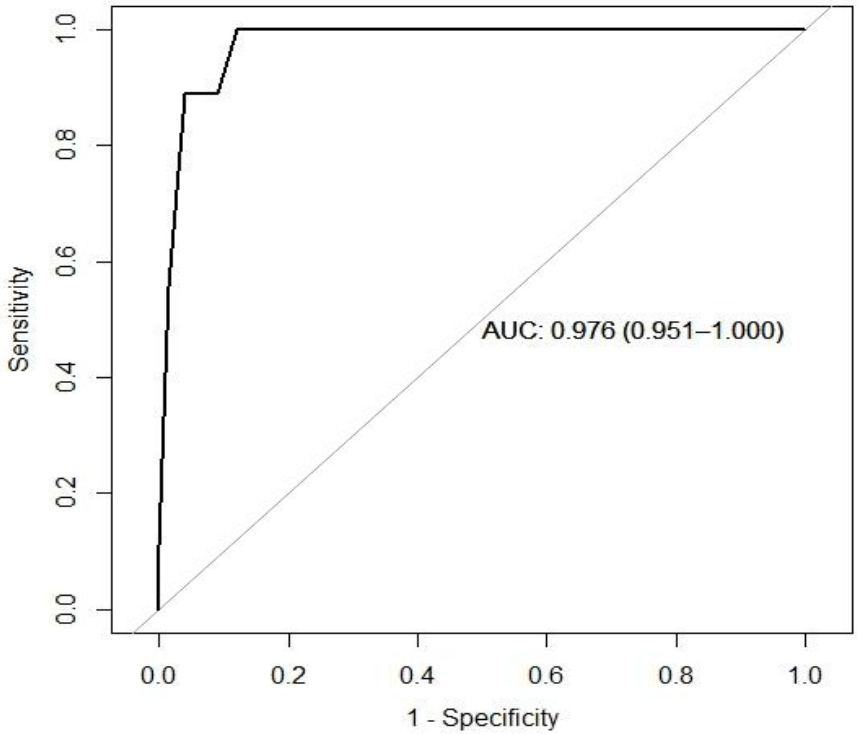

Figure 5.5. ROC curve: performance of Sectors in detection of CSME confirmed during the DFE.

Number of Sectors	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
1	100%	87.9% (83.3% - 92.4%)	27.3% (21.4% - 37.5%)	100%
3	88.9% (66.7% -100%)	96.5% (93.4% - 98.9%)	53.3% (37.5% - 80.0%)	99.5% (98.4% - 100%)

Table 5.2. Summary of the screening test characteristics: Number of sectors affected by hard exudates vs. CSME presence confirmed during an OCT-assisted dilated fundus exam.

An increase by one Sector was associated with the odds ratio of 2.07 for being diagnosed with CSME when holding the level of retinopathy and ERG implicit time constant. A one millisecond increase in the ERG implicit time was associated with a 46% increase in the odds of being diagnosed with CSME when holding the level of retinopathy and Sectors constant. We have evaluated whether this multivariable model would perform better as a screening tool for the detection of CSME when compared to using Sectors alone. The ROC curve based on this multivariable regression model is shown in Figure 5.10. The screening test characteristics of this model (AUC = 91.2%) are not better than when using Sectors alone (AUC = 93.2%).

