Title: Immunomodulatory Biomimetic Device to Treat Myocardial Stunning in ESRD Patients

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Clinical Study Protocol

Title: Immunomodulatory Biomimetic Device to Treat Myocardial Stunning in ESRD Patients

Drug/Device: Selective cytopheretic device (SCD)

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List ofAbbreviations

AKI	Acute Kidney Injury
AMI	Acute Myocardial Infarction
AV	Arteriovenous
BP	Blood Pressure
Ca_i	Ionized Calcium
CBC	Complete Blood Count
CHF	Congestive Heart Failure
CPB	Cardiopulmonary Bypass
CRF	Case Report Form
CRRT	Continuous Renal Replacement Therapy
CRP	C-Reactive Protein
CKD	Chronic Kidney Disease
CVD	Cardiovascular Disease
ESRD	End-Stage Renal Disease
HD	Hemodialysis
HgB	Hemoglobin
HR	Heart Rate

IDWG	Inter-Dialytic Weight Gain
IL	Interleukin
IRB	Institutional Review Board
LE	Leukocytes
LVEF	Left Ventricular Ejection Fraction
Mϕ	Marcophage
MO	Monocyte
MOD	Multi-Organ Dysfunction
MS	Myocardial Stunning
NE	Neutrophils
NIH	National Institute of Health
PMN	Polymorphonuclear
RCA	Regional Citrate Anti-Coagulation
RR	Respiratory Rate
SAE	Serious Adverse Event
SCD	Selective Cytopheretic Device
SHH	Standard Heparin Hemodialysis
T2D	Type II Diabetes
TNF-α	Tumor Necrosis Factor-alpha
UF	Ultrafiltration
WBC	White Blood Cell Count

Study Synopsis

Study Objectives	❖ To diminish the ischemia induced myocardial stunning events observed in ERSD patients on chronic hemodialysis requiring aggressive ultrafiltration
Study Design	❖ This is a Phase II trial. ❖ Myocardial stunning events and biomarker measurements will be compared pre and post hemodialysis with standard heparin anticoagulated hemodialysis treatment and after one hemodialysis treatment with the regional citrate anticoagulated SCD device.
Study Population	❖ The subject population to be studied are those who have been on chronic hemodialysis for ≥ 3 months ❖ It is estimated that up to 10 Subjects will be studied in this protocol.
Entry Criteria	❖ **Inclusion Criteria** 1. End-stage renal disease (CKD Stage 5) 2. Receiving chronic hemodialysis thrice weekly for ≥3 months 3. Current dialysis access is a well-functioning arteriovenous (AV) graft, AV fistula, or cuffed tunneled catheter 4. Patient can safely receive chronic hemodialysis thrice weekly with heparin anticoagulation 5. Patient has achieved Kt/V≥ 1.2 over the preceding 2 months 6. Patient has no contraindication for regional citrate anticoagulation 7. Baseline blood pressure before hemodialysis has been ≥100/50 over preceding 4 weeks 8. Able to and have given informed consent 9. Recurrent intradialytic weight gain >0.04 kg/kg body weight at the *beginning* of the week (i.e. Monday or Tuesday). Recurrent is defined as at least two of eight sessions the past 60 days or at least three of twelve sessions the past 90 days. *OR* Intradialytic hypotension (IDH) prone (IDH greater than 30% of dialysis sessions in month before session) IDH defined as systolic blood pressure (SBP) less than 100 mmHg or SBP decrease greater than >10% of predialysis with symptoms 10. Age ≥ 18 years ❖ **Exclusion criteria:**

	1. Severe heart failure (NY Heart Association functional class greater than or equal to 3) 2. Cardiac transplant recipient 3. Mental incapacity to consent 4. Human immunodeficiency virus (HIV) 1 or 2 Ab (+) 5. Any active inflammatory condition (e.g., gout, systemic lupus erythematosus flare, hepatitis B or C infection, allograft rejection, subcutaneous injection of illicit drugs, "skin popping") 6. Treatment with immunosuppressive therapy (e.g anti-neoplastic agents, glucocorticoids, calcineurin inhibitors) within 30 days of the treatment program 7. Hemoglobin (HgB) <9 g/dL 8. Platelet count <50,000/mm^3 9. WBC <4,000/mm^3 10. Acute coronary syndrome, including myocardial infarction within 90 days of the treatment period 11. Ischemic cardiomyopathy 12. Participation in an experimental drug or device study, within 30 days of the treatment period 13. Woman who is pregnant, breast feeding a child, or try to become pregnant 14. Inability to comply with Study Procedures 15. Exposure to hemodialysis for less than 90 days 16. Positive test for active infection with COVID-19 virus
Treatment Plan	Each Subject will receive one standard study hemodialysis treatment with heparin anticoagulation during which all procedures noted in Appendix 1 will be performed. Subsequently each subject will receive hemodialysis with the added SCD filter anticoagulated with regional citrate.
Data Safety Monitoring	This is a small feasibility study. A safety analysis will be performed on each patient prior to their next hemodialysis treatment by the PI.
Key Measurements	❖ Adverse events ❖ Regional wall abnormalities and myocardial strain on Echocardiogram
Exploratory Measurements	❖ Soluble Markers (Elastase, Hs CRP, IL-6, IL-10, IL-17, IL-23, G-CSF, TNF-α, MCP-1, L-selectin, neopterin, IL-1β, IL-8) ❖ Leukocyte flow cytometry (circulatory monocyte subsets and circulatory activated neutrophils) ❖ WBC and platelet counts

Primary Analyses	Descriptive analysis will be conducted on the following parameters in all Subjects exposed to any study hemodialysis treatment: ❖ Adverse events ❖ Regional wall abnormalities and myocardial strain ❖ Clinical laboratory and physiological measurements
Secondary Analyses	A number of secondary analyses will be conducted, including the following: ❖ Mean change from baseline in cytokines at the end of each treatment ❖ Mean change from baseline in markers of inflammation at the end each treatment
Study Periods and Study Duration	❖ The total duration of participation will be approximately 3 weeks.

1. Background and Rationale

More than 400,000 people are currently treated by hemodialysis (HD) in the US end stage renal disease (ESRD) program. The mortality in this group of patients is unacceptably high with almost half of the deaths due to cardiovascular disease (CVD). Strategies to reduce the mortality of HD patients with directed interventions at cardiovascular risk factors have largely been ineffective. It is well recognized that HD patients suffer excess morbidity and mortality, mainly due to cardiac failure and sudden death rather than traditional thrombotic occlusion leading to acute myocardial infarction (AMI).[1,2] The excess cardiovascular risk is not the result of the traditional factors associated with CVD but a combination of non-traditional factors unique to chronic kidney disease (CKD).

There is a growing body of evidence to suggest that a combination of multiple hemodynamic and inflammatory factors contribute to this elevated risk. These factors operate simultaneously and sequentially, contributing to the poor outcomes. They include the repetitive hemodynamic insult precipitated by rapid volume removal with HD and the immune dysregulation accentuated by dialysis. It has been shown that dialysis is associated with a repetitive ischemic injury termed myocardial stunning (MS) [3]. A subsequent observational study over 12 months has shown that MS is associated with 30% mortality and 10% reduction in left ventricular ejection fraction [4]. Larger inter-dialytic weight gain (IDWG) with the requirement for larger ultrafiltration volume is strongly associated with MS and increased mortality [4-6], specifically for ultrafiltration (UF) requirements exceeding 12.37 ml/h/kg. It seems that HD itself may contribute to the development of heart failure and the risk of sudden death. In addition to the hemodynamic instability precipitated by HD, these patients are subject to substantive metabolic and inflammatory stress [7].

Since treatment of traditional risk factors for CVD have been ineffective, focus on non-traditional risk factors, especially acute and chronic inflammation, has become a focus of therapeutic interventions in dialysis patients. In this regard, inflammation and its cellular immunologic effector, the activated monocytes (MO), are central to the accelerated progression of CVD in patients with CKD and patients with ESRD on HD. Consequently, a focus on non-traditional and non-modifiable risk factors has emerged with MO activation as a prime therapeutic target. A pivotal pathobiologic role of MO and macrophages (Mϕ) on development and progression of atherosclerosis has been clearly established [8-10]. In this regard, heterogeneous subpopulations of MO have been identified in humans. Cell surface expression of CD14 and CD16 identifies 3 functionally distinct subsets of MO: classical ($CD14^{hi}$, $CD16^{-}$, pro-inflammatory), intermediate ($CD14^{hi}$, $CD16^{+}$, pro-inflammatory) and non-classical ($CD14^{low}$, $CD16^{++}$, anti-inflammatory/reparative). Intermediate MO play an instrumental role in the development of atherosclerosis in the general and CKD populations [11-14]. This subset of M has a greater degree of pro-inflammatory phenotype compared to the other subsets. Furthermore, epidemiologic studies have reported that the number of intermediate MO increases with worsening renal function and higher cell numbers predict adverse cardiovascular outcomes in ESRD patients undergoing chronic dialysis [14-17]. This proposal plans to evaluate a novel immuno-modulatory device, the selective cytopheretic device (SCD), on the immune dysregulated state of ESRD and to assess the benefit of this innovative strategy to improve cardiac performance and reduce progressive congestive heart failure (CHF).

The SCD has shown to has shown to improve clinical outcomes of critically ill patients with multiorgan dysfunction (MOD) by mitigating the inflammatory cascade. Benefit has also

been indicated with chronic inflammation, such as that observed in end stage renal disease (ESRD). This technology has proven to be effective in pre-clinical animal models of disease for which inflammation is present, such as cardiopulmonary bypass (CPB), septic shock and stroke. This proposal plans to further evaluate therapeutic impact of a novel device (SCD) specifically toward ischemic cardiac events during hemodialysis (HD) in ESRD patients.

