Research Study Protocol

Study Title: Monocytes in Subjects with Type 1 Diabetes and Chronic Kidney Disease

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Sponsor, if Applicable (include NIH and other funding sources here): Stanford K12 DiabDocs and the NIH (K12 award)

BACKGROUND AND SIGNIFICANCE

Chronic kidney disease (CKD) is prevalent in patients with type 1 diabetes (T1D) with rates of 30-40%.[1] The current treatment of T1D CKD targeting hemoglobin A1c (A1c) and the renin-angiotensin system reduces the progression of CKD, but does not completely abolish this risk suggesting additional factors contribute to the residual risk. Proinflammatory monocytes activated by episodes of acute hyperglycemia may contribute to this risk. In humans with T1D CKD, macrophage infiltration correlates with renal dysfunction.[2] The mechanisms driving monocyte infiltration in T1D CKD are incompletely studied in humans. A potential therapy is glucagon-like peptide-1 (GLP-1) agonists. Exposure of THP-1 cells, a human monocytic line, to GLP-1 in hyperglycemia reduced TNF-α secretion and podocyte apoptosis. Currently, it is unclear if baseline time in range (TIR) on continuous glucose monitor (CGM) 70-180 mg/dl affects the degree of acute hyperglycemia required for monocyte activation, and if GLP-1 agonists can reduce this monocyte activation. Further, predictive markers of T1D CKD progression reflective of monocyte activation are not well-defined. Understanding these mechanisms can lead to development of more effective therapies which can reduce the residual risk of CKD in T1D.

SPECIFIC AIMS

Our proposal will fill existing gaps by testing the central hypothesis: T1D CKD subjects with lower TIR have monocyte activation at a lower degree of hyperglycemia to drive podocyte injury, which is attenuated by GLP-1 agonists. To test our hypothesis, we propose the following aims:

- **Aim 1:** Determine if lower TIR in subjects with T1D CKD requires a lower degree of hyperglycemia for proinflammatory monocyte activation (defined by the classical monocyte phenotype, inflammatory signaling, and TNF-α secretion) to drive podocyte injury. We will then assess if monocyte activation is attenuated by GLP-1 agonist.

- **Aim 2:** Explore if monocytes are a potential source of serum TNFR 1, 2 and TNF-α due to higher monocyte iRHOM2, ADAM17 expression. We will do this by testing if monocyte iRHOM2, ADAM17 mRNA correlate with serum TNFR 1, 2 and TNF-α.

These aims will be conducted in subjects with T1D CKD compared to monocyte response in T1D subjects without CKD as controls. This will help us define which of our results are specific to patients with T1D CKD or is seen in individuals T1D as well.

STUDY DESIGN

This is a cross-sectional study in patients with T1D CKD to test if TIR affects the degree of hyperglycemia required for monocyte activation, podocyte injury, and assess if monocyte activation is attenuated by GLP-1 agonist treatment ex vivo. The monocyte response from patients with T1D CKD will be compared to monocytes from T1D subjects without CKD as a control. Potential subjects will be recruited from existing Cleveland Clinic patients or the community. The study will entail an initial phone screen followed by a formal screening visit to determine eligibility. Eligible subjects will return for a study visit within 4 weeks for a blood draw. There is no intervention. This will complete the study for subjects unless we do not obtain enough monocytes from the initial blood draw to complete the requisite lab studies. If this occurs, we will ask the patient to return for a repeat study visit. All endpoints evaluating monocyte and podocyte injury will be conducted in the lab ex vivo.

Residual blood may be requested from the core lab up to (80 ml) up to twice per week to isolate human monocytes to refine/optimize assays involved with this study. No data will be extracted from these tests, residual blood samples will be used simply to optimize the lab tests and assays.

Inclusion:

1. **T1D CKD subjects:**
    a. Adults, males or females diagnosed with T1D
    b. Age 18-65 years
    c. Diagnosed with CKD (eGFR 60-90 ml/min/1.73 m2)
    d. Diagnosed with albuminuria (UACR 30-500 mg/g)
    e. On insulin injections or pump
    f. On CGM

    Based on baseline CGM metrics, we will stratify subjects to 2 groups
    Group A (Lower TIR group): TIR≤60%, A1c 7.5-9.5
    Group B (Higher TIR group): TIR≥70% A1c 5.0-7.0

2. **Controls: T1D subjects without CKD**
    a. Adults, males or females diagnosed with T1D
    b. Age 18-65 years
    c. No CKD (eGFR >90 ml/min/1.73 m2)
    d. No albuminuria (UACR <30 mg/g)
    e. On insulin injections or pump
    f. On CGM