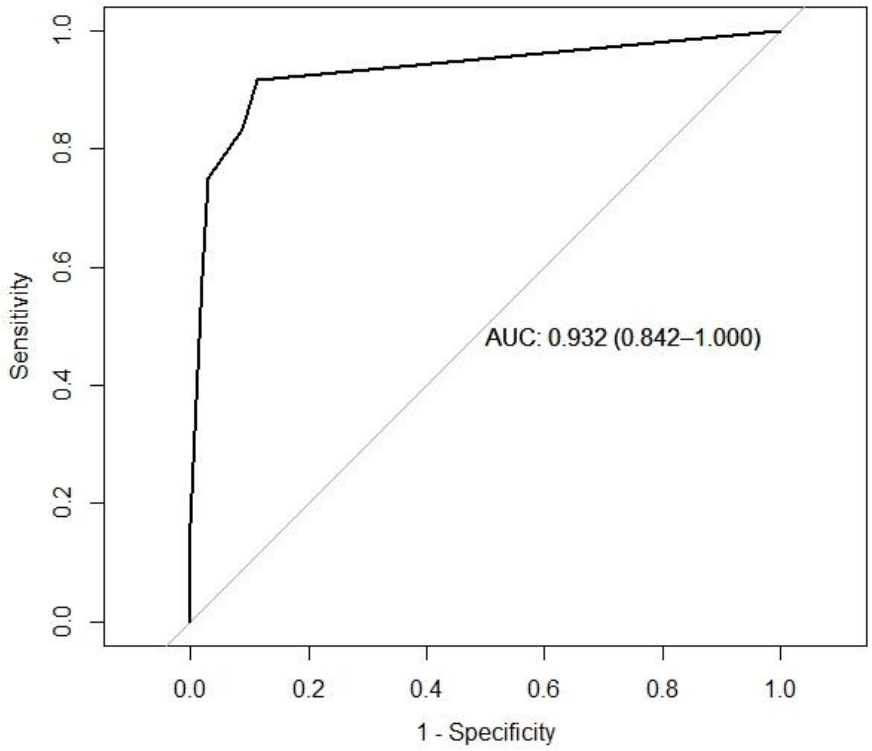

Figure 5.6. ROC curve: performance of Sectors in detection of CSME confirmed by the evaluation of the stereoscopic photographs.

Number of Sectors	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
1	91.7% (75.0% - 100%)	88.7% (84.1% - 92.8%)	33.3% (25.6% - 45.5%)	99.4% (98.3% - 100%)
3	75.0% (50.0% -100%)	96.9% (94.4% - 98.9%)	60.0% (40.0% - 83.3%)	98.1% (96.9% - 100%)

Table 5.3. Summary of the screening test characteristics: Number of sectors affected by hard exudates vs. CSME presence confirmed by stereoscopic photograph grading.

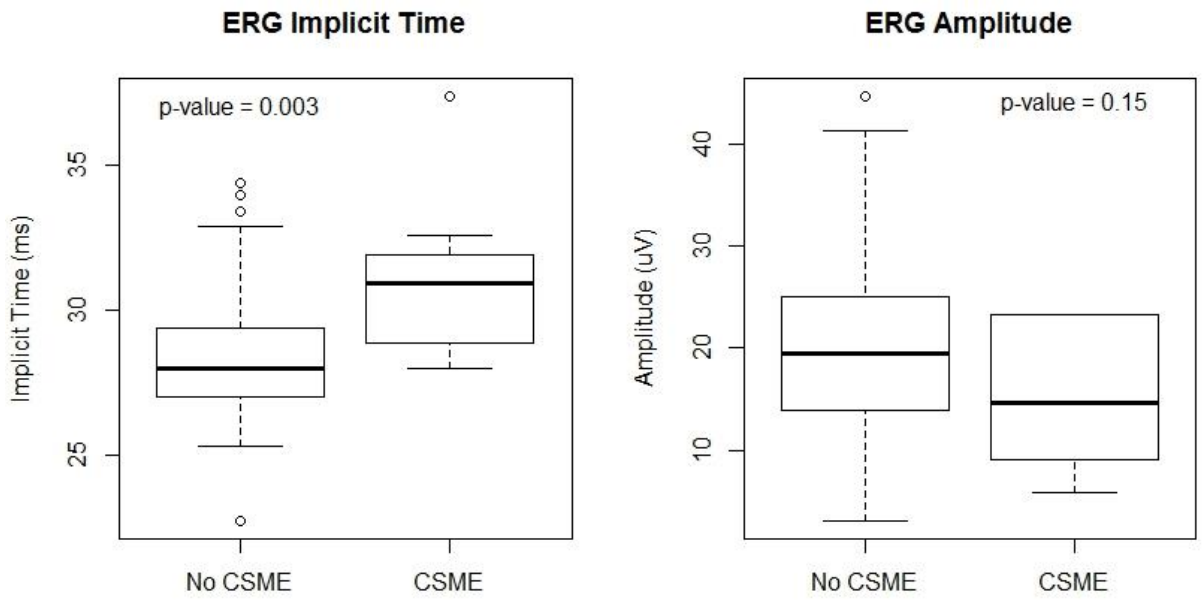

Figure 5.7. Plot of the ERG-derived parameters compared by CSME group.

