The Effect of Loop Diuretics on Severity and Outcome of Acute Kidney Injury

RESEARCH PLAN

A. Specific Aims

The goal of the study is to determine the severity of acute kidney injury (AKI) early in the clinical course of the disease. Patients who suffer from acute kidney injury can have one of four potential outcomes (1) recovery of kidney function without need for dialysis (2) recovery of kidney function with need for dialysis (3) need for dialysis without renal recovery, and (4) death. The goal of the study is to determine if we can identify which clinical course will occur early in the course of the disease. 5) We seek to determine how well physicians caring for those with AKI can predict the clinical course compared to novel biomarkers of AKI. 6) we seek to determine the association between this clinical course and 3 year patient outcomes.

B. Background and Significance

AKI is a very common disease in patient admitted to the hospital. However, despite advances in supportive care, patients with AKI carry a high mortality rate (50% to 70%).[1] AKI affects nearly 5 percent of all hospitalized persons and as many as 15 percent of critically ill patients. [1, 2] Currently, there are no FDA approved therapeutic agents for the treatment or prevention of AKI.

Recently, at a large international consensus meeting held in Vancouver, BC,[3] the single most important question that was raised by this was the question of when to initiate renal replacement therapy (e.g dialysis). Retrospective studies suggest that the early initiation of renal replacement therapy (RRT) improves outcome. [4-7]

However, a significant subset of patients with AKI recovers without the need for RRT. Many clinicians tend to take a “wait and see” approach because they do not want to dialyze a patient who is destined to recover renal function without the need for RRT. Therefore, it is vitally important to know early in the course which patients with AKI are likely to progress to RRT.

C. Preliminary Studies

The majority of studies on the timing of initiation of dialysis have been case series with historical controls or retrospective case-control series that were performed early after the routine clinical use of dialysis was first introduced. For this reason, the BUN at the initiation of dialysis in the "early" treatment group in these previous studies were high by today’s standards.

More recently, Gettings et al.[8] conducted a retrospective study of patients with posttraumatic AKI and stratified patients on the basis of timing of the initiation of dialysis into "low" and "high" degree of azotemia groups using a BUN cutoff of 60 mg/dl. Mortality rates among patients who initiated dialysis at the lower BUN cutoff was 61%, compared with 80% among those who started dialysis with higher BUN levels (P = 0.04).

Additional studies[9, 10] examined the timing of initiation of dialysis for AKI after cardiac surgery and demonstrated a benefit to earlier initiation of dialysis. These studies used a definition of AKI to justify the provision of early dialysis of a urine output <100 ml during the first 8 h after bypass surgery regardless of solute clearance. Demirkilic et al.[9]studied a total of 61 patients; the overall mortality rate in those who were treated with early dialysis was 24%, compared with 56% in control subjects (P = 0.016). Elahi et al. [10] reported the results of their analysis of 64 patients with postbypass AKI; the overall mortality rate in

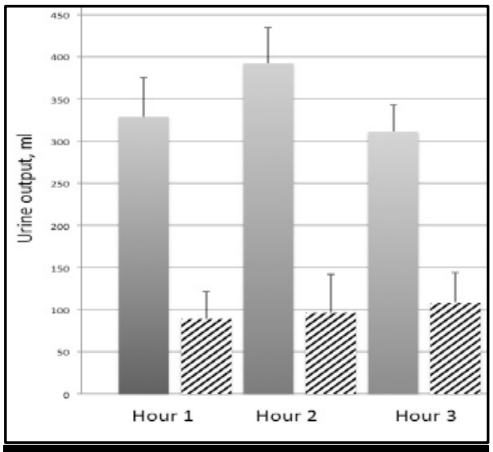
those who were treated with early dialysis was 22%, compared with 43% in control subjects (P<0.05). However, the relevance of these data to nonpostoperative patients or to patients with nonoliguric AKI is unclear.

Liu et al.[3] showed that even with adjustment for confounding and selection effects, patients with higher BUN concentrations at the start of dialysis had worse outcomes as compared to those patients with lower BUNs.