SCD Mechanism of Action

The SCD is an immuno-regulating, extracorporeal fiber membrane device targeted to modulate the cardiac ischemic events occurring during HD. The platform of SCD technology is the ability of polysulfone fibers to selectively sequester activated systemic leukocytes (LE), primarily neutrophils (NE) and MO, as they flow through the fiber casing via an extracorporeal circuit. The bound LE are deactivated via exposure to a pharmacologic agent (citrate), resulting in lower ionized calcium (iCa) which resets the excessive/dysregulated activation kinetics of LE to a more normal state, while also providing regional anticoagulation. The reset LE are then released back to the systemic blood. The SCD has demonstrated to ameliorate acute MOD in four clinical trials resulting in a relative 50% reduction in mortality rates of ICU patients with MOD [18-20], as well as shifting the circulating MO pool to a less inflammatory phenotype in ESRD patients on chronic HD. The SCD has the potential to transform the therapeutic approach to a disorder that results in progressive cardiac failure in the ESRD population on chronic HD [21].

Preliminary Clinical Observations

Acute Renal Failure

This approach has been initially evaluated in ICU patients with acute kidney injury (AKI) and multiorgan failure requiring CRRT. These trials were initiated after preclinical experiments in a porcine model demonstrated that SCD therapy had a significant ameliorative effect on sepsis induced organ dysfunction, including cardiac, pulmonary and renal parameters along with an immunomodulatory effect on circulating LE. The clinical evaluation of SCD therapy has been completed in 3 exploratory clinical trials in ICU adult patients with AKI requiring continuous renal replacement therapy (CRRT) and multi organ dysfunction (MOD). These trials have demonstrated an excellent safety profile and suggested efficacy impact. Leukopenia and sustained thrombocytopenia were not observed; accelerated renal recovery with RRT discontinuation and an approximately 15-20 percent or greater improvement in survival rates has been observed compared to conventional RRT. This device is added in series to the CRRT after the standard HD filter. Of note, in all 3 trials SCD therapy was well tolerated, without significant effects on hematological parameters, including white blood counts (WBC) and platelet counts, and with no unanticipated serious adverse events related to SCD therapy. Due to these favorable results in 3 trials, a Phase III controlled, randomized, multicenter clinical trial was undertaken. The control group received standard CRRT with RCA and the SCD treated group received up to 7 days of device therapy. An analysis of per protocol patients demonstrated

no device related serious adverse events. The SCD therapy group had a 60-day mortality rate of 16% compared to 41% in control. The SCD therapy group had no patients with dialysis dependency at day 60 compared to 23% of control patients. The composite end point of either death or dialysis dependency at day 60 was 16% for SCD therapy vs. 58% for control (p=0.01).

Acute Heart Failure and CHF

Over the past decade, a number of novel pharmacologic approaches have failed to prove clinical efficacy, accentuating the need to discover new, safe approaches to treat CHF. Inflammation is central to the development of a variety of acute organ failures, including heart, kidney, lung and brain, as well as chronic organ dysfunction, including heart, kidney and Type 2 Diabetes. CHF and acute decompensated heart failure have been increasingly recognized as associated with chronic systemic inflammation [22]. MO and tissue Mϕ have been identified as critical sources of systemic inflammation in CHF [23] and cause a decrease in cardiac myocyte contractility [24]. In this regard, the SCD has been assessed in a preclinical model of CHF. SCD efficacy to dampen the cardio-depressant effects associated with the chronic pro-inflammatory state of CHF was evaluated in a preclinical canine CHF model [25] developed and fully characterized by Dr. Hani Sabbah. This model manifests many of the sequelae of CHF in humans, including profound systolic dysfunction, increased systemic vascular resistance and decreased cardiac output [26]. It has been used to predict efficacy of new therapies for treatment of CHF [26-28]. Five dogs with advanced CHF were used in the study set. The effect of SCD therapy on LVEF in this model, demonstrates that this therapy converts viable but non-contracting myocardium to contracting myocardium. The renal effects were also substantive with doubling of urine sodium excretion with SCD therapy compared to baseline. With respect to safety profile, no episodes of arrhythmias or hypotension were observed during the treatment period. Recent ongoing studies supported with NIH funding in this model has demonstrated that this SCD related improved myocardial performance lasts up to 4 weeks after treatment.

Ischemia/Reperfusion Injury of Myocardium

Inflammation with LE infiltration into injured myocardium [29] plays a critical role in the degree of myocardial ischemia reperfusion injury after AMI. To evaluate the ability of SCD therapy to reduce infarct size of AMI, dogs underwent complete occlusion of the circumflex coronary artery with a balloon catheter for 3h followed by 2h of reperfusion after balloon deflation. Control IRI dogs (n=4) received no treatment; SCD therapy was initiated 20-30 minutes prior to reperfusion and continued for the entire 2h reperfusion period (n=3). As demonstrated in Figure 3, SCD therapy had a substantial effect to reduce infarct size reducing the percent of infarct area to total LV mass from control of 19 ± 1.35 to 10.2 ± 2.6 % ($p<0.05$) in SCD therapy animals. The degree of NE infiltration was markedly diminished in the area of injury. Serum troponin levels were 10x lower in SCD animals compared to controls (data not shown).

Pilot Feasibility Trial of SCD Therapy in ESRD Patients

A pilot safety trial was undertaken by Szamosfalvi et al. to evaluate SCD therapy in a more stable renal failure patient population. Fifteen ESRD patients with CRP levels above

5mg/L were enrolled to assess the safety and early efficacy signals on inflammatory biomarkers in these patients. After a Non-Significant Risk designation by the Henry Ford Health System (HFHS) IRB, this study was performed [21]. The clinical results demonstrated an excellent safety profile with no decline in total WBC, NE or platelet counts. To evaluate the effect of SCD therapy on M phenotypes in these patients, determination of $CD14^{hi}$ fluorescent intensity on peripheral blood M was analyzed utilizing flow cytometry. SCD therapy promoted a shift in MO phenotype from predominantly $CD14^{hi}$ expressing MO at baseline/pre-SCD therapy to $CD14^{low}$ expressing MO post-SCD therapy (Figure 4, A/B). In the 13 ESRD patients evaluated, a significant shift in MO population phenotype afforded by a single SCD therapy session was observed ($p<0.013$). In a subset of patients (n=7) presenting with T2D, indicative of an even greater chronic inflammatory state, this persistent decline in M CD14 expression was sustained as long as 2 weeks post therapy (Figure 4, C). The average number of cells bound to the SCD membrane following 4 hours of therapy showed $1.13x10^9$, with 68% identified as NE, 23% M, 7% eosinophils and 0% lymphocytes. The eluted M were almost all of $CD14^{high}$ phenotype; with the differential percentage being more than double the average 9% circulating M pool. Of note, two patients underwent SCD treatment with standard heparin HD (SHH), as opposed to RCA. No change in M phenotype was observed, but symptomatic and biochemical adverse events were seen after SHH+SCD treatment, including a greater than doubling of CRP levels. No further studies were undertaken with SHH+SCD treatment due to these adverse events.

Myocardial stunning in ESRD patients undergoing chronic hemodialysis

The mortality rate in ESRD patients requiring chronic hemodialysis treatment is unacceptably high with almost half of the deaths due to cardiovascular disease (CVD). This excessive CVD mortality is not due to classical atherosclerotic disease complications but mainly due to cardiac failure and sudden death, often during the dialysis procedure[1,2]. The pathogenesis of heart failure in this patient population appears to be due to intradialytic recurrent myocardial stunning (MS) as defined as ischemia mediated transient reduction in myocardial function. Overtime these recurrent ischemic insults results in irreversible fibrotic changes, chronic heart failure, arrhythmias, and sudden cardiac death[3,30,31]. In this regard, hemodialysis-induced myocardial ischemia and MS has been found to be common in these patients and is significantly associated with high rates of ultrafiltration and intradialytic hypotension[4]. Of note, pediatric patients devoid of atherosclerotic coronary artery disease also have had a high incidence of MS[32]. MS in HD patients promotes both ischemia induced areas of hypokinesis and akinesis. The majority of these areas of MS improve within 30 minutes post hemodialysis[33,34] More frequent HD regimes are associated with lower UF volumes and reduction of intradialytic hypotension and MS events. This relationship may explain in part improved outcomes associated with more frequent HD, such as nocturnal or home HD, compared to the conventional 3 times a week HD treatment program[35]. MS may be the critical pathophysiologic factor promoting the CV related deaths predominated by heart failure and sudden death in ESRD patients on chronic hemodialysis.