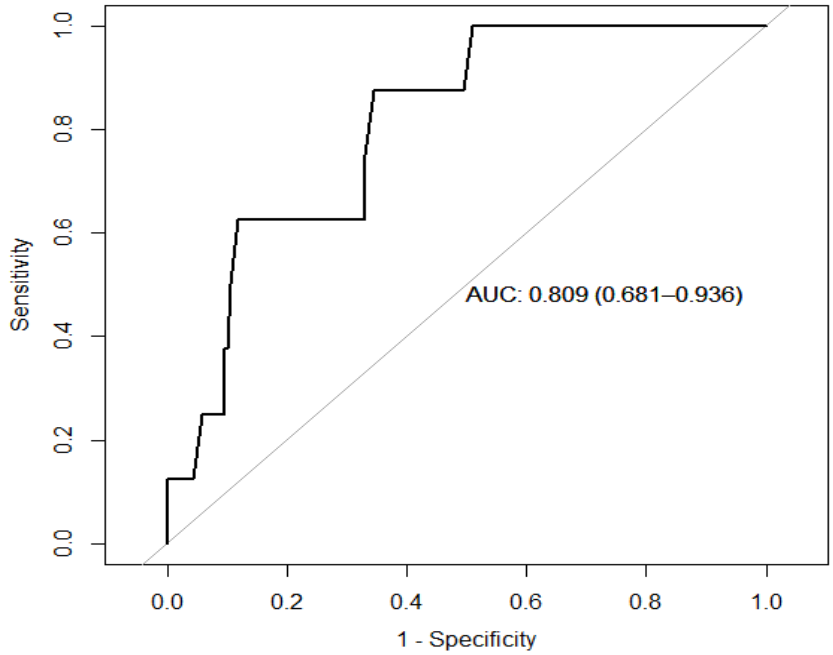

Figure 5.8. ROC curve: performance of ERG IT in detection of CSME confirmed by the evaluation of the stereoscopic photographs.

Odds Ratio (95% CI)		
Sectors	**Implicit Time**	**Retinopathy Level**
2.07	1.46	1.29
(1.14, 4.56)	(1.02, 2.24)	(0.42, 4.1)

Table 5.4. Summary of the odds ratios derived from the multivariable logistic regression model evaluating the association between the stereoscopically determined diagnosis of CSME and clinical variables.

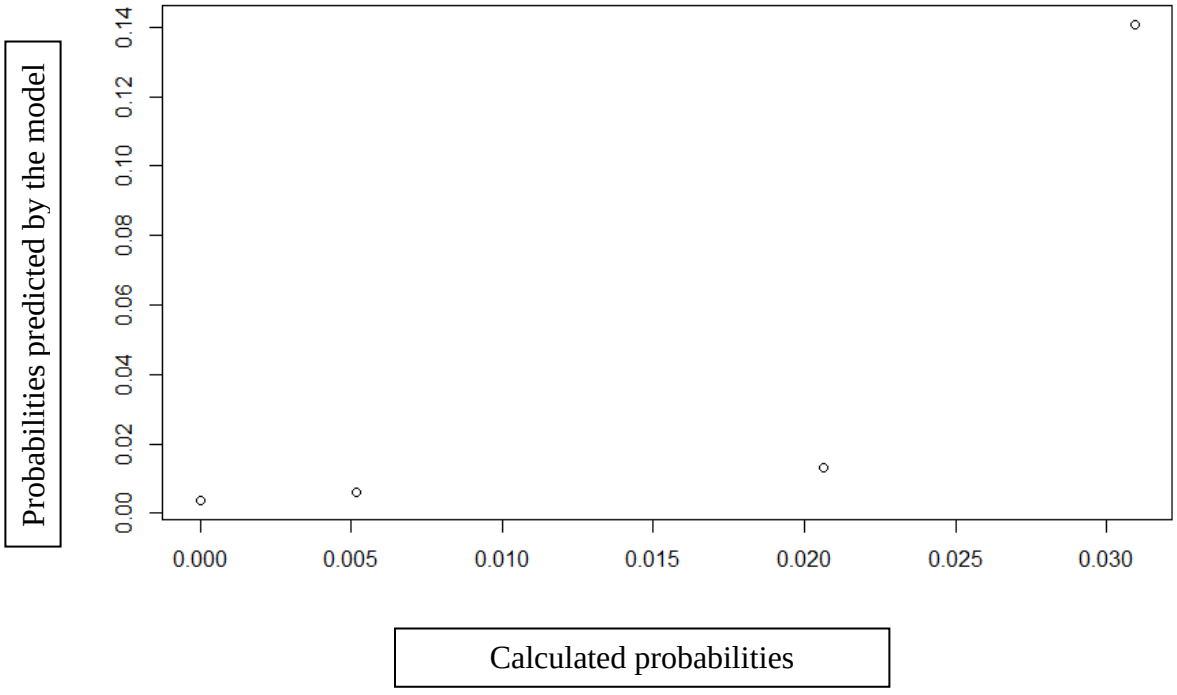

Figure 5.9. Goodness of fit of the multivariable model.