Since the initiation of this protocol, we have recruited subjects with early Stage 1 AKI (a 0.3 mg/dL absolute or 50% relative increase in serum creatinine or 0.5 ml/kg/hr of urine output for 12 hours – AKIN Stage 1) to prospectively receive a one-time furosemide bolus. We have recently published our preliminary data describing patient outcomes following the Furosemide challenge [11]This Renal Stress test was prospectively administered to 54 ICU patients (across 2 centers) in the setting of early AKI and was dosed at Furosemide 1 mg/kg for loop diuretic naïve patients (1.5 mg/kg for those who had previous loop exposure). Over the first 6 hours post FST, UOP was able to significantly predict the development of Stage 3 AKI (AUCs 0.82-0.87) as well as a composite endpoint of Stage 3 AKI or In-Hospital Death (AUC 0.74- 0.77). (**Table 1** These results were similar to those of a 23 subject
retrospective cohort from the Southern AKI Network (SAKI-Net) who were noted to have previously received an FST in the setting of Stage 1 AKI: AUC for AKIN-3 (0.83-0.87) and 0.85-0.89 for AKIN-3 or Death. **Figure 1** demonstrates the UOP in progressors (those reaching Stage 3, n=25) and non-progressors (n=52) in the first 3 hours following FST (n=77). ***In our 77 subject cohort, total UOP of less than 200 mls during the first 2 hours post-FST was both a sensitive (87.1%) and specific (84.1%) marker for the progression to Stage 3 AKI****.[11]* This cutoff of 200 mls remained extremely sensitive for the composite endpoint of AKIN-3 and death (90%) although the specificity did drop (74.2%). *Finally and most importantly, the FST was extremely well tolerated with no episodes of hypotension or any adverse event attributable to the diuretic challenge.* This stems from the pre-enrollment selection of patients who were euvolemic and stable for FST as deemed by their treating physicians. Additionally, treating teams were given the option to replace urinary losses after FST with the IV fluid of their choosing.

Table 1: Prospective Cohort - FST ROC-AUCs for Progression to AKIN -3 and Composite Endpoint (n=54)

	AKIN-3	AKIN-3 or Death
One Hour	0.82(0.07)*	0.74(0.08)*
Two Hour	0.87(0.07)*	0.76(0.08)*
Three Hour	0.87(0.07)*	0.76(0.08)*
Four Hour	0.87(0.07)*	0.76(0.08)*
Five Hour	0.87(0.07)*	0.77(0.08)*
Six Hour	0.86(0.07)*	0.76(0.08)*
*p<0.01		



after each of the first 3 hours following FST (p<0.001 for all

Future studies are needed to validate our preliminary data as well as assess the post-hospital discharge outcomes in this cohort. Once the furosemide challenge is validated and we have a method to identify severe early AKI then and only then can we focus on randomized prospective studies on early versus late RRT in patients with AKI.

D. Research Design and Methods

We plan to enroll up to 200 patients in a study to determine the feasibility of determining the severity of AKI early in the disease process. Patients with AKI often have tubular injury and therefore renal tubular dysfunction. We hypothesize that patients with severe AKI (AKI that will require RRT) will have more severe tubular dysfunction than patients with less severe AKI (AKI that improves without the need for RRT). Because patients with AKI are often treated with furosemide we plan to test the hypothesis that a poor response to furosemide will identify patients with severe AKI. Furosemide works in the renal tubules, and the ability of furosemide to cause an effective diuresis is contingent on a functioning renal tubule.

Study Design

Patients who are suffering from AKI will be eligible. For purposes of this study, AKI will be defined using the Acute Renal Injury Network(AKIN) criteria[12]: an increase in serum creatinine of greater than or equal to 0.3 mg/dL over 48 hours or increase to greater than or equal to 150 to 200 percent from baseline or sustained oliguria (mean output of <0.5 cc/kg/hr for 6 hours).. For patients who meet entry criteria based on serum creatinine, those subjects must be enrolled within 48 hours of their initial increase in serum creatinine. Patients who have a clinical syndrome consistent with pre-renal or post-renal AKI will be excluded.

