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Inflammatory Mediators in the Pathophysiology of Diabetic Retinopathy (INSPIRE): Study Design and Baseline Characteristics

Sapna Gangaputra[1], Jinsong Sheng[1] and Stephen J Kim[1*]

Abstract

Purpose: To evaluate inflammatory intraocular and systemic cytokines associated with diabetic retinopathy (DR) and diabetic macular edema (DME).

Methods: The INflammatory MediatorS in the PathophysIology of Diabetic REtinopathy (INSPIRE) study is a prospective, placebo-controlled, interventional study that enrolled 328 eyes of 164 adult patients with type II diabetes. 118 (236 eyes) diabetic patients with moderate nonproliferative diabetic retinopathy (NPDR) were randomized to either topical ketorolac 0.45% (Acuvail®) or placebo (Refresh Optive ®, preservative free) in double-masked fashion. A cohort of 23 (46 eyes) patients with diabetes but no retinopathy (No DR) and 23 patients with proliferative diabetic retinopathy (PDR) were also enrolled. All patients underwent complete ophthalmic examination including bilateral aqueous biopsies to test for 24 inflammatory cytokines and 5 classes of prostaglandins. A group of 107 patients without diabetes who were undergoing elective vitrectomy for non-inflammatory conditions had blood and aqueous fluid collected at the time for surgery for cross-comparison analysis.

Results: There was similar age and sex distribution across cohorts. The study population was predominantly white. Longer duration of diabetes was observed in the NPDR and PDR groups. The ocular characteristics of all patients were similar for visual acuity, IOP, lens status and median central subfield thickness.

Discussion: The INSPIRE study may provide greater understanding of the role of inflammation in DR and allow for the discovery of novel biomarkers and therapeutic targets that can better predict risk of vision loss and prevent progression of DR and DME.

Keywords: Inflammation; Ketorolac; Diabetic Retinopathy; Diabetic macular edema; DME; Randomized controlled trial; Intraocular cytokines.

Introduction

Nearly 300 million individuals have diabetes worldwide and prevalence is rising. Over one-third of diabetic patients will develop diabetic retinopathy (DR) during their lifetime and 5-10% of these individuals will suffer from vision-threatening complications [1]. DR progresses in many patients despite preventable measures such as blood sugar and blood pressure control [2,3]. To date the only validated prognostic DR biomarker is the circulating glycemia marker glycated hemoglobin (HbA1c), but HbA1c accounts for only 11% of the risk of DR progression. Once DR threatens or causes vision

Affiliation:
[1]Vanderbilt Eye Institute, 2311 Pierce Avenue Nashville, United States

***Corresponding author:**
Stephen J Kim, Vanderbilt Eye Institute, 2311 Pierce Avenue Nashville, United States

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loss, treatments involve invasive eye injections that are often repeated long term, laser photocoagulation that can result in central scotomas and constricted peripheral visual field, and vitrectomy surgery for late-stage complications. Due to its rising prevalence, DR is the leading cause of legal blindness among working-age adults [1]. Current diagnostic tests fail to identify early disease stages or predict disease progression. Consequently, new biomarkers and therapeutic strategies are needed.

Inflammatory mediators are candidates for direct biomarkers that may predict DR progression as well as treatment response. HbA1c screening reflects glucose control, which indicates disease risk as opposed to DR pathology [4]. It is now well established that PGE2 is the predominant inflammatory prostaglandin (PG) class within the eye. PGE2 is increased by 40% in the retinal vasculature of diabetic rats and is significantly reduced by treatment with insulin [5]. PGE2 promotes vasodilation, disrupts the blood-ocular barrier, and facilitates leukocyte migration. The latter would further disrupt the blood-ocular barrier and potentially lead to a self-propagating inflammatory cascade. Nonsteroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of cyclooxygenase (COX) enzyme and thereby the synthesis of all downstream PGs [6]. NSAID treatment shows favorable clinical effects on inflammation and macular edema [7-9]. In streptozotocin-induced diabetic rats the NSAID celecoxib significantly inhibits PGE2 secretion, retinal VEGF expression and vascular leakage [9,10].