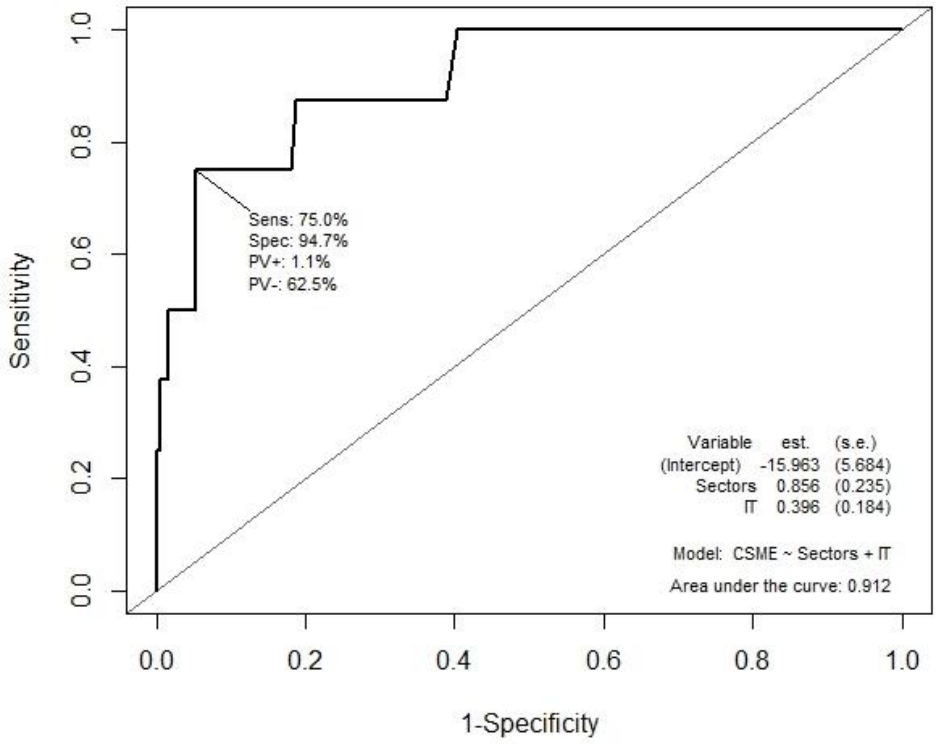

Figure 5.10. ROC curve evaluating the screening test characteristics calculated from the multivariable regression model which includes the following predictor variables: Sectors and ERG implicit time.

5.6 Discussion

We have evaluated the performance of a new screening approach, the number of sectors affected by hard exudates within the central 1-disc diameter zone of the macula, for the detection of CSME. Sectors showed good screening test characteristics in detection of CSME when compared against the clinical standard test (stereo biomicroscopy with OCT) as well as the standard test used in research. Good agreement in the screening test characteristics derived based on these two diagnostic methods suggests that the results can be generalized to a range of clinical settings. While standardized grading of stereoscopic photographs by multiple graders provides an accepted, rigorous and repeatable evaluation of the CSME presence, it is not generally performed in a clinical setting. More frequently, a decision to initiate a treatment for the sight-threatening diabetic macular edema is made, at least in part, based on the dilated fundus biomicroscopy and the central subfield thickness on OCT[13]. The proposed screening method allows for an easy threshold optimization to aid in decision-making with respect to the timing as well as the type of service (general vs. sub-specialty) to be specified in the referral. For example, an already implemented DRS program which utilizes digital fundus photography would maximize the detection of CSME by using 1 Sector as a cutoff value and would refer patients meeting this criterion, but not meeting the 3 Sector criterion, to a general eye care service which could easily handle up to 12% of patients who were incorrectly identified as having CSME. Similarly, such a program would use a 3 Sector cutoff value to refer patients directly to a retina specialist within the appropriately short period of time. This will assure a low over-referral of approximately 4% and prioritize patients with more severe cases of diabetic macular edema, as