Intervention

Once patients are identified and consented, at the time of study entry 7 cc of whole blood and 50 cc of urine will be collected. Thereafter, a single bolus of furosemide will be given to the patient.

Furosemide - If the patient is furosemide naive, they will receive a dose of 1.0 mg/kg. If patient takes a loop diuretic at home, or has been receiving furosemide previous to study entry, the dose of furosemide will be 1.5 mg/kg. The patient will be assessed for 6 hours following the furosemide administration (with a second blood draw/urine collection at 2 hours). All blood draws / urine collections will be 1 tube of blood (5-7 ccs) and 25-50 ccs of urine (when possible)

Baseline urine output and post-furosemide urine output will be recorded. Urinary sediment will also be assessed at both 0 and 2 hours .

Blood Studies - Blood samples (plasma) will be spun down (15 minutes at 1,600 x g) and aliquoted (0.5 ml for plasma) and placed in -80 freezer. These samples will be

assessed for plasma levels of neutrophil gelatinase lipocalin (NGAL) and potentially for other AKI biomarkers.

Urine Studies - Urine will be spun (1,000 x g at 4°C. for 10 minutes)) and aliquoted. Urine will be assessed for Tissue Inhibitor Metalloprotease -2 (TIMP2), insulin-like growth factor binding protein 7 (IGFBP7) NGAL potentially other biomarkers AKI biomarkers. All of these proteins are evolving potential AKI biomarkers.

Long Term Follow-up

Subjects will be contacted monthly for the first 3 months following their hospital admission and then annually for 3 years to collect kidney health, dialysis, and hospitalization and mortality outcomes. Patients will sign a release of information form at the time of consent that will permit the study team to contact their outside physicians for any / all relevant lab work collected (complete blood counts , comprehensive metabolic panel and any urine studies). They will also be asked questions to ascertain the incidence of any Major Adverse Renal –Cardiac Events (MARCE) – these include death, new dialysis, worsening of kidney function (CKD), myocardial infarction, stroke or heart failure. [13] Finally, subjects will be providing consent for linkage with administrative database such as the Social Security Death Index (SSDI) and the United States Renal Data System (USRDS).

Physician Assessment:

At the study enrollment, 24 hour, ICU discharge and hospital discharge timepoints we will ask members of the treating team (attending, fellows, residents and nurses) a simple 8 question survey (with only questions 5-7 being asked at hospital discharge) All answers (accept question 1 will be given on a 5-point Likert scale (strongly disagree, disagree, neutral, agree, and strongly agree). :

1) What is the source of the patients AKI? (Multiple choices: Pre-renal azotemia, Acute tubular necrosis acute interstitial nephritis, athero-emboli, glomerulonephritis, multi-factorial or other Ischemia, Sepsis, Hypotension, Contrast, Other Nephrotoxin)?
   1b) Contributing causes of AKI (circling all that apply) – decreased perfusion pressure , inflammation, contrast, pigment(myo/hemoglobin), aminogylcosides, NSAIDs, amphotericin, other)
2) This patient's AKI will progress from Stage 1 to Stage 3 (200% increase in serum creatinine)
3) This patient will require renal replacement therapy during this hospital stay
4) This patient will die during this hospitalization
5) This patient will require renal replacement therapy in the next 3 months
6) This patient will return to living at home, independently, over the next 3 months
7) This patient will be readmitted to the hospital in the next 3 months
8) This patient will die during the next 3 months

Safety

The greatest risk in administering a diuretic is the potential to make the patient volume depleted via diuresis. In order to ensure that this does not happen, the urine output after the furosemide bolus will be matched ml for ml with isotonic saline. The treating team has the option of replacing less isotonic saline if the treating team wants volume to be removed. However, the default procedure will conduct this matched replacement for six hours after the furosemide bolus. Since furosemide induces the production of hypotonic urine, a milliliter for milliliter matched repletion of normal saline guarantees that the patient cannot become volume depleted.

Furosemide is routinely given to patients with AKI, particularly those with acute tubular necrosis. Furosemide has not been shown to be harmful nor helpful in patients suffering from AKI despite its widespread use in patients with acute and chronic kidney disease. Because we are excluding patients who are pre-renal, we are minimizing the risk of volume depletion.