<u>Immunomodulatory Biomimetic Device to Treat Myocardial Stunning in ESRD Patients</u>

This clinical protocol will evaluate the influence of the SCD on myocardial stunning events in ESRD patients in chronic hemodialysis patients. Regional citrate anticoagulation

protocols are routinely used during continuous renal replacement therapy for critically ill patients in intensive care units in many medical centers. A citrate protocol will be adapted for use in this research clinical study in patients with ESRD during one hemodialysis treatment with the added SCD cartridge. We have used this protocol with variations in blood flow/dialysate flow in previous research studies with SCD in both hemodialysis and CRRT treatments. With the currently available citrate intravenous solutions, blood flow through the dialysis system will be 300 mL/min rate. The delivered dose of clearance will be measured online using conductivity dialysance measurements available on Fresenius 2008 T machine. Citrate infusion into the blood will lower the blood ionized calcium level, so that blood ionized calcium levels must be monitored closely. Possible differences may occur in ionized calcium levels in the blood from the extracorporeal circuit obtained at the pre-citrate infusion site compared to the systemic levels may occur. This difference would only occur due to recirculation of blood around the blood access site used for dialysis treatment. Accordingly, assessment of recirculation will be performed using online recirculation module available on Fresenius 2008 T machine. If citrate use results in hypocalcemia this may be associated with paresthesia, tetany, lethargy. If hypocalcemia is not corrected and continues to worsen it may result in severe laryngospasm, seizures, and in rare cases death. Due to these rare potential risks during citrate infusion, ionized calcium levels in the blood from the extracorporeal circuit obtained at the pre-citrate infusion will be carefully monitored, with rapid treatment for protocol subjects in the rare event of developing symptomatic hypocalcemia. Clinical use of regional citrate anticoagulation to prevent circuit clotting (see sections 6.3.4 and 6.3.5) is established and routinely used in clinical hemodialysis at many medical centers. The authors conducted a study of SCD in ESRD patients using citrate anticoagulation at Henry Ford Health System (HFHS) where HFHS IRB deemed the study Non-Significant Risk.

2. Potential Risks and Benefits

The hemodialysis treatment with SCD is comprised of two dialysis cartridges in series within an extracorporeal blood circuit and its associated blood pump systems and blood tubing. The first dialysis cartridge is a conventional high-flux, high surface area Fresenius polysulfone hemofilter (Optiflux 250NR) and the second cartridge is the SCD, which is identical to an FDA approved conventional Fresenius polysulfone F40 filter, Fresenius Medical Care North America, Waltham, MA, U.S.A. approved by the FDA for use in acute and chronic hemodialysis. The extracorporeal perfusion circuit uses standard dialysis arteriovenous blood tubing and is schematized in the Figure 1 with two hemodialysis cartridges in series. The cartridges and blood tubing are placed in a dialysate delivery pump system.

The risks of this device system are similar to risks associated with hemodialysis treatments and include clotting of the perfusion circuit, air entry into the circuit, catheter or blood tubing kinking or disconnection, and temperature dysregulation. The dialysis machines and associated dialysis blood perfusion sets have been designed to identify these problems during treatment with alarm systems and to mitigate any clot or air embolism to the patient with clot filters and air bubble traps. These pump systems and blood tubing sets are FDA approved for this treatment indication.

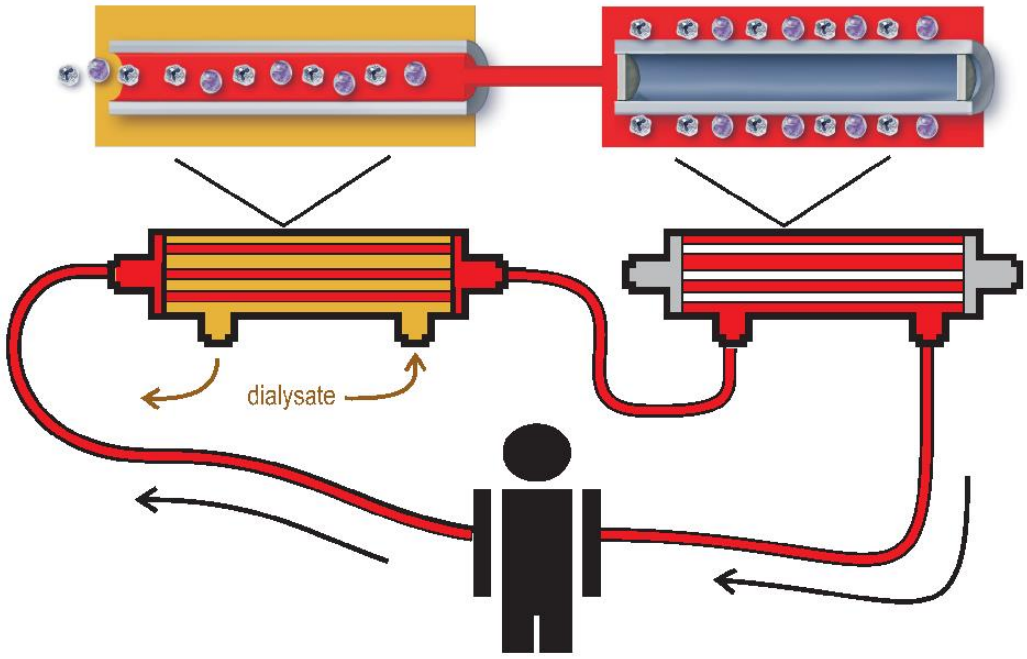


Fig. 1. SCD device.

The blood flow path is changed in the second cartridge compared to conventional use, with blood flowing outside the hollow fibers in the extracapillary space of the hemofiltration cartridge. The lumens of the hollow fibers of the second cartridge will be filled with normal saline, and the entry and exit ports will be capped. This formulation is a reversal of conventional fluid flow mechanics of the dialysis membrane. The risk of this change in flow geometry has proven to be minimal. The use of this second cartridge in this extracorporeal perfusion circuit has been evaluated in over 100 large animals in pre-clinical testing and in 123 patients in Phase I, II, and III clinical studies with no unanticipated adverse events related to the second cartridge and the perfusion circuit. These studies have used both systemic heparin and regional citrate anticoagulation treatments. In fact, in the clinical experience, if clotting occurs within the circuit, it has always been initiated in the first dialysis cartridge. The anticoagulation protocols, both systemic heparin and regional citrate in general (see sections 6.3.4 and 6.3.5), are currently established and routinely used in clinical hemodialysis at many medical centers. Potential benefits may be a short-term improvement of the chronic pro-inflammatory state of ESRD.

For our intended use population the following risk are: cardiac arrhythmias, atrial fibrillation, atrial flutter, bradycardia, sinus tachycardia, ventricular fibrillation, ventricular tachycardia. these risks are all associated with myocardial ischemia. This risk is higher in the intended use population since the enrolled patients will have a tendency to myocardial stunning/ischemia promoting intradialytic hypotension.

3. Study Objectives

The objective of the study is to evaluate the efficacy and safety of SCD in diminishing ischemia induced myocardial stunning events observed in ERSD patients on chronic

hemodialysis requiring aggressive ultrafiltration. Our aim is to decrease myocardial stunning events in hemodialysis patients.

4. Study Design

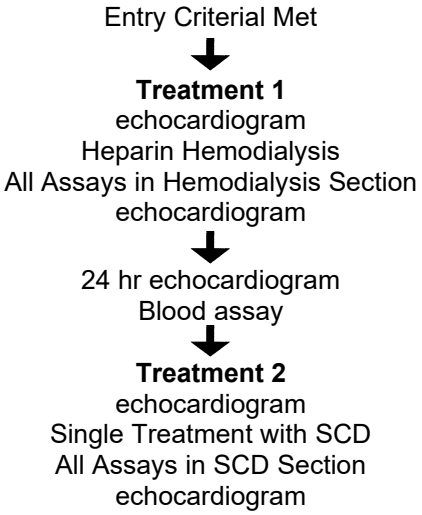
4.1 Design

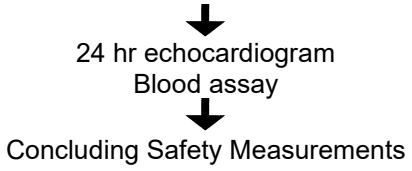
This is an exploratory pilot trial. The subject population to be studied are those who have been on chronic hemodialysis for ≥ 3 months with recurrent intradialytic weight gain >0.04 kg/kg body weight.
Regional myocardial wall abnormalities and biomarker measurements will be compared pre and post hemodialysis with standard heparin anticoagulated hemodialysis treatment and after one hemodialysis treatments with the regional citrate anticoagulated SCD device.

Two different treatment regimens are planned for this clinical trial.

Treatment 1 will be a 5 hour hemodialysis treatment session utilizing heparin anticoagulation. The first hour will utilize a blood flow rate (BFR) of 300 mL/min and a dialysate flow rate (DFR) of 600 mL/min with no ultrafiltration (UF). After the first hour, the blood flow rate will be adjusted to 200 mL/min with a dialysate flow rate of 400 mL/min. This BFR and DFR will be maintained for the remaining 4 hours of the 5 hour session.

Treatment 2 will be a 5 hour hemodialysis treatment session. The first hour will be undertaken with heparin anticoagulation utilizing a BFR of 300 mL/min and a dialysate flow rate of 600 mL/min with no UF. After the first hour, the SCD will be integrated into the circuit with regional citrate anticoagulation and BFR adjusted to 200 mL/min with a DFR of 400 mL/min. This BFR and DFR with RCA will be maintained for the last 4 hours of the 5 hour session.
Of importance, this 5 hour session is planned to ensure adequate solute clearance but with only 4 hour high UF to achieve the patients dry weight.





The hemodialysis treatments will be performed at the Acute Dialysis Unit, University of Michigan.

4.2 Number of Subjects

10 Subjects will be studied in this protocol.

4.3 Duration of Study

The total duration of participation will be approximately 3 weeks.