    Based on baseline CGM metrics, we will stratify subjects to 2 groups
    Group A (Lower TIR group): TIR≤60%, A1c 7.5-9.5
    Group B (Higher TIR group): TIR≥70% A1c 5.0-7.0

Exclusion for T1D CKD and T1D controls:

1. Hemoglobin <9
2. On GLP-1 agonist or DPP4 inhibitor or sodium-glucose co-transporter-2 inhibitors use within 30 days
3. pregnancy or plans to become pregnant
4. On steroids
5. Diagnosed with cancer, immunosuppression/autoimmune conditions
6. Reported heavy alcohol use or recreational drug use
7. Any condition which jeopardizes patient safety or affects monocytes at physician's discretion

Study Recruitment:

The study team will recruit subjects from the electronic health record (EHR) and by word of mouth. For subjects identified through the EHR, the research team will send a MyChart message followed by follow up phone calls to potential research subjects. Interested subjects will be contacted for a 10-minute phone screen to review potential eligibility. Potential subjects will then be scheduled for the screening visit at the F20 clinic at main campus. At the screening visit, a formal consent will be reviewed, and the patient will decide if they wish to participate. Those who decline will receive parking validation and leave the visit. Those who consent to the study will proceed to have review of medical history relevant to inclusion/exclusion criteria, vitals, height, weight. T1D subjects will have download of CGM data, and screening labs unless they had these labs previously which can be shared with the study team (see table below). In this case, their previous labs can be used as screening labs. Eligible subjects based on results of the screening visit will return within 4 weeks for the study visit, which entails a blood draw.

Study Procedures:

We will be isolating monocytes from T1D CKD and T1D without CKD as controls. Potentially eligible subjects have a screening visit at the F20 clinic at main campus.

Screening Visit:

Subjects will meet the study team to obtain informed consent, review medical history. We will obtain vitals, anthropometrics (height, weight, body mass index (BMI)), and urine pregnancy test for women of childbearing potential. We will obtain point of care A1c, CGM data, a blood draw for basic metabolic panel (BMP) and urine microalbumin/creatinine ratio (UACR) unless the patient can provide records of these as part of routine medical care.

Study Visit:

Subjects meeting inclusion/exclusion criteria will return for a study visit within 4 weeks and obtain up to 5.5 tablespoons (80 ml) of blood. The patient will be complete with the visit, and a visit stipend will be issued to the patient. The patient will be complete with enrollment in the study unless we ask the patient to return for a blood draw at a later data for additional data collection. This would occur if the quantity of monocytes isolated from the initial visit is inadequate to complete the planned testing in the lab studies. The monocytes will be isolated and treated to varying degrees of hyperglycemia ex vivo to assess their inflammatory response and ability to

injure podocytes. We also treat monocytes with GLP-1 to see if this can reduce the monocyte response. The patient will not receive any treatment. Total expected time for all visits is <3 hrs.

Procedures	**Screening**	**Study Visit**	**Potential Repeat Study Visit ***
BMP, UACR, CGM data, A1c, urine pregnancy test, vitals, anthropometrics (height, weight, body mass index (BMI))	X		
Medical history and Exam	X		
Blood draw for monocytes		X	X

*Only if inadequate monocytes are isolated from the initial study visit. Up to 3 additional visits maximum, no more frequently than every other week.

Study Intervention/procedures:
This is not an interventional study. The only procedure will be obtaining screening labs and blood draw for monocytes. There will not be any genetic testing. Samples obtained for this study will be destroyed once the work for this study is completed.

Recruitment Procedures:
Potential subjects will be identified from medical records, and word of mouth referrals. From the medical records, we will access the patient's demographics, medical history, medications, labs, and ICD-10 codes relevant to the study. Personal health information (PHI) will not be reused or disclosed to others outside the scope of the study.

Consent Process:
Informed consent will be obtained by the study team at the F20 clinic at main campus in a private room during the screening visit. Patients will have ample time to review study materials and opportunity to address questions. Discussion includes purpose of the study, risks and benefits, voluntary nature, rights and protections, and protocol. We will ascertain subject's comprehension by verbal testing of content discussed including risks and benefits. The consent will be documented in paper format with a copy provided to the patient. We will not use vulnerable subjects in this study.

DATA ANALYSIS PLAN

Data Collection:
Each screened patient will be assigned a unique study number. Phone screen and data from the EMR will be entered into a Redcap database. Patient research binders will be stored in a locked cabinet with the key in a separate location.