Sample Size:

The study team aims to enroll 200 patients with AKI.

First and foremost we are looking to determine if we can replicate the success of our pilot data in a larger multi-center investigation. In powering the overall validation study we assumed 80% power and 5% alpha with this analysis being performed using SAS, Cary, NC, USA. [14] We used a conservative approximation to the variance that assumes independence of the binary variables to calculate power. To investigate the utility of the FST to predict the progression from Stage 1 AKI to Stage 3 we have provided a **Table 6** which demonstrates the change in the True Positive Rate (ΔTPR) based on the event rate and the risk threshold for defining high risk. Recall that the TPR is equivalent to the number of True Positives divided by the True Positives plus the False Negatives (TPR= ((TP)/(TP+FN)) and that this is the equivalent of McNemar's test. [15] Moreover it is important to note that ΔTPR is the equivalent of the Net Reclassification Index (NRI) for those with the event (Progression to Stage 3 AKI). Practically, this same Table demonstrates the minimum detectable change in the False Positive Rate (ΔFPR). The FPR is the number of False Positives divided by the false positives plus the True Negatives (FPR = ((FP)/(FP+TN)). Additionally, the FPR= 1-Specificity and perhaps more importantly, ΔFPR is

Table 2 - Detectable change in the Percentage of cases classified as high risk by adding biomarkers to the clinical model.

Total Sample Size	Event Rate	Expected Number of AKI Progressors (Cases)	10% Classified as High Risk without biomarker	20% Classified as High Risk without Biomarker	50% Classified as High risk Without biomarker
150	15%	22.5	0.21	0.30	0.36
150	30%	45	0.16	0.20	0.26
150	45%	67.5	0.13	0.17	0.22
200	15%	30	0.20	0.28	0.34
200	30%	60	0.15	0.19	0.25
200	45%	90	0.12	0.16	0.20
250	15%	37.5	0.19	0.26	0.32
250	30%	75	0.14	0.18	0.23
250	45%	112.5	0.11	0.15	0.19
300	15%	45	0.18	0.24	0.30
300	30%	90	0.13	0.17	0.21
300	45%	135	0.10	0.14	0.17

equivalent to the NRI Non Event (non-progression to Stage 3). Thus one could look at Table 6 and view these numbers as the NRI for Non-Events.

For the purpose of this analysis we have varied the cohort sample size as well as the event rate (progression). Event rate variation accounts for the limited data on progressive AKI in the existing literature. While our pilot data (n=77) demonstrated an event rate 32% [11], previously published studies have had varied rates from TRIBE AKI's (13%) [16], EARLY-ARF's (33%) [17]and Siew's et al (29%) [18]. As such a rate progression rate of 30% seems appropriate. To interpret Table 2 if we assume we have 200 individuals with a 30% AKI progression rate (60 cases /AKI progressors) we will be able to detect improvements of 0.15-0.25 in the % of cases classified as high risk (ΔTPR=NRI events). Inversely, for the same cohort and event rate we would be able to detect identical improvements in the ΔFPR (= NRI for non-events). **Thus with 200 subjects and a 30% event rate we would be able to detect small to medium sized improvements in reclassification (NRI events + NRI non-events =Total NRI 0.30-0.50)[19]**

Endpoints and Definitions

AKI – an increase in serum creatinine of >0.3 mg/dL or 50% increase from baseline over 48 hours or an increase to greater than or equal to 150 to 200 percent from baseline or sustained oliguria (mean output of <0.5 cc/kg/hr for 6 hours).