We previously demonstrated that PGE2 and several inflammatory cytokines are significantly elevated in the vitreous of patients with proliferative diabetic retinopathy (PDR). In a prospective trial, we also demonstrated that topical ketorolac tromethamine (ketorolac) 0.45% (Acuvail®, AbbVie Inc. North Chicago, IL) achieves therapeutic vitreous levels and significantly reduces IL-8 and platelet-derived growth factor (PDGF)-AA in the vitreous of diabetic patients [11,12]. In 2009, the Food and Drug Administration (FDA) approved the preservative-free 0.45% ketorolac formulation (Acuvail) with enhanced pharmacokinetics due to incorporation of the vehicle carboxymethylcellulose with increased ketorolac adhesion and permeation in the eye [13-15]. Topical administration minimizes systemic exposure, and the potential for cardiovascular, renal and gastrointestinal toxicity associated with long-term oral administration [16].

The INflammatory MediatorS in the PathophysIology of Diabetic REtinopathy (INSPIRE) study was designed to expand upon these preliminary results. INSPIRE is a 3-year prospective, randomized, double-blinded, placebo-controlled trial currently being conducted at the Vanderbilt Eye Institute, Nashville, TN. Successful enrolment of 328 eyes of 164 patients with diabetes has already been completed. One hundred and seven eyes of 107 patients without diabetes were also concurrently enrolled. The study is expected to conclude in late 2025 with final analyses conducted in 2026 for publication and dissemination to the scientific community.

The INSPIRE study has three primary aims:

Measure the association between aqueous PGE2 and inflammatory cytokines with DR and DME in a natural history study.

Investigate the long-term effects of daily topical application of ketorolac 0.45% (Acuvail®) on PGE2 and intraocular cytokine levels.

Determine how consistent topical application of ketorolac 0.45% influences DR progression and development of DME.

Materials & Methods

The Vanderbilt University Medical Center Institutional Review Board approved all aspects of this study, and all patients gave written informed consent prior to any study activity. The study complies with all aspects of the Health Insurance Portability and Accountability Act and is being conducted in accordance with the tenets of the Declaration of Helsinki. The trial is registered at ClinicalTrials.gov Identifier: NCT04505566 and funded by the National Eye Institute: R01-EY031315. An Investigational New Drug (IND) application was submitted to the FDA and was approved for long-term topical use of ketorolac. INSPIRE is a single center trial and all participants were recruited and enrolled at the Vanderbilt Eye Institute, Vanderbilt University Medical Center, in Nashville, TN from November 20, 2020, until May 12, 2022. All data was deidentified and collected on a Research Electronic Data Capture (REDCap) database maintained by the Vanderbilt University Medical Center [17,18].

Study Population I (Diabetic Patients)

A total of 164 adult patients, 18 years or older, with type II diabetes agreed to enroll (Figure 1). The International Clinical Disease Severity Scale [19,20] is a standard classification system consisting of two categories and five stages: the nonproliferative category, stages 1-4 and proliferative category, stage 5. Stage 1 is characterized as “no apparent diabetic retinopathy.” The nonproliferative stage is further grouped into stage 2 (mild nonproliferative diabetic retinopathy, NPDR), stage 3 (moderate NPDR), and stage 4 (severe NPDR). Stage 5 is final stage, proliferative DR, PDR. Diabetic patients corresponding to stages 1, 3, and 5 were enrolled (23 patients with no DR, 23 patients with PDR, and 118 patients with moderate NPDR), assigned according to grading of baseline fundus photographs [20]. Patients with moderate NPDR were required to have a recorded HbA1c ≥ 8.0 within 12 months of enrollment to increase the likelihood of observing progression and complications of DR during the 3 years of this study.

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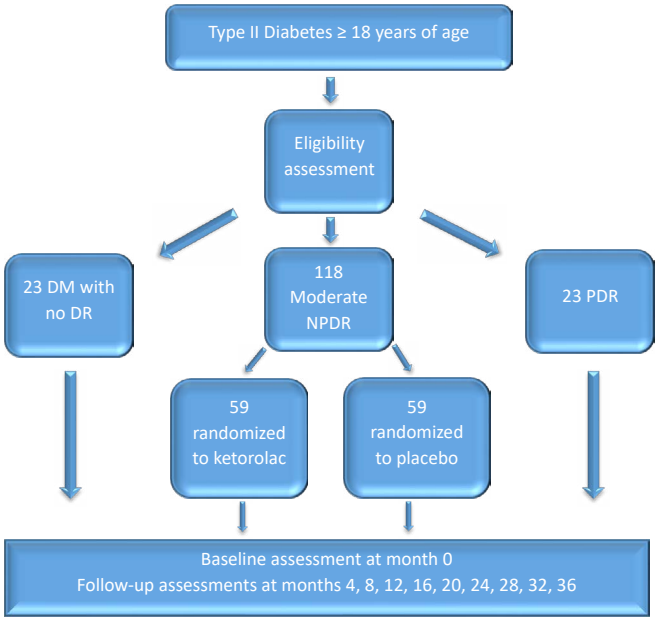

Figure 1: Participant flowchart in the INSPIRE study.