shown in our previous study[14]. This grading approach will keep false positives and false negatives to a desired minimum, while requiring no additional resources for DRS programs. Our results suggest that regardless of the diagnostic method used, this screening test will miss up to 8% of CSME cases (100% – best Sensitivity, Table 5.3), an acceptable false negative level in most screening situations. In addition, this screening approach will result in approximately 4% of unnecessary referrals (100% - best Specificity, Table 5.3), an acceptable false positive level. The strength of Sectors as a screening approach for CSME detection as well as its improved performance, especially with respect to the specificity when compared to the existing methods, is in its ability to parallel the relationship between the extent and the proximity of retinal thickening in the macula captured by the CSME definition. Specifically, according to ETDRS data, the smaller area of retinal thickening closer to the fovea carries an increased risk for vision loss as does the larger area of retinal thickening located farther away from the fovea, with center-involving edema carrying somewhat greater risk[4,19,38]. To parallel that, it would require a larger patch of hard exudates to occupy 3 Sectors if the patch is located farther away from the fovea than if it was located closer to the fovea. If HE are assumed to approximate the location and the extent of retinal thickening, then Sectors give greater weight to the HE, and therefore edema, closer to the fovea. This approach is best suited to capture all cases of CSME with desired sensitivity and specificity.

In addition to Sectors, we have evaluated the association between the amplitude and the implicit time (IT) of a 30Hz photopic flicker ERG as well as a number of other clinical variables, in their association with CSME diagnosed in stereoscopic photographs (Table 5.1). While Sectors, IT and the level of retinopathy were significantly associated with CSME in a bivariate analysis, only Sectors and IT were significantly associated in a multivariable model (Table 5.4). Further analysis of the model revealed no interaction among the selected variables. We have also tested whether the combined predictors, Sectors and IT, perform better as a screening tool than Sectors alone. We discovered that the multivariable model does not perform better than Sectors alone, offering the sensitivity of only 75% (Figure 5.4).

A potential limitation of this study is the relatively low number of CSME cases (Table 5.1). This contributed to somewhat wider confidence intervals around the sensitivity estimates than desired, although still within reasonable range. The confidence intervals around the specificity estimates, however, are desirably narrow. We considered it important to recruit consecutive diabetic patients presenting to an optometric clinic (rather than artificially recruiting a sample of patients enriched with CSME) in order to evaluate our screening approach in a real-life situation which favors generalization of the findings to a wider population[39].

The strength of this study is the inclusion of two diagnostic methods for the diagnosis of CSME and a substantial agreement between the two methods. In addition, even though this inter-method agreement is not perfect, the results of the screening test accuracy analysis calculated for each of the two diagnostic methods separately are very similar, offering further support to the validity of the proposed screening method.

There were five cases of disagreement between the two modalities of CSME diagnosis. Out of those cases, there was only one case where CSME was detected by OCT-assisted eye exam but not detected on stereoscopic photography evaluation. In that case, retinal thickening was noted on stereoscopic photographs and was located on the border of one-disc diameter away from the fovea.

The extent of thickening was deemed to be less than one-disc area in size. Out of the four cases of CSME diagnosed on stereoscopic photographs but not detected during the OCT-assisted eye exam, one case had definite retinal thickening within 500 microns of the fovea which was clearly visible on OCT b-scans and topographic map with central subfoveal thickness of 300 microns and the area of retinal thickening extending into the temporal and inferior inner quadrants. The second case may be considered borderline CSME with retinal thickening seen on OCT scan definitely within one-disc diameter from the fovea but the area of thickening apparent on OCT scans was less than one-disc area in size. The third case showed definite retinal thickening seen on OCT that is likely greater than disc area in size but is right at the border of one-disc diameter away from the fovea. The fourth case was a diffuse, non-central thickening, definitely visible on OCT scan within one-disc area from the center of the macula and of one-disc area in size. These diagnostic discrepancies are not entirely surprising given that stereoscopic photography allows for careful examination by several graders which presumably results in increased diagnostic accuracy of the borderline cases. These findings also emphasize the lack of objective guidelines in the diagnosis and management of non-center involved and subclinical CSME based on OCT scans[21].

Finally, when compared with the screening strategy used by NHANES which, in part, relies on the detection of rings of exudates within 500 microns of the fovea or rings of exudates of 1 disc area in size located within 1 disc diameter from the fovea[13], the use of Sectors has the potential to reduce the percentage of center-involving DME cases missed from approximately 39% to about 6%[14]. We have not tested this hypothesis directly in the current or previous work but it should be considered in the future work with the special attention devoted to preserving sufficiently high specificity. This may be achieved by improving on the proposed utilization of the Sector approach by incorporating automated HE detection algorithms which can assess the effect of systematic rotation of the eight or more sector grid centered on the fovea on the number of sectors affected by HE. For example, a single large exudate that falls on the sector border may be counted as affecting two sectors. However, if the same exudate is located away from the sector border, it will be deemed to occupy one sector. By systematically changing the orientation of the sectors, a value of the minimum number of sectors occupied by HE can be determined. This value may play a role in further improving the accuracy of the proposed screening approach.