5. Subject Selection

5.1 Inclusion Criteria

5.1.1 End-stage renal disease (CKD Stage 5)

5.1.2 Receiving chronic hemodialysis thrice weekly for ≥3 months

5.1.3 Current dialysis access is a well-functioning arteriovenous (AV) graft, AV fistula, or cuffed tunneled catheter (average blood flow >350 ml/min in the last 3 HD sessions)

5.1.4 Patient can safely receive chronic hemodialysis thrice weekly with heparin anticoagulation

5.1.5 Patient has achieved Kt/V≥ 1.2 over the preceding 2 months

5.1.6 Patient has no contraindication for regional citrate anticoagulation

5.1.7 Baseline blood pressure before hemodialysis has been ≥ 100/50 over preceding 4 weeks

5.1.8 Recurrent intradialytic weight gain >0.04 kg/kg body weight at the beginning of the week (i.e.Monday or Tuesday). Recurrent is defined as at least two of eight sessions the past 60 days or at least three of twelve sessions the past 90 days.
OR
Intradialytic hypotension (IDH) prone (IDH greater than 30% of dialysis sessions in month before session) IDH defined as systolic blood pressure

(SBP) less than 100 mmHg or SBP decrease greater than >10% of predialysis with symptoms

5.1.9 Able to and have given informed consent

5.1.10 Age ≥ 18 years

5.2 Exclusion Criteria

5.2.1. Human immunodeficiency virus (HIV) 1 or 2 Ab (+)

5.2.2. Any active inflammatory condition (e.g., gout, systemic lupus erythematosus flare, hepatitis B or C infection, allograft rejection, subcutaneous injection of illicit drugs, "skin popping")

5.2.3. Treatment with immunosuppressive therapy (e.g., anti-neoplastic agents, glucocorticoids, calcineurin inhibitors) within 30 days of the treatment program

5.2.4. Hemoglobin (Hgb) <9 g/dL

5.2.5. Platelet count <50,000/mm^3

5.2.6 WBC <4,000/mm^3

5.2.7. Acute coronary syndrome, including myocardial infarction within 90 days of the treatment period

5.2.8. Ischemic cardiomyopathy

5.2.9 Participation in an experimental drug or device study, within 30 days of the treatment period

5.2.10. Woman who is pregnant, breast feeding a child, or is trying to become pregnant

5.2.11 Inability to comply with Study Procedures

5.2.12 Exposure to hemodialysis for less than 90 days

5.2.13 Severe heart failure (NY Heart Association functional class greater than or equal to 3)

5.2.14 Cardiac transplant recipient

5.2.15 Mental incapacity to consent

5.2.16 Positive test for active infection with COVID-19 virus

6. Study Materials

6.1 Study Materials Packaging and Storage

All non-routine supplies and components needed to administer SCD treatments will be stored in a secure, limited-access area.

6.2 Use of the SCD

Personnel at the University of Michigan dialysis unit will be responsible for administering SCD treatments under the supervision and guidance of the clinical investigators.

In this trial, "SCD treatment" using a two-cartridge system will be delivered using a type of dialysis equipment commonly used for conventional hemodialysis therapy. The SCD cartridge will be connected in line with the conventional hemodialysis system, and treatment will be delivered for the usual duration of patient treatment. Blood exchange will occur using the patient's existing hemodialysis access (i.e., arteriovenous graft, arteriovenous fistula, or dialysis catheter).

6.2.1 SCD Set-up

Consult The Set Up Manual of Operations For Fresenius 2008T.

6.2.2 Vascular Access

The Subject's current hemodialysis vascular access will be used for study hemodialysis treatments. The vascular access will be cannulated or accessed only by persons who are trained and experienced in this procedure.

6.2.3 Dialysate Fluid

The dialysate fluid during SHH and during the first hour of SCD treatment arm will be chosen by the Principal Investigator based on the Subject's laboratory tests and clinical status. The content of the dialysate fluid may be adjusted as needed. For the next four hours of SCD treatment with citrate anticoagulation, 0 mM calcium dialysate will be used.

6.3 Selective Cytopheresis System Procedures

6.3.1 Description of SCD System

Dr. H. David Humes, Professor of Internal Medicine, and his group have developed a biomimetic membrane cell processing device, named the Selective Cytopheretic Device (SCD). This developing commercial product licensed to CytoPherx, Inc. (a University of Michigan spinoff) is identical to hemodialysis cartridges (F series) manufactured by Fresenius, AG except for a cartridge casing with closed end caps (U.S. patent 9,341,626). This device, when incorporated into an extracorporeal blood circuit, preferentially binds activated leukocytes (LE), and in the low calcium environment afforded by regional citrate anticoagulation (RCA), the bound LE are deactivated and released back to the systemic circulation. This device is identical to a polysulphone membrane dialyzer but with the blood flow path directed to the outside of the hollow fiber membrane rather than the inside of the membrane. The blood flow path results in low shear forces similar to capillary shear so the membrane has selectivity to bind activated LE. This continuous cell processing activity results in measurable diminution of excessive inflammatory responses in a variety of preclinical and clinical disorders.

The Selective Cytopheretic Device (SCD) investigational device to be used in the execution of this clinical trial protocol consists of two (2) devices packaged separately: 1) SCD Blood Tubing Set (Product Code 8X64R1) 2) SCD-F40 (Fresenius F40S). These two devices are connected in series to a Renal Replacement pump (a Fresenius 2008T ® RRT device). Blood from the Renal Replacement pump circuit is diverted after the hemodialysis dialyzer through the extra capillary space (ECS) of the SCD-F40. Blood circulates through this space and it is returned to the patient via the venous return line of the renal replacement therapy circuit. Regional citrate anticoagulation is used for the entire hemodialysis circuit and SCD blood circuits. When utilized with this flow pathway and in the presence of regional citrate anticoagulation, the SCD membrane binds activated leukocytes and modulates inflammation. Thus, the F40-SCD, and blood tubing are investigational- not FDA approved or cleared for marketing. The remainder of the system is commercially available, FDA cleared.

6.3.2 Intended Use

The SCD is intended to be used in conjunction with FDA-approved dialysis systems on ESRD patients who are already receiving chronic dialysis in the dialysis unit of the University of Michigan.

6.3.3 SCD System Perfusion Initiation Protocol

Procedural Overview

The preparation for hemodialysis with SCD integration is similar to that of the conventional dialysis blood lines by adding the SCD in series with the initial hemodialysis cartridge.

Aseptic technique must be used throughout the entire integration procedure. The SCD cartridge will be brought to the patient's bedside where the SCD blood line will be connected in series to the existing dialysis circuit. After one hour of hemodialysis the SCD will be integrated into the extracorporeal circuit, the dialysate pump system will be initiated and maintained by the trained personnel of the dialysis unit.

6.3.4 Citrate Anticoagulation Therapy

- Citrate acts as an anticoagulation agent by binding with calcium. The bound calcium is then unavailable to trigger clotting factors. A fixed blood flow to citrate flow ratio will be used to ensure strong anticoagulant activity (circuit ionized Ca < 0.4 mM) throughout the entire circuit

- The specific clinical protocol developed at Henry Ford Hospital by Balazs Szamosfalvi (co-investigator) for citrate anticoagulation will be used. About 90-95 % of the free [$citrate^-$ and complex [$citrate\text{-}calcium]^-$ ions are cleared on the high flux and high efficiency OF250NR dialyzer using a commercial Fresenius calcium-free dialysis fluid at 400 ml/min dialysate flow rate. The remaining clinically negligible citrate enters the patient with the venous blood return and is metabolized in the liver. The very high citrate removal on the dialyzer is a unique feature of the IHD-RCA protocol and will prevent citrate accumulation in the patient even in the absence of body metabolism of citrate.

- The filter performance in removing citrate is indirectly monitored by online measurements of conductivity dialysance and by visual filter inspections for clotting.

- Anticoagulation is only reversed when calcium is added to the circuit venous bloodstream just before it is returned to the patient in order to restore systemic ionized calcium levels that will allow adequate systemic blot clotting and cardiac function.

- The initial calcium rate is selected from a table based on patient online hemoglobin (Crit-Line III) and last measured albumin level. The calcium infusion is adjusted hourly based on online hemoglobin values and every time a systemic ionized calcium measurement is performed.