In cases of disease asymmetry, the stage of the more severe eye determined recruitment. In the landmark United Kingdom Prospective Diabetes Study (UKPDS), roughly 20% of diabetic patients had retinopathy in 1 eye but not the other [21].

Exclusion criteria included:

previous vitrectomy in either eye

prior intravitreal injection within 3 months

prior intraocular surgery other than cataract surgery

co-existent macular, retinovascular, or inflammatory disease

history of ocular trauma

current use of prescription topical or systemic NSAIDs

blood pressure > 180/110 mmHg

risk for corneal melting

regular aspirin use (defined as taking on average ≥ 1300 mg per week)

inability to comply with follow-up

Clinical Examination

All enrolled diabetic patients underwent complete baseline ophthalmic examination of both eyes including best corrected ETDRS visual acuity, intraocular pressure (IOP) measurement, slit-lamp examination, dilated funduscopic examination, OCT and OCT angiography (OCTA), Schirmer's testing, and color fundus photography (Figure 2).

Vision was tested by masked, trained certified ophthalmic technicians. Wide-field fundus photography (Optos, Inc, Marlborough, MA, USA) and Enhanced Depth OCT (Heidelberg Spectralis) were obtained by trained ophthalmic photographers who were masked to study assignment. Retinal thickness scans were acquired using 30-degree X 30-degree cube scan with 61 raster scan protocol and enhanced depth imaging to capture the choroid. OCTA (Optovue Angiovue) images were taken by trained ophthalmic photographers with five repeated 3mm x 3mm cube scans centered on the fovea of each eye. All ophthalmic examinations and testing, as described above, were repeated every 4 months for 3 years (total of 10 study visits per patient). Specular microscopy (Konan Medical CellChek) and Oxford corneal exam were performed at baseline and will be repeated yearly for 3 years.

Blood Glucose Levels

Serum blood glucose and HbA1c of enrolled diabetic patients was measured using an FDA approved point of care device (Afinion™ 2, Abbott, Lake Forest, IL) from blood drawn at baseline and every 4 months for 3 years.

Aqueous Sample Collection

Three applications of topical antibiotic spaced by 5 minutes were administered to each eye. Topical (Akten® 3.5%, Akorn, Lake Forest, IL) or subconjunctival 2% lidocaine anesthetic was administered for anesthesia. A 30-gauge needle on a 1 ml tuberculin syringe was inserted into the anterior chamber under direct visualization and used to collect 0.1 ml or more of aqueous fluid from each eye. All aqueous samples were immediately processed, aliquoted into smaller volumes, and kept on dry ice until they were individually stored at -80°C for subsequent testing of inflammatory cytokines and PG levels.

Serum and Whole Blood Collection

Blood was drawn manually using a 23-gauge butterfly needle attached to a 10 ml syringe. The collected blood was then transferred into a standard "red" top vacutainer tube for serum collection after clotting or into a standard "purple" top vacutainer tube with K2 EDTA to prevent clotting. Serum and whole blood were then aliquoted into smaller volumes and individually stored at -80°C for subsequent testing of inflammatory cytokines and PG levels.

Measurement of PG Levels

The Eicosanoid Core Laboratory at Vanderbilt will use liquid chromatography electrospray ionization tandem mass spectrometry, that provides greater accuracy than ELISA testing, to analyze thawed aqueous and serum samples for all 5 PG classes (PGE2, PGD2, PGF2α, PGI2, Thromboxane A2) [22].