5.7 References

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Chapter 6: Conclusions and Future Directions

6.1. Summary and Conclusions

The Center for Disease Control and Prevention (CDC) has referred to the spread of diabetes in the United States as an epidemic. Over the last three decades the number of people diagnosed with diabetes in the United States has quadrupled, from 5.5 million to 21.3 million[1]. This reflects a global trend where the estimated prevalence has increased from 108 million in 1980 to 422 million in 2014[2]. CDC projects that 1 in 3 adults in the U.S. have prediabetes and 1.7 million new cases of diabetes are diagnosed each year. If sustained, this increase will result in 1 out of every 3 adults in the United States living with diabetes[1]. This rapid growth in prevalence of diabetes presents a significant public health challenge. Already, an estimated $245 billion is spent annually in direct medical costs as well as indirect costs related to disability and loss of productivity[3]. Ocular complications of diabetes are the leading cause of vision impairment in working-age adults in the United States[4]. Clinically significant macular edema (CSME) is the main cause of vision loss in persons with diaetes[5]. CSME can be successfully treated when detected in time[6,7,8]. The three clinical studies presented in this dissertation share a common goal of improving the accuracy of CSME detection.

The results of the study presented in Chapter 3 provide evidence supporting the use of hard exudates within one-disc area from the center of the macula for the detection of CSME by diabetic retinopathy screening (DRS) programs. The study offered new information which helps clarify the pre-existing discrepancy regarding the specificity of this approach in CSME detection. Finally, it has evaluated the implementation of this approach in a "real world" scenario, testing its effectiveness under the constraints of the existing DRS infrastructure.

The study presented in Chapter 4 tested whether the proximity of hard exudates to the fovea was associated with the severity of the detected CSME, thus requiring a more aggressive management approach. We found that the proximity of hard exudates alone was not significantly associated with the presence of severe cases of CSME. We did, however, find that a combined measure of the areal extent of exudation and its proximity to the fovea, measured as a number of radially arranged sectors affected by hard exudates (Sectors), is a significant indicator of severe CSME presence. In fact, we found that an increase in one Sector was associated with a two-fold increase in the odds of being diagnosed with severe CSME. In this chapter, we also developed an OCT-based adaptation of the ETDRS-derived DME severity scale. This allowed us to objectively classify edema cases into severe and non-severe CSME. An important factor in this classification scale is that eyes with severe CSME are expected to have reduced visual acuity of 20/30 or worse, on average. This OCT-based severity scale may be an important step forward in exploring reliable structure-function correlations in DME. This has clinical implications since central subfield thickness, an OCT-derived parameter commonly used in clinical trials, has only modest correlation with visual acuity complicating the interpretation of results regarding clinical management of patients at risk for vision loss[9,10,11].

We built on the knowledge gained from the earlier two studies and investigated whether Sectors were significantly associated with any type of CSME and if Sectors may be used as a new CSME detection approach by DRS programs. Finally, we tested whether the abnormalities in the electrical retinal response to the photopic 30 Hz flicker ERG stimulus improves our model of CSME

detection. The results presented in Chapter 5 support the use of Sectors as a new approach for the detection of CSME by DRS programs. While latency in the photopic 30 Hz flicker ERG response was significantly associated with CSME, this information did not improve the performance of our CSME detection model.

In summary, the work presented in this dissertation has the potential to improve the accuracy of CSME detection, a leading cause of vision loss in persons with diabetes, by DRS programs.

6.2. Future Directions

This work also offers new avenues for exploration. Among other things, we have learnt that there are barriers to treatment access by patients who were referred by a DRS program. Future work could improve the management of patients with sight-threatening microvascular complications of diabetes by identifying such barriers and exploring the most-effective means to address them. The OCT-derived CSME severity scale has the potential to offer a more repeatable and objective measure of edema assessment that correlates well with visual acuity. This severity scale will need to be validated and, perhaps refined, on a new set of study participants. Finally, the emphasis of future work should be on identifying early, reliable predictors of vision loss related to retinal complications of diabetes with the ultimate goal of evaluating new therapeutic approaches before the devastating late stages of diabetic retinal disease occur.

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