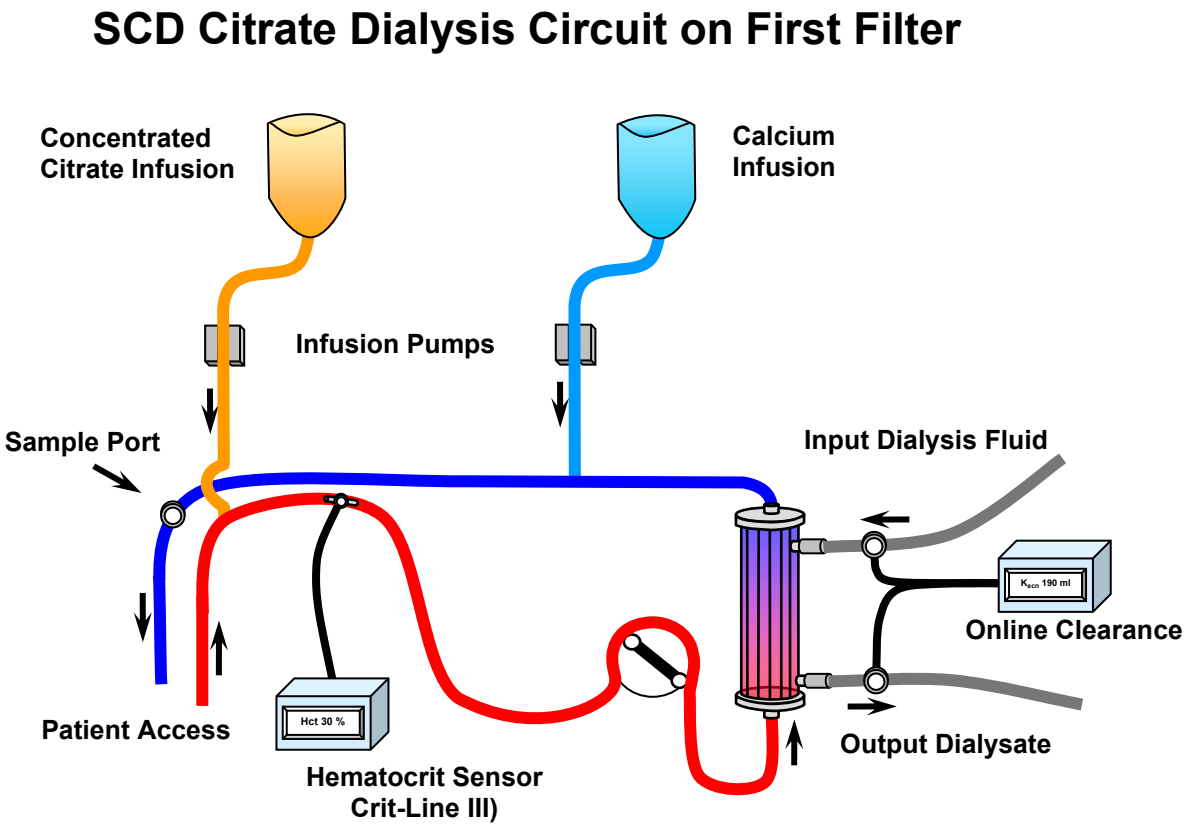


Figure 2. **Diagram of the first filter circuit for the HFH citrate protocol**

- **<u>IHD-RCA Protocol at QB=200: Ca-infusion (200 ml 10% CaCl2 + 0.8 L 0.9% saline) dosing</u>**

1. Select the base Calcium-infusionTable l Rate at QB=200: The amount of calcium removed from the circuit blood by the dialyzer is altered by the hemoglobin (Hb) and albumin level. The initial calcium infusion rate is prescribed by the PI based on the last laboratory hemoglobin and serum albumin using Table 1. At start the nurse adjusts the initial Ca-infusion Table l Rate using the Crit-Line online optical hemoglobin value and the last serum albumin and confirms any change with the PI. The systemic ionized calcium at 1 hour is charted but not used to alter the initial rate. An offset is always applied to the Table l Rate based on the hourly net ultrafiltration rate (see below).

Table 1 ACD-A (Citrate) Infusion Fixed Rate, Blood flow = 200 ml/min, ACD-A flow = 400 ml/hour

Rate in ml/hour of the Ca-infuson (20 gm CaCl2 + 0.8 L 0.9% saline). The rate is defined by the systemic albumin and hemoglobin at a blood flow of 200 ml/min.

Hb	6-6.9 g/dL	7-7.9 g/dL	8-8.9 g/dL	9-9.9 g/dL	10-10.9 g/dL	11-11.9 g/dL	12-12.9 g/dL	13-13.9 g/dL	14-14.9 g/dL	15-15.9 g/dL
Albumin										
0.0-0.7 g/dL	110	106	102	98	93	90	86	82	78	73
0.8-1.2 g/dL	116	112	108	103	99	95	91	87	82	78
1.3-1.7 g/dL	122	118	113	109	105	100	96	91	87	82
1.8-2.2 g/dL	129	125	120	115	110	106	101	96	91	87
2.3-2.7 g/dL	136	131	126	121	116	111	106	101	96	91
2.8-3.2 g/dL	142	137	132	127	121	116	111	106	101	96
3.3-3.7 g/dL	149	143	138	132	127	121	116	111	105	100
3.8-4.2 g/dL	155	149	144	138	132	127	121	116	110	104
4.3-4.7 g/dL	162	156	150	144	138	132	126	120	115	108
4.8-5.2 g/dL	168	162	156	150	144	137	131	125	119	113

2. Adjust the base Ca-infusion Table l Rate at QB=200 using systemic hemoglobin changes The systemic hemoglobin (Hb) level in the arterial limb of the blood circuit will be continuously measured and displayed by an optical sensor (Crit-Line III).
The Hb level may decrease due to bleeding and increase due to blood transfusions or hemoconcentration due to excessive ultrafiltration. If the Hb increases, the circuit plasma flow will decrease and the dialyzer calcium losses will decrease, therefore the calcium infusion rate must be decreased. If the Hb decreases, the circuit plasma flow will increase and the dialyzer calcium losses will increase, therefore the calcium infusion rate must be increased.
The Hb based adjustments are done when a Hb-range change is observed or at least every 20 minutes independent of other adjustments. Select the new base Ca-infusion rate for the hemoglobin range (column) corresponding to the last optical Hb value from Table 1. Use the most recent albumin level to find the appropriate row. Apply the Total Current Offset to the base Ca-infusion Table l Rate to get the final Ca-infusion rate (see below).

3. Offset the base Ca-infusion Table l Rate at QB=200 using the hourly net ultrafiltration rate: Circuit Ca losses due to net ultrafiltration need not be replaced. Calculate the hourly net ultrafiltration rate: [Pre-

dialysis weight (kg) — estimated dry weight (kg)] * 1000 / 4 (hours) = Net ultrafiltration rate (ml/hour). The base Ca-infusion Table l Rate is always decreased by 1 ml/each 100 ml/hour net ultrafiltration rate, e.g in table 2:

Table 2 QB=200: Use the net ultrafiltration rate to adjust the base Table l Rate of the Ca-infusion (200 ml 10% CaCl2 + 0.8 L 0.9% saline) at a blood flow of 200 ml/min

The Patient's Hourly Net Ultrafiltration Rate	**Calcium Infusion Table l Rate Offset for Hourly Net Ultrafiltration**
Net UF 100 ml/hour	Offset Table l Rate DOWN by 1 ml/hr
Net UF 700 ml/hour	Offset Table l Rate DOWN by 7 ml/hr
Net UF 1300 ml/hour	Offset Table l Rate DOWN by 13 ml/hr

6.3.5 Heparin Anticoagulation therapy

- Heparin is a naturally occurring anticoagulant that binds to the enzyme inhibitor antithrombin III (AT-III) causing a conformational change which results in its active site being exposed. The activated AT-III then inactivates thrombin and other proteases involved in blood clotting, most notably factor Xa.

- 1000 units of heparin will be used as a loading dose with a maintenance dose of 1000U heparin/hr via the heparin infusion port, with the syringe on the 2008T machine.

- The heparin dose may be adjusted to the patient’s stable dialysis regimen.

6.3.6 Sampling

Blood samples will be drawn from sampling sites along the extracorporeal blood circuit. Ionized calcium samples will be drawn from extracorporeal blood circuit before citrate infusion site.

6.3.7 Required Support Equipment for SCD Treatment

(Refer to individual equipment manual for proper operating instructions.)

6.3.8 Operating SCD Circuit During Normal Treatment Period

Observe pressure monitors for any consistent, large pressure fluctuations (this could indicate a clotting problem or line occlusion). If the problem persists, call the Principal Investigator. If pressure build up is such that Circuit lines could fail, SCD therapy will be discontinued immediately.

6.3.9 Conclusion of Normal SCD Perfusion

At the conclusion of SCD therapy the patient's blood in the SCD system will be returned to the patient under conventional dialysis and hemodialysis treatment procedures.

6.3.10 Removal of the SCD

After the conclusion of SCD therapy the SCD circuit system will be flushed by research personnel with normal saline to clear the SCD cartridge of residual blood. Elution buffer will be infused into the cartridge and all ports capped.

6.3.11 Discontinuation of SCD Therapy Due to Adverse Event

If the Principal Investigator or Principal Investigator delegate determines that SCD therapy should be discontinued prior to the scheduled end of treatment, the following procedure should be followed:

A. The dialysate pump system is turned off.

B. A decision to return the blood in the extracorporeal blood circuit to the patient is made.

C. The SCD and its circuit are removed from the dialysis system.

D. The PI decides to reinstate hemodialysis.

If there is clotting of the cartridge per se unaccompanied by any clinical prohibitions, the clotted cartridge is to be removed and replaced with a new device. See section 6.3.14 below.

6.3.12 Warnings and Precautions

The following warning and precautionary signs could indicate potential system failures:

A. Changes in patient status should be referred to the clinical investigator.

B. Any leakage of blood from system lines.

C. Any sudden large (>40%), persistent (>2 minutes) change in monitored pressures.

D. Air observed in lines.

E. Development of clots in bubble traps or SCD cartridge extracapillary space.

6.3.13 Required Documentation

The following items need to be recorded:

A. Time of SCD cartridge hook-up.

B. Time of SCD system integration with hemodialysis system.

C. Visual inspection of SCD system upon initiation of perfusion.

D. Calcium levels in blood samples and other parameters as required during Citrate anticoagulation therapy per University of Michigan protocol.

E. System pump speeds every hour.

F. Adjustments in IV pump infusion rates.

J. Time of cessation of SCD therapy.

K. Any unanticipated occurrences should be recorded in hourly comment sections.

6.3.14 Hemodialysis Cartridges

A new SCD cartridge will be used for each treatment.

In the event that either hemodialysis cartridge is not usable for the entire treatment for any reason, a new cartridge will be placed within the perfusion circuit using procedures similar to replacing cartridges during standard hemodialysis.

6.3.15 Dialysis Prescription

- Subjects will have their routine hemodialysis treatment with the added SCD filter.
- All “chronic hemodialysis treatments” will be conducted according to usual clinical practice, using standard hemodialysis machines.
- The schedule of chronic dialysis will be consistent with the research subjects’ routine treatment day.

- ❖ The Principal Investigator will adjust net ultrafiltration to achieve the patient's established stable dry weight.