Measurement of Inflammatory Cytokines

Due to limited volumes, a microparticle bead-based multiplex assay was used to measure inflammatory aqueous cytokines as previously described [23]. In brief, aqueous samples will be thawed and prepared using the Millipore Multiplex Human Cytokine/Chemokine/Growth Factor Panel A (MilliporeSigma, Burlington MA, USA). Bead samples will be analyzed with Luminex 200™ Analyzer

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Figure 2: Visit and Examination Schedule

Month	BL*	4	8	12	16	20	24	28	32	36
Consent, eligibility, and randomization	X									
Visual acuity**, blood pressure, IOP	X	X	X	X	X	X	X	X	X	X
Medical, ophthalmic, and eye drop treatment history	X	X	X	X	X	X	X	X	X	X
Schirmer's test	X	X	X	X	X	X	X	X	X	X
Specular microscopy	X			X			X			X
Ocular surface disease index (OSDI) questionnaire	X			X			X			X
Optical coherence tomography (SDOCT, OCTA)	X	X	X	X	X	X	X	X	X	X
Color fundus photographs (Optos)	X	X	X	X	X	X	X	X	X	X
Blood draw, blood glucose, HbA1c	X	X	X	X	X	X	X	X	X	X
Eye exam	X	X	X	X	X	X	X	X	X	X
Bilateral aqueous biopsies	X	X	X	X	X	X	X	X	X	X
Oxford grading cornea scale	X	X	X	X	X	X	X	X	X	X

* BL = Baseline
** Evaluation of best corrected visual acuity with ETDRS

following the manufacturer's protocols for 24 inflammatory cytokines: Fibroblast growth factor (FGF)-2, Eotaxin, Granulocyte colony stimulating factor (G-CSF), FMS-like tyrosine kinase 3 ligand (FLT-3L), Growth-regulated alpha protein (GROa), Interleukin (IL)-10, Monocyte chemotactic protein (MCP)-1, MCP-3, Macrophage-derived chemokine (MDC), soluble CD40L (sCD40L), IL-17A, Interleukin-1 receptor antagonist (IL-1RA), IL-1β, IL-2, IL-4, IL-6, IL-8, Interferon gamma-induced protein 10 (IP-10), Macrophage inflammatory protein (MIP)-1β, Tumor Necrosis Factor (TNF)-α, Vascular endothelial growth factor A (VEGF-A), Regulated on activation normal T expressed and secreted (RANTES), Platelet-derived growth factor (PDGF)-AA and PDGF-AB/BB. Stored serum will be thawed and analyzed for the same 24 cytokines in identical fashion. Triplicate testing of cytokines will be performed.

Randomization

Patients with moderate NPDR had both eyes randomized in permuted block fashion to either ketorolac 0.45% or placebo-control (Refresh Optive ®, preservative free, AbbVie Inc. North Chicago, IL) in double-masked fashion. Randomization was done using an online module within the REDCap application. Patients were instructed to apply 1 drop of their assigned medication 2 times daily in each of their eyes and then to close their eyes for 5 minutes after each application to improve absorption. Subjects continued with their assigned medication for 3 years, the duration of the study. Both drugs were clear in appearance and provided to patients in identical single-use vials with the end of each vial (that contained written drug information) removed to maintain masking. Ketorolac and placebo medications were prepared and packaged by a single study coordinator who was unmasked and not involved in patient care. 4-month supply of prepared medications in medical-grade reclosable plastic bags were then placed in either a "Red" box or a "Blue" box to be distributed to patients based on their randomized assignment: Red or Blue at each of their study visits.

Study Population II (Non-diabetic Patients)

This study population consisted of 107 consenting patients without diabetes who were undergoing elective unilateral vitrectomy surgery for non-inflammatory conditions such as epiretinal membrane or macular hole. Aqueous fluid and serum were collected at the time of surgery and stored at -80°C and will be tested for inflammatory cytokines and PGs as detailed above, to provide a reference level for comparison analysis.

Adverse Event Monitoring

All patients were re-examined immediately after the biopsy for bleeding, hypotony, cornea integrity, and lens trauma. Patients were contacted by phone 1 day after aqueous sampling and asked about changes in vision or pain. Patients were given a handout listing warning signs and provided a direct number to call if they experienced unusual or persisting symptoms. A Data and Safety Monitoring committee met annually to review data integrity and to monitor for any adverse events. As specified by the US FDA, an adverse event is considered serious if it results in any of the following: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, disability, or permanent damage, or a congenital anomaly/ birth defect.

Double-Masking of the Randomized Control Trial

All personnel involved in patient care including the principal and co-principal investigator, study coordinators, nurses, and photographers were masked to randomization. Study participants were masked to their drug assignment for the 3-year duration of the study.