Power Analysis:
The primary outcome in Aim 1 is the difference in podocyte apoptosis after exposure to 5 vs 16.7 mM D-glucose monocyte media. The preliminary data in 5 subjects had a standardized difference (mean/standard deviation) of 1.23. A sample size of 12 provides >95% power to detect a difference

at a 2-sided α-level of 0.05. In Aim 2, our sample size is based on the correlation between monocyte iRhom2 and TNF-α (0.68). A sample size of 14 per group will give >95% power to detect a correlation of 0.68 at a 2-sided α of 0.05. To fulfill both aims, a sample size of 30 would provide >95% power to detect a difference at a 2-sided α of 0.05. Assuming 50% screening failure based on our prior study experience, we will need to screen 60 patients (30 per group) to meet recruitment goals.

Statistical Analysis:
Each subject will be assigned a study number which will be stored on a password encrypted computer and charts stored in a locked cabinet with key in a separate location. De-identified demographic data will be collected on the data collection sheet with the study number only. In Aim 1, to find the change in podocyte apoptosis on exposure to 5, 10, 16.7 mM D-glucose +/- GLP-1, we will use a repeated measure, mixed model ANOVA. A similar analysis will be done for all outcomes in Aim 1. In Aim 2, we will assess the correlation between baseline monocyte IRHOM and ADAM17 mRNA with serum TNF-α levels, TNFR 1 or 2, and UACR by Pearson correlation. The statistical analysis will be conducted by statisticians who work with the Endocrine division at Cleveland Clinic. All ex vivo results will be identified by subject study number only. See above for statistical power to test hypotheses.

ALTERNATIVES
The only alternative is to not participate.

RISKS
Risks of participation include loss of personal health information. Risks of study procedures from the blood draws include pain, bleeding, decrease in hemoglobin, bruising, infection, or a vasovagal event. These risks will be decreased via the use of trained personnel in the presence of a study physician. Only the minimal amount of blood necessary for the study will be obtained to decrease the risk of anemia.

The risk of loss of confidentiality will be minimized by assigning a de-identified code to each patient stored on a password encrypted computer accessible to only the research team. All data will be stored in a secure website Redcap, and charts will be stored in a locked cabinet with the key in a separate location. All information will be deleted in a HIPAA-safe manner at the earliest time upon study completion.

BENEFITS
We will discuss during the informed consent that subjects will not benefit directly from the study. However, the information gained from understanding the effects of acute hyperglycemic on inflammation driving CKD and the impact of GLP-1 agonists on this process is significant. The risks posed in this study will be mitigated by trained personnel and any adverse reactions will be promptly treated. Due to the potential generalizable impact of this study and mitigated risks, we believe the benefits outweigh the risks to participation.

COSTS
There is no cost to participating to the patient.

PAYMENT
You will receive a $20 stipend for completion of the screening visit. You will receive $50 for V1/initial blood draw plus a $10 parking voucher. If you are asked to return for a re-draw, you will receive a $50 stipend and a $10 parking voucher. The stipend will be sent to you 1-2 weeks after the study visit via a check to your home address.

PRIVACY AND CONFIDENTIALITY
The risk of loss of confidentiality will be minimized by assigning a de-identified code to each patient stored on a password encrypted computer accessible to only the research team (PI, co-investigator, study coordinators). Only de-identified data will be provided to the statisticians for analysis. Charts will be stored in a locked cabinet with the key in a separate location. All information will be deleted in a HIPAA-safe manner at the earliest time upon study completion.

DATA/SAMPLE SHARING
Data nor specimens will be shared with any 3rd parties. Post analyzed, summarized data will be shared with study sponsor, Stanford Diabdocs K12 program.

ADVERSE EVENTS AND DATA MONITORING COMMITTEE (DMC)
Any adverse events will be recorded in the study chart and reported to the IRB per IRB reporting guidelines. Additionally, adverse events will be monitored during the study period and participation stopped for reactions as deemed by the PI. Participants are eligible to withdraw participation at any time. For adverse reactions which develop during the study, patients will be directed to the appropriate medical care including their primary care physician (PCP) or emergency care. Subjects will be directed to their PCP for any incidental findings which arise during the study.

Given the non-intervention nature, limited sample size, and short duration of the study, there will not be a DMC used or interim analysis conducted.

ADDITIONAL DOCUMENTS
- MyChart initial message
- Consent form
- Phone script and screening questions
- Data collection sheet

REFERENCES
1.) Perkins, BA et al. Risk Factors for Kidney Disease in Type 1 Diabetes. *Diabetes Care.* 2019;42:883-890.

2.) Nguyen D, Ping F, Mu W, Hill P, Atkins RC, Chadban SJ. Macrophage accumulation in human progressive diabetic nephropathy. *Nephrology*. 2006;11(3):226-231.