6.3.16 Duration of Treatment

Each Subject will receive one SCD treatment.

6.3.17 Disconnecting From the SCD Cartridge

- ❖ All blood in the Study Hemofiltration Circuit will be returned to the Subject (unless contraindicated due to presence of clot, Subject's volume status, etc.).

- ❖ If the access being used is a tunneled catheter, flush each lumen with 10 mL of 0.9% NaCl solution USP and then instill a heparin flush (5,000 units/mL) into the ports.

- ❖ If the access being used is an AV fistula or AV graft, remove the dialysis needles or vascular catheters (i.e. angiocaths) used to cannulate the access according to the hospital's dialysis unit protocol.

7. Concomitant Therapy

7.1 Permitted Medications and Therapies

The Subject should continue receiving his usual chronic medications during the Study without limitation in addition to any other needed medications. Routine medications will be given after SCD treatment.

8. Study Methods

8.11 Key Study Measurements

- ❖ Adverse events, vital signs, and laboratory data
- ❖ Regional wall abnormalities and myocardial strain on spectral tracking on echocardiogram (see section 8.4.1)

8.12 Exploratory Measurements

- ❖ Soluble Markers (Elastase, Hs CRP, IL-6, IL-10, TNF-α, L-Selectin, MCP-1, IL-17, IL-23, IL-8. G-CSF, IL-1β, WBC, platelet count, Neopterin)
- ❖ Leukocyte flow cytometry (circulatory monocyte subsets)
- ❖ SCD integrity monitoring during treatment will include visual inspection for clotting and hemolysis, and additional testing following return of hemofilter to the research laboratory.

8.2 Screening Procedures (See also Appendix 1)

Initial screening of Subjects for eligibility may be performed within 4 weeks prior to the start of treatment. Informed consent should be obtained prior to initiation of any study-specific procedures.

- ❖ Obtain informed consent.
- ❖ Review CBC with Differential and Platelet Count that would fall into the Screening Period window.
- ❖ Review Entry Criteria, including Medical History and Concomitant Medications. If the subject meets the entry criteria, including Concomitant Medications, contact the study monitor to confirm admission to the study.
    - ❖ Perform a pregnancy test on women of child bearing potential (serum quantitative bHCG)

8.3 Hemodialysis Procedures – (See also Appendix 1)

Subjects will enter the Hemodialysis Period within 4 weeks of completing the screening process.

8.3.1 Study Treatment

Each Subject will have the treatments described below, during which identical study procedures will be performed during each study treatment.

Treatment 1: Initial standard hemodialysis treatment with heparin (SHH) anticoagulation.

Treatment 2: A single hemodialysis treatment with SCD treatment and regional citrate anticoagulation one week after Treatment 1.

Each subject's safety data will be monitored by the PI on an ongoing basis.

Prior to start of Study Treatment (SHH or SCD Treatment)

8.3.1.1 Perform 2 D echo

8.3.1.2 Prepare a heparin or citrate intravenous infusion per hospital routine to use with a standard volumetric programmable pump and Crit-Line III online hematocrit measurement device if required by the citrate protocol.

8.3.1.3 Assemble blood drawing supplies, including ice bucket, and label tubes.

8.3.1.4 Assemble and set-up Study Circuit.

8.3.1.5 For Subjects with AV fistula or graft, access with needles or venous cannula (i.e., angiocatheters) according to Investigator orders or per hospital routine.

8.3.1.6 For Subjects with tunneled catheters, aspirate heparin flush according to Investigator orders or per hospital routine.

8.3.1.7 Review medical history.

8.3.1.8 Review concomitant medications.

8.3.1.9 Obtain BP, RR, and HR, weight without shoes or over clothes, and oral temperature. Perform physical exam.

8.3.1.10 Obtain blood for albumin according to instructions in **Appendix 2.**

8.3.1.11 Obtain blood for basic metabolic panel according to instructions in **Appendix 2.**

8.3.1.12 Obtain blood for CBC with differential and platelets according to instructions in **Appendix 2.**

8.3.1.13 Obtain blood for systemic soluble markers according to instructions in **Appendix 2.**

8.3.1.14 Obtain blood for flow cytometry according to instructions in **Appendix 2**.

Initiate the Study Hemodialysis Treatment (SHH or SCD Treatment)

8.3.1.15 Begin intravenous heparin infusion as prescribed by standard protocol (*for SHH treatment, heparin will be used for the entire duration of the procedure. For the SCD treatment, heparin will be used only during the first hour of the SCD treatment.)*

8.3.1.16 Turn on blood pump at the speed prescribed by the Principal Investigator. Turn on the calcium infusion per protocol.

8.3.1.17 Obtain BP, HR, and RR.

Repeated Procedures During Entire Study Hemodialysis Treatment (SHH or SCD Treatment)

8.3.1.18 Record Adverse Events.

8.3.1.19 Record concomitant medications.

8.3.1.20 Adjust heparin flow rate as needed for SHH or calcium chloride administration rate for SCD treatment protocol to maintain systemic iCa level between 1-1.3 mM during the citrate anticoagulant therapy. Document the pre-SCD filter ionized Ca as required.

8.3.1.21 Observe cartridges for evidence of hemolysis, clotting, and leakage hourly.

At 1 Hour into Study Treatment Protocol (For SCD Treatment Only)

8.3.1.22 Obtain blood for iCa with i-STAT, a bedside diagnostic device (Abbott Labs).

8.3.1.23 At 1 hour, stop heparin, change dialysate to zero calcium and start citrate and calcium infusion. After 3 minutes of citrate and calcium infusion, stop the blood pump, clamp the venous return line. Please refer to Fresenius Operator's Manual on how to integrate the SCD to circuit. Within the first 10 minutes of initiating citrate with the integrated *SCD* obtain blood from sampling port of arterial segment of extracorporeal circuit (pre-citrate infusion) and send for iCa using a bedside i-STAT Analyzer (**Appendix 2**).

At 2 Hour Into Study Treatment (For SCD Treatment Only)

8.3.1.24 For *SCD treatment protocol,* obtain blood for iCa from extracorporeal blood circuit (pre-citrate infusion) arm using the bedside i-STAT Analyzer.

At 3 Hours Into Study Treatment (For SCD Treatment Only)

8.3.1.25 For *SCD treatment protocol*, obtain blood for iCa from extracorporeal blood circuit (pre-citrate infusion) using the bedside i-STAT Analyzer.

At 4 Hours Into Study Treatment (For SCD Treatment Only)

8.3.1.26 For *SCD treatment protocol*, obtain blood for iCa from extracorporeal blood circuit (pre-citrate infusion) using a bedside i-STAT Analyzer.

At 5 Hours Into Study Treatment (SHH and SCD Treatment except if indicated otherwise)

8.3.1.27 Obtain blood for basic metabolic panel according to instructions in **Appendix 2.**

8.3.1.28 Obtain blood for CBC with differential and platelets according to instructions in **Appendix 2.**

8.3.1.29 Obtain blood for systemic soluble markers according to instructions in **Appendix 2.**

8.3.1.30 Obtain blood for flow cytometry according to instructions in **Appendix 2**.

8.3.1.31 For *SCD treatment protocol*, obtain blood for iCa with i-STAT Analyzer.

End the Study Treatment Period

8.3.1.32 Discontinue heparin infusion in heparin anticoagulant treatment or citrate infusion in citrate anticoagulant treatment.

8.3.1.33 Remove any peripheral intravenous catheter(s).

8.3.1.34 For Subjects with AV fistula or graft, remove needles or venous cannula (i.e. angiocatheters) and obtain hemostasis according to Investigator orders or per hospital routine.

8.3.1.35 For Subjects with tunneled catheters, disconnect tubing and instill heparin flush according to Investigator orders or per hospital routine.

8.3.1.36 Obtain oral temperature, BP, pulse, and weight without shoes or overclothes.

8.3.1.37 Disassemble the Study Circuit.

8.3.1.38 Perform 2 D echo

8.3.1.39 The Subject may be discharged after at least 1 hour of observation following completion of his/her last study treatment. At the Investigator's discretion, the Subject may be kept for observation beyond 1 hours, if needed. During this 1 hour observation period, additional iCa may be obtained post SCD treatment session, upon PI's determination.

24 hours after Treatment (SHH or SCD Treatment)

8.3.1.40 Perform 2 D echo

8.3.1.41 Obtain blood for systemic soluble markers according to instructions in **Appendix 2.**

8.3.1.42 Obtain blood for flow cytometry according to instructions in **Appendix 2**.

Before Initiation of SCD Treatment

8.3.1.43 The PI must review all safety data of the Subject and determine their suitability to move to the SCD treatment. Further, the WBC count must be >4000/mm^3, neutrophil count >500 and platelet count >50,000/mm^3 in prior treatments.

Ionized calcium during SCD Treatment

8.3.1.44 The PI may draw more frequent iCa using i-STAT device if deemed necessary for safety concerns.

8.4 Detailed Study Measures

8.4.1 **Echocardiographic Methods**

Complete transthoracic echocardiograms including structural imaging, color Doppler for evaluation of valvular regurgitation, spectral Doppler for hemodynamic quantitation and longitudinal strain in 3 views for calculation of global longitudinal strain (GLS) will be performed at each time point as per the specified protocol.

All echocardiograms will be performed on an identical echocardiographic platform (Epiq, Philips Ultrasound, Andover MA) using identical platform upgrades and software. Standard measures of left ventricular dimensions and volume from which ejection fraction will be calculated will be

performed off-line using the digitized images. The calculation of GLS will be performed using the integrated software of the platform.