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Sample Size Calculations

The sample size and power calculation were performed for the moderate NPDR group using simulations as well as the preliminary data for the primary outcome, PGE2 and one of the major secondary outcomes, IL-8. We assumed that the outcomes would follow a log-normal distribution and intra-class correlation was conservatively assumed to be 0.8. We considered a 30% decrease in the mean of outcomes in ketorolac treatment group compared to placebo group as a clinically meaningful difference. We determined that a total of 100 diabetic patients randomized to placebo or topical ketorolac treatment will provide at least 90% power with the type I error rate at α=0.05. We, therefore, planned to randomize a total of 118 diabetic patients (59 per group), assuming a 15% total attrition rate during the 3 years of follow-up.

Statistical Analysis Plan

The primary and secondary outcomes (e.g., PGE2, VEGF-A, IL-6, and IL-8 levels) will be repeatedly measured every 4 months over 3 years from placebo and topical ketorolac-treatment groups. A linear mixed effect model will be used to examine whether the mean level for each mediator is different between placebo and topical ketorolac-treatment groups eyes after adjusting for baseline measurements for each mediator and confounding factors (such as HbA1c > 10 and < 7.9, systemic NSAID use including aspirin, systemic corticosteroid use including prednisone, intraocular anti-VEGF drug treatment, and intraocular corticosteroid treatment). We will also examine the association between the outcomes (e.g., PGE2, VEGF, IL-6, IL-8 levels) and ketorolac levels with adjustment of potential confounding factors. Proportional odds models with 95% confidence intervals and robust standard errors to account for correlation of both eyes within each study patient will be used to assess the relationship between DR severity and cytokine levels.

The statistical team will apply regression analyses to model for possible confounding of inflammatory cytokine levels due to intravitreal injections of anti-VEGF agents for PDR and DME. We do not plan to stop patients from getting standard of care treatment for diabetic eye disease. We would use "Last Observation Carried Forward" analysis for non-compliance to study visits. We would analyse aqueous for ketorolac levels in randomized eyes to determine adherence to treatment.

Results

Table 1: Demographic Characteristics of the Diabetic Cohort at Enrollment.

	No DR (N=23 subjects)	Moderate NPDR (N=118 subjects)	PDR (N=23 subjects)
Median age at enrollment, years (range)	64 years (46 – 74 years)	63 years (27-79 years)	59 years (40- 74 years)
Male, N (%)	9 (39.1%)	61 (51.7%)	12 (52.2%)
Race/ Ethnicity			
White	19 (82.6%)	87 (73.7%)	16 (69.6%)
Black	4 (17.4%)	24 (20.3%)	5 (21.7%)
Asian	0	1 (0.8%)	1 (4.3%)
Hispanic or Latino	0	1 (0.8%)	0
Others*	0	5 (4.2%)	1 (4.3%)
Duration of DM			
Less than 5 years	2 (8.7%)	8 (6.8%)	0
6 – 10 years	8 (34.8%)	10 (8.5%)	1 (4.3%)
11 – 20 years	9 (39.1%)	48 (40.7%)	7 (30.4%)
More than 21 years	4 (17.4%)	52 (44.1%)	15 (65.2%)
Random blood glucose (median)	176.0 mg/dl	176.5 mg/dl	163.0 mg/dl
HbA1c, median (range)	6.6% (5.7 – 10.4%)	7.5% (5.4 – 12.9%)	7.8% (5.5 – 12.0%)

DR: diabetic retinopathy
NPDR: nonproliferative diabetic retinopathy
PDR: proliferative diabetic retinopathy
DM: diabetes mellitus
HbA1c: Hemoglobin A1c

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*Others include American Indian/Alaska native (2), Native Hawaiian/Pacific islander (1), Middle Eastern/North

African (1) and unknown (2)