Specific echocardiographic parameters to be quantified at each time point are as noted on the echocardiographic case report form.

Because the majority of echocardiographic parameters which reflect left ventricular contractility are both pre-and afterload dependent a substantial range of findings across patients is unavoidable and it is not feasible within the scope of a study this size to identify "normative" values nor is it possible to accurately identify thresholds of change in parameters such as ventricular size or ejection fraction in patients in whom intravascular volume and systemic systolic pressures vary. Each patient will therefore serve as his or her own control comparing pre-and post-dialysis echocardiograms following standard dialysis compared to pre-and post-echocardiograms following investigational dialysis using the SCD device[36,37].

Assessment: Echocardiographic parameters which are less (but not entirely) preload and afterload dependent include quantitation of regional wall motion abnormalities as well as calculation of GLS. Normative values for regional wall motion abnormalities are defined as normal thickening and endocardial excursion in all regions and any deviation from normal thickening or excursion is considered abnormal. Regional wall motion will be scored using a standard wall motion scoring scheme. (1= normal; 2=hypokinetic; 3= akinetic; 4=dyskinetic). Scores will be tabulated for each of 16 predefined segments and a global wall motion score calculated as the sum of individual scores divided by the number visualized segments. For this scheme a score of 1 represents normal ventricular function with progressively higher numbers representing progressively worse systolic function. Scores from pre HD, post HD and 24 hour post HD Echos from all 10 patients will be collated and analyzed for statistical significance between Treatment 1 and Treatment 2 groups.

Global longitudinal strain will be obtained using tissue tracking methodology[38]. There is a significant range within the left ventricle of normal values for regional strain depending on wall location. Values for normal GLS in normal controls have ranged from -16.0 to -22.0. Patients with systemic diseases including hypertension, diabetes and renal disease have all been described to have reduced GLS in the absence of overt systolic dysfunction. As "normal" values for GLS cannot be predicted in the patient population under consideration each patient will serve as his or her own control for tracking improvement or deterioration in global longitudinal strain.

Assessment: The largest body of data regarding using global longitudinal strain for tracking myocardial performance is in the patients receiving cardiotoxic chemotherapeutic agents where a threshold of 10-15% change has been considered as significant variation from baseline[39]. This will be used as a dichotomous threshold for defining either improvement or deterioration in GLS.

Echocardiographic images will be interpreted at the Echo Laboratory, Frankel Cardiovascular Center, Floor 3. Area B, University of Michigan, Ann Arbor, MI

8.4.2 **Soluble Markers Assay Methods**

Assays for soluble markers (Table 3) are either sandwich or competitive ELISAs' and will be performed according to manufacturer's suggested protocols. High sensitivity Quantikine kits from R&D Systems will be run with Quantikine® Immunoassay Control Group 10 Catalog Number: QC41 which provides high, medium and low reference for high sensitivity kits, including IL-1β, IL-6, IL-8, IL-10 and TNFα. MCP-1 Quantikine kit will be run with Immunoassay Control Group 1 Catalog Number: QC01-1, which provides high, medium and low reference for a wide range of soluble markers including MCP-1. Inter assay controls are provided within the kits for CD62L, elastase, hsCRP, and neopterin. The 96 well format plates will be read using a Molecular Devices Spectramax M5 plate reader, and acquired data analyzed using SoftMax Pro 6.5.1 Software. Inter and Intra Assay precision will be calculated and criteria for acceptance established using expected values provided by the kit manufacturers. Non-compliant analysis will be repeated until criteria are met.

The slated soluble markers are expected to deviate from reported normal values with in patient populations with myocardial stunning. Baseline values will be compared to reported normal and diseased values as reported in the literature and where reported by kit manufacturers. Each patient's pre-treatment value will be used as its own baseline and this value compared to those obtained with the normal hemodialysis session and with SCD therapy. Statistical significance of assay values will be determined using Anova for repeated measures and paired student t test where appropriate.

Table 3. Assays for Soluble Markers

Soluble Marker	Assay Kit	Assay Type	Inter Assay Controls
Elastase	ThermoFisher BMS269	Sandwich ELISA	High/Low Included in Kit
Hs CRP	ALPCO 30-9710s	Sandwich ELISA	High/Low Included in Kit
IL-1β	R&D Systems HSLB00D	Sandwich ELISA	R&D Systems QC41
IL-6	R&D Systems HS600B	Sandwich ELISA	R&D Systems QC41
IL-8	R&D Systems HS800	Sandwich ELISA	R&D Systems QC41

IL-10	R&D Systems HS100C	Sandwich ELISA	R&D Systems QC41
TNFα	R&D Systems HSTA00E	Sandwich ELISA	R&D Systems QC41
CD62L (L-Selectin)	Abcam	Sandwich ELISA	Single Control
MCP-1	R&D systems DCP00	Sandwich ELISA	R&D Systems QC01-1
Neopterin	TECAN IBL RE59321	Competitive ELISA	High/Low Included in Kit

8.4.3 **Flow Cytometry Methods**

Blood will be drawn into EDTA tubes and processed immediately on wet ice. Erythrocytes will be lysed with ammonium chloride buffer, and remaining leukocytes washed by centrifugation and suspended in protein free buffer for live dead labelling to allow for the elimination of false positives. Leukocytes will be transferred at 106 cells/100□L/test into tubes containing the following antibody cocktails and incubated for 30 minutes. Tubes will be simultaneously fixed and undergo secondary lysis with BD FACS lyse buffer which has the added benefit of allowing leukocyte cell populations to be identified using scatter profiles.

In Panel 1, antibodies targeting human CD91, CD14, CD16, HLADR, CD192 and CD197 will be used to determine changes in the circulating monocyte population. Neutrophils and monocytes mobilize intracellular stores of CD11b to the cell surface as they become activated, allowing a real-time measurement of systemic acute neutrophil and monocyte activation. Blood treated with a saturating amount of anti-human CD11b allowing for the quantitative analysis of expression to be reported as relative fluorescence intensity.

Systemic Blood Panel 1: CD11b acute activation and MO subtypes

Target	Channel	Vendor	Catalog#	Clone
CD11b	BL1-FITC	BioRAD	MCA551F	ICRF44
CD91	BL2-PE	ThermoFisher	12-0919-42	A2MR-a2
CD14	BL3-PerCP Cy5.5	BioRAD	MCA1568	Tük4
CD192	RL1-Alexa Fluor 647	BD Biosciences	558406	48607
CD16	RL3-APC H7	BD Biosciences	560195	3G8
CD197	VL1-BV421	BioLegend	353208	Go43H7
Live Dead	VL2-Fixable Aqua	ThermoFisher	L34966	NA
HLA-DR	V3-BV605,	BD Biosciences	562845	TU36

Using panel 2, platelet activation will be measured by the percentage of platelets associated with monocyte using the pan monocyte marker CD91 and the platelet membrane glycoprotein recognized by anti-human CD42a. Neutrophil life cycle will be evaluated using anti-human CD10, CD184 (CXCR4) and CD177. Immature neutrophils do not express CD10, while

CD177 and CD184 are expressed on aging neutrophils. A saturating amount of CD62L (L-selectin) will be used to allow for comparison of this activation marker across neutrophil subsets.

Systemic Blood Panel 2: CD62 on NE, monocyte platelet aggregates

Target	Channel	Vendor	Catalog#	Clone
CD62L	BL1-FITC	BD Biosciences	555543	DREG-56
CD91	BL2-PE	ThermoFisher	12-0919-42	A2MR-a2
CD10	BL3-PERCP Cy5.5	BD Biosciences	563508	HI10a
CD42a	RL1-Alexa Fluor 647	BioRAD	MCA1227A647	GRP-P
CD177	VL1-BV421	BD Biosciences	564240	MEM-166
Live Dead	VL2-Fixable Aqua	ThermoFisher	L34966	NA

Events will be collected using the ThermoFisher Attune flow cytometer and data analyzed using FlowJo software. Gates will be confirmed using fluorescence minus one controls, and a blue laser control bead will be used to ensure inter assay consistency with CD11b and CD62L that will be expressed as relative fluorescence in the blue laser line. Specificity of the antibodies will be confirmed using matched isotype controls. Statistical significance of differences in population percentages and relative fluorescence intensity values will be determined using Anova for repeated measures and paired student t test where appropriate.

8.5 Study Exit Procedures

A Study Termination form will be completed for all patients who have completed the Study Procedures according to the protocol as well as for those who are terminating early for any cause

9. Statistical Considerations

Sample size determination for primary outcome regional wall abnormalities: Our consultant, C. McIntyre has previously demonstrated a difference in this variable in 8 to10 patients using a similar protocol to this proposal when comparing standard and biofeedback dialysis and dialysis cooling [33,34]. Sample size of 10 would appear to be sufficient to detect a difference of 20% and is based upon a standard deviation of 14%, significance level of 0.05 and 80% power. In the McIntyre studies, differences of greater than 20% were observed. We plan to recruit 10 patients in this study, to better ensure an adequate sample size to obtain statistically relevant primary and secondary endpoint comparisons. The patients will serve as their own controls, comparing BL-SHH parameters to SCD therapy parameters. Independent t-test will be used to compare the peak frequency of MS between SHH and SCD sessions. Global LVEF and wall motion in 16 segments in 8 regions at each time point in the dialysis session will be compared using repeated measures and Bonferroni's test for multiple comparisons. IDWG will be compared using paired and unpaired non-parametric testing or t-test if the variable is

normally distributed. These approaches have shown significance in prior studies for MS in HD. Statistical correlations are planned for regional wall abnormalities, inflammatory indices, monocyte subsets and pre-dialysis blood pressures [33,34]

10. Adverse Events

10.1 Definitions

An **Adverse Event** is any change from the Subject's baseline and usual (pre-treatment) condition, whether or not it is believed related to the experimental treatment. An adverse event, therefore, is any unfavorable and/or unintended sign, symptom, or disease that is temporally associated with the use of the experimental treatment and occurs for the first time after treatment initiation or, if present at the time of enrollment and treatment initiation, intensifies or indicates a worsened clinical condition..

Any Adverse Event, whether considered study-treatment related or not, which fits any of the criteria below, is considered a **Serious Adverse Event** (SAE):

- Results in death.
- Is life-threatening (meaning that the Subject was at risk of death at the time of the event; this does not refer to an event which might have caused death if it had occurred in a more severe form).
- Requires inpatient hospitalization or prolongs the existing hospitalization.
- Is a persistent disability/incapacity.
- Is a congenital anomaly or birth defect.
- Is considered an important medical event by the Investigator (e.g., surgery, return to ICU, emergency procedures, etc.)

Medical judgment should be exercised in deciding whether other medical events may be considered serious. Important medical events which may not be immediately life threatening or result in death or hospitalization but which may jeopardize the Subject or require intervention to prevent one of the serious outcomes listed above should be considered serious.

Any Adverse Event, which is not consistent in specificity or severity with the current Investigator's Brochure including all amendments, is considered "**unexpected"**.

Causality is defined as the probability that the adverse event may have been caused by the study treatment. The following definitions for causality assessment will be used in this study:

- **DEFINITELY RELATED**: A clinical event, including a significant change in a laboratory test, that occurs in a plausible time relationship to the study hemofiltration treatment, and which cannot be explained by concurrent disease or other drugs, chemicals, or procedures.

- **PROBABLY RELATED**: A clinical event, including a significant change in a laboratory test, occurring within a reasonable time sequence in relationship to the study hemofiltration treatment that is unlikely to be attributed to concurrent disease, other drugs, chemicals, or procedures, and that follows a clinically reasonable response upon withdrawal of the study hemofiltration treatment.

- **POSSIBLY RELATED**: A clinical event, including a significant change in a laboratory test, occurring within a reasonable time sequence in relationship to the study hemofiltration treatment that could also be explained by concurrent disease, drugs, chemicals, or procedures. The clinical course after withdrawal of the study hemofiltration treatment may be unclear with respect to the contribution of the study hemofiltration treatment to the adverse event.

- **DEFINITELY NOT**: An adverse event, including a significant change in a laboratory test, with a temporal relationship to the SCD treatment that makes an association with the study hemofiltration treatment improbable, and in which other drugs, procedures or underlying disease(s) provide likely explanation.

The **intensity** of all adverse events should be evaluated using the following definitions:

- **SEVERE**: An event requiring immediate intervention; causes significant discomfort

- **MODERATE**: An event that requires non-routine intervention; i.e., a new clinical treatment or diagnostic procedure, administered within an hour of the event; causes annoying discomfort

- **MILD**: An event that requires minimal clinical treatment, or an adverse event requiring monitoring but no intervention or treatment; causes slight discomfort

10.2 Reporting Requirements

All adverse events occurring from Time 0 through Day 14 must be recorded on the Adverse Event page of the Case Report Form (CRF).

Serious Adverse Events

In addition to recording data on the CRF, any SERIOUS adverse event (regardless of relationship to the study hemofiltration treatment) that occurs during the first study hemofiltration treatment will be reported to the IRB. The clinical site will be required to complete a written Serious Adverse Event report, which must be signed by the Sponsor-Investigator.

For any serious adverse event that is ongoing at the time of the initial report, periodic follow-up information will be required until the adverse event is resolved or is considered to be chronic and stable.

Sponsor Investigator Responsibilities

The Sponsor-Investigator will promptly review documented adverse effects and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse effect; 2) if there is a reasonable possibility that the adverse effect was caused by the investigational device or, if applicable, other study treatment or diagnostic product(s); and 3) if the adverse effect meets the criteria for a serious adverse effect.

In accordance with 21 CFR Part 812.150(a)(1) and (b)(1), the sponsor shall promptly report the results of an evaluation of any serious and unanticipated adverse device effect to FDA, the University of Michigan IRBMED and participating investigators (if any) as soon as possible, but not later than 10 working days after the sponsor first receives notice of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effect as the FDA requests. Complications and non-serious or anticipated adverse events should be documented and tabulated but need not be submitted by the sponsor to the FDA as individual reports.

Independent Data Review and Data and Safety Monitoring Committee

To protect the interests of research subjects and ensure that they are not exposed to undue risk, this trial will be monitored by an independent Data and Safety Monitoring Committee (DSMC). The DSMC will been appointed by the Sponsor-Investigator, and shall have no formal involvement with the subjects or the investigation and function independently of Sponsor-Investigator. The DSMC will be members independent from the sponsor-investigator and co-investigators. The DSMC will provide an independent, and unbiased assessments of adverse events and any need for study termination. The Committee will consist of one nephrologist and one cardiologist with expertise in heart failure and who are independent of the study team.

The DSMC will monitor the progress of the trial with a scheduled meeting or conference call. In addition to reviewing Serious Adverse Events (SAEs), the first DSMC meeting will focus on over all safety of the trial and study device and will make a determination as to whether or not the study should proceed.

The DSMC will also serve as the Clinical Events Committee (CEC), and will adjudicate all adverse events for clinical accuracy and relatedness to the treatment, and will also ensure a unified evaluation by applying standardized even criteria. The CEC will perform final adjudication of adverse events.

10.3 Clinical Research Monitoring

To assure adequate protection of the rights of human subjects, per 21 CFR §812.40, 812.43 and 812.46, this study will be monitored by the University of Michigan Institute of Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. A site activation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Sponsor-Investigator.

The established monitoring plan will ensure the quality and integrity of the data throughout the study conduct to verify adherence to the protocol, completeness and accuracy of study data and samples collected, dispensing and inventory of the device, and compliance with regulations.

11. Investigator Responsibilities and Statement

By my signature, I confirm that my staff and I have carefully read and understand this protocol and agree to comply with the conduct and terms of the study specified therein. In particular, we have agreed to:

- ❖ Conduct the study according to the protocol, its amendments, and study guides.
- ❖ Obtain Institutional Review Board approval of the study, any amendments to the study, and periodic re-approval, as required.
- ❖ Obtain witnessed, written informed consent from each study participant or their legal representative. Affirm each Subject meets the inclusion and exclusion criteria with study monitor.
- ❖ Report all serious adverse events as required by the protocol and IRB regulations to the IRB.
- ❖ Maintain confidentiality and assure security of confidential documents such as the protocol, consent form, case report form, final study reports, manuscript, and/or unpublished data and correspondence.

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Principal Investigator Date

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Appendix 1. Schedule of Events

Event	4-week Screening Period	Hemodialysis (Procedures to be repeated on days study hemodialysis treatment(s) are given)							24 hour after (± 4 hours)
		Pre-Rx Baseline (time 0)	Rx start >0–<1h	1h	2h	3h	4h	5h	
Informed consent	X								
Review of entry criteria	X								
Echocardiogram[1]		X						X	X
albumin		X							
Basic Metabolic Panel[6]		X						X	
CBC/diff/plt[6]		X						X	X
Pregnancy test[4]	X								
Systemic soluble markers [2]		X						X	X
Leukocyte flow cytometry		X						X	X
Physical examination		X							
Review/update medical history		X							
BP, HR, RR[5]		X	X	X	X	X	X	X	X
Weight/temp		X						X	
Concomitant medications	X	X	X	X	X	X	X	X	x
Adverse events		X	X	X	X	X	X	X	x
iCa[3,6]				X[7]	X	X	X	X	
Visual inspection for hemolysis and clotting in blood circuit				X					

Notes:

1. Hemodialysis procedures should begin within 2 hours of completion of echocardiogram and completion of baseline procedures (physical examination, review/update medical history, vital signs (BP, HR, RR Weight/temp) and recording and review of concomitant medications.
2. Elastase, Hs CRP, IL-6, IL-10,IL-17, IL-23, G-CSF, TNF-α, , MCP-1, IL-17, IL-23, IL-8, G-CSF, IL-1β, Neopterin.
3. For SCD Treatment Protocol only, iCa will be drawn. Levels of iCa will be determined from extracorporeal circuit before citrate infusion site. Additional iCa may be obtained if clinically indicated and post SCD treatment session, upon PI's determination.
4. Pregnancy test: Quantitative bHCG test will be obtained in the screening period.
5. Vital signs (BP, HR, RR) would be recorded within 20 minutes of indicated times.
6. iCa, CBC/diff.plt, basic metabolic panel would be collected within 15 minutes of indicated times.
7. For SCD Treatment Protocol only, iCa will be drawn during this hour just prior to initiation of therapy, and within 10 minutes of starting therapy.

Appendix 2. Laboratory Analyses

Test	Specimens	Volume (mL)	Total volume (mL)
Albumin	No additive	1 ml	1 ml
Basic metabolic panel	No additive	2 ml	8 ml
iCa*	No additive	0.5 ml	3 ml
CBC and plt	EDTA	3 ml	18 ml
Systemic soluble markers	EDTA	11 ml	66 ml
Leukocyte flow cytometry	EDTA	1 ml	6 ml
Quantitative bHCG	Red top or SST tube	0.5 ml	0.5 ml

***Ionized calcium will only be sampled during SCD treatment. Ionized calcium will be drawn with i-STAT Device**