A total of 408 eligible diabetic patients were offered enrollment and 164 participants (40%) consented and were included in the study. Most of the patients were white (around 80% in each of the groups) with the remainder being Black or African American, which is reflective of the population of the state of Tennessee during the time of enrollment (Table 1). The ages of our patients were quite similar, with the median age at enrollment being 64 years (range 46-74 years) in the No DR group, 63 years (range 27-79 years) in the NPDR group and 59 years (40-74 years) in the PDR group. There were lesser males in the No DR group (39.1%) as compared to nearly equal males and females in the NPDR and PDR groups. The duration of diabetes of the subjects enrolled varied by group. The majority of subjects (17 subjects, 74%) in the No DR group had a history of diabetes from 6 years to 20 years. The moderate group had 85% (100 subjects) with more than a 10-year history, of which 44% (52 subjects) had diabetes for more than 21 years. In the PDR group, there was only one subject with a history of less than 10 years, and 65% (15 subjects) had diabetes for more than 21 years. The median HbA1c at enrollment was lower in the No DR Group (6.6%) and similar between NPDR (7.5%) and PDR (7.8%).

The ocular characteristics of the groups were very similar (Table 2), with almost all eyes with very good visual acuity (20/25 or better) and intraocular pressure in the 15-17mm Hg range. None of the eyes exhibited signs of severe dry eye with median Schirmer's test results of 10mm or higher for all groups. The majority of eyes were phakic (64% or higher) in all groups. A small proportion of eyes had baseline OCT central subfield thickness of 320 microns or higher. Median central subfield thickness and macular volume in each group was similar at enrollment.

Table 2: Ocular Characteristics of the Diabetic Cohort at Enrollment

	No DR		Moderate NPDR		PDR	
	Right eye	**Left eye**	**Right eye**	**Left eye**	**Right eye**	**Left eye**
BCVA (median ETDRS visual acuity)	20/20	20/25	20/25	20/25	20/25	20/25
IOP (median)	15 mmHg	15 mmHg	15 mmHg	15 mmHg	16 mmHg	17 mmHg
Schirmer's test (median)	13mm	13mm	10mm	11mm	12mm	12mm
Lens status (phakic, N, %)	16 eyes (69.6%)	16 eyes (69.6%)	75 eyes (63.6%)	75 eyes (63.6%)	15 eyes (65.2%)	15 eyes (65.2%)
Median central subfield thickness (SD)*	278 μ (18 μ) N=20 eyes	273 μ (22 μ) N=21 eyes	281 μ (52 μ) N=113 eyes	285 μ (65 μ) N=112 eyes	266 μ (57 μ) N=21 eyes	262 μ (52 μ) N=21 eyes
Median macular volume (SD)*	8.26 μ^3 (0.38 μ^3) N=20 eyes	8.30 μ^3 (0.36 μ^3) N= 21 eyes	8.60 μ^3 (0.82 μ^3) N=113 eyes	8.62 μ^3 (0.98 μ^3) N=112 eyes	8.41 μ^3 (1.21 μ^3) N=21 eyes	8.23 μ^3 (0.83 μ^3) N=21 eyes
Number of eyes with central subfield thickness >320 microns*	0 eyes	0 eyes	18 eyes	25 eyes	6 eyes	5 eyes

DR: diabetic retinopathy

NPDR: nonproliferative diabetic retinopathy

PDR: proliferative diabetic retinopathy

BCVA: best corrected visual acuity

ETDRS: early treatment diabatic retinopathy scale

IOP: intraocular pressure

*Eyes with obvious epiretinal membrane, vitreomacular traction, and artifacts that confound measurement were excluded

Discussion

The INSPIRE Study is the first clinical trial to examine the association of intraocular inflammatory cytokines with DR progression. Ultimately, intraocular cytokine profiles may better risk stratify diabetic patients who are more likely to experience DR and its complications.

Currently, DR is treated after pathology and tissue damage has occurred. Demonstrating that specific intraocular cytokine levels mirror DR grade would aid in risk-stratifying patients and shift clinical practice paradigms by allowing earlier identification of patients more likely to experience progression or complications from DR. A biomarker that

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closely reflects DR activity and accurately predicts risk of progression is an innovation that could help millions of patients. Our hypothesis is that aqueous PGE2 and cytokine levels are markers of both systemic diabetes and DR activity and therefore predict risk of DR progression.

Determining the long-term effects of chronic topical ketorolac treatment on intraocular cytokine levels, DR progression and DME incidence may provide an immediate avenue for preventing disease, reducing disability, and relieving economic burden. A prospective controlled trial, conducted by the National Eye Institute, demonstrated that the oral NSAID celecoxib significantly reduced vascular leakage in patients with DR despite early treatment stoppage due to cardiovascular toxicity concerns [24]. Topical administration minimizes systemic exposure, and the potential for cardiovascular, renal and gastrointestinal toxicity associated with long-term oral NSAID application, morbidities which diabetic patients are already at greater risk for [7,13-16,25].

Confirming that PGE2 contributes to the pathogenesis of DR could impact clinical practice immediately as PGE2 inhibitors (NSAIDs) are commercially available [26,27]. Notably, ketorolac treatment may circumvent or delay the need for corticosteroids, laser surgery or anti-VEGF injections. Strictly inhibiting VEGF neither addresses the inflammatory mechanism of DR nor the underlying cause of VEGF upregulation. Better understanding of inflammatory pathways and the relationship of inflammatory cytokines with DR progression may provide novel therapeutic targets that drive the field towards preventative anti-inflammatory-based therapies.

Cytokines are promising candidates for novel predictive DR biomarkers. Intraocular cytokine levels reflect real-time biochemical activity: production from leukocytes and retinal cells and degradation via proteases. A recent small study in Japan reported that the aqueous humor concentrations of 9 immune mediators - bFGF, CD40 ligand, fractalkine, G-CSF, IL-6, IL-8, MIP-1α, MIP-1β, and VEGF were significantly elevated in patients with DR as compared to controls [28]. Cytokines within the intraocular space more likely correlate with disease state than systemic markers. The Diabetic Retinopathy Clinical Research (DRCR) network demonstrated that treatment of DME with intraocular corticosteroid significantly delayed progression of DR [27]. Currently, several corticosteroid formulations (Ozurdex and Iluvien) are FDA approved for DME treatment [29,30]. A growing body of scientific evidence indicates that corticosteroids treat DME by inhibiting inflammation, which plays a pivotal role in the development and progression of DR. We hypothesize that anti-inflammatory NSAID based therapies may delay progression of DR and vision-threatening complications if applied early in the disease course without the typical risks associated with corticosteroid treatment.

We have already overcome major potential procedural and technical challenges in INSPIRE. Aqueous biopsy with needle insertion and withdraw is difficult in some patients. Consequently, proficiency and first-hand experience with this technology is required. Both authors (SJK, SG) routinely perform aqueous biopsies and have had no adverse events to date with the procedure. We also have considerable experience testing aqueous samples for cytokines using a microparticle bead-based multiplex assay that allows small volume (50µl) testing of multiple cytokines [23]. Further, INSPIRE may demonstrate that repeat, longitudinal aqueous sampling is clinically feasible and safe and can therefore provide the opportunity to measure inflammatory biomarkers to monitor treatment response.

A potential problem for any large, multi-year, study is recruitment and retainment of study participants. INSPIRE was able to successfully recruit all 164 participants within 18 months of trial initiation despite the enormous challenges faced with the COVID 19 pandemic (recruitment began in November 2020). Compliance has been outstanding with 95% of participants (including those lost due to death) having completed 2-years of follow-up. Another potential foreseeable problem involves confounding of our results due to anti-VEGF or other treatments during the 3 years of follow-up. While we excluded any patient receiving anti-VEGF treatment within 3 months of enrollment, it was anticipated that some patients will need to receive treatment during the study and our biostatistical team is prepared to model for this. We excluded patients who had any known systemic autoimmune diseases or concurrent use of systemic or topical corticosteroids and immunosuppressive medications or regular use of NSAIDs. We also excluded any known retinal or macular disease that may alter the intraocular cytokine profile; however, it is possible that other life style choices such as diet and smoking may also affect the cytokines, which we are unable to document or control. As such we hope randomization will distribute the patients equally into receiving drug or placebo. Finally, our study design and population provide a unique opportunity to study longitudinal microvascular changes in diabetic patients in relationship to intraocular and systemic inflammatory mediators using OCTA. This non-invasive imaging modality allows detailed vascular imaging of the retina and choroid that can be serially repeated over time for comparative analysis.

Conclusions

The INSPIRE study should meaningfully contribute to our understanding of the role of inflammation in diabetic eye disease. Importantly, identification of novel biomarkers and therapeutic targets will greatly add to our knowledge and may aid in risk-stratification, improved assessment of treatment response, and better predicting those patients with DR who are at the greatest risk of vision loss.

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None of the authors have any financial conflict of interest.

Trial registration:

ClinicalTrials.gov NCT04505566. Registered on August 10, 2020.

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