Non-renal recovery

(1) Patients who require RRT within 14d of enrollment
(2) Patients who die before 14d, and whose serum creatinine remains >2.0 mg/dl over baseline creatinine at the time of death
(3) Patients who die before 14d and whose urine output is less than 400cc/24 hours at the time of death

Renal Recovery

(1) Patients who do not require RRT and survive 14d or are discharged prior to 14 days
(2) Patients who die before 14d with a serum creatinine>2.0 mg/dl above their baseline AND with a urine output >400cc/24 hours

Statistical Analysis

Patients will be followed for 30 days or until hospital discharge, whichever occurs first. Need for dialysis, serum creatinine, urine output, medication regimens, and mortality will be assessed. Primary analysis will be based on the net reclassification index as discussed in the sample size section above, additionally we will look at the more traditional receiver operating characteristics (ROC) of urine output and urinary sediment score and biomarkers after furosemide challenge to predict AKI progression. Additionally, time to AKI progression and renal recovery/non-recovery will be assessed. Patient outcomes will be stratified by entry criteria and baseline renal function. We will be looking at the long term, (3 year) outcomes in this cohort as well. However given the focus of this study is to identify severe AKI early in the clinical course and that rates of morbidity and mortality in our ICU- AKI cohort are extremely high, we are not sufficiently powered to look at long term outcomes and this aspect of the study is somewhat exploratory and will serve as pilot data for future studies.

Study Procedures

1. Identify appropriate patients via inclusion/exclusion
2. Consent patient or legal representative
3. Draw 7cc of blood, collect 50cc of fresh urine
4. Bolus dose of furosemide
5. Assess urine output response over the next 6 hours
6. Match urine output with intravenous isotonic saline, l ml for 1 ml for 6 hours (at the discretion of the clinically treating team – if they feel diuresis is needed clinically they can decide to hold the intravenous fluids – this has not changed from prior submission)
7. Draw 7cc of blood, collect 50cc of fresh urine 2 hours after the furosemide
8. Draw 7 cc of blood; and another 50cc of urine <u>the following morning at 24 hours post dose</u>
9. Draw 7 cc of blood; and another 50cc of urine at the time of ICU discharge
10. Draw 7 cc of blood; and another 50cc of urine at the time of hospital discharge
11. Patient to receive phone call 30 days following the administration of Furosemide – with ascertainment of any recent laboratory samples outside of the University of Chicago
12. Patient to receive phone call 60 days following the administration of Furosemide - with ascertainment of any recent laboratory samples outside of the University of Chicago
13. Patient to receive phone call 90 days following the administration of Furosemide - with ascertainment of any recent laboratory samples outside of the University of Chicago 14. Telephone contact yearly following furosemide administration for 3 years

E. Study Population

The study population will be all patients who develop AKI in the hospital. A wide range of ages over 18 years old and different ethnic background will be included.

Inclusion criteria

- Age: 18 years and older

- Gender: Both
- Increase in serum creatinine of 0.3 mg/dl within 48 hrs or an increase of greater than or equal to 150 to 200 percent from baseline or sustained oliguria (mean output of <0.5 cc/kg/hr for 6 hours).
- Written informed consent
- Patients who already have a indwelling bladder catheter

Exclusion criteria

- Patients under 18-year-old
- Voluntary refusal or missing written consent of the patient or the designated legal representative
- Patients with advanced Chronic Kidney Disease – as defined by a baseline GFR of < 30 ml/min as calculated by the MDRD equation
- Patients with renal transplantation
- Patients with an allergy or sensitivity to loop diuretics
- Patients with a serum K < 3.0 meg/L
- Patients with a serum Mg < 1.0 meq/L
- Patients with a history of hearing loss
- Patients receiving aminoglycosides
- Pregnancy
- Patients with nephrostomy tubes or any type of urinary diversion
- Patients with a clinical syndrome consistent of pre-renal AKI
    - Defined by fractional excretion of Na of <1% and no evidence of the urinary casts, or
    - Patients that are under-resuscitated as deemed by treating clinical team, or
    - Patients who are actively bleeding
- Patients with a clinical syndrome of post-renal AKI
    - Any radiological study that shows hydro-ureter, or
    - Clinical scenario wherein urinary obstruction is considered a likely possibility for the cause of AKI

Gender and Minority Inclusions

AKI affects men and women approximately equally, and there is no known racial susceptibility to AKI. Thus, the study is expected to include both male and female subjects, and a significant number of minority subjects.

F. Human Subjects

The study aims to enroll 200 subjects with AKI that are admitted to the hospital. The proposed study requires an intervention as well as collection of human specimens, urine and blood samples. As such, approval from the IRB will be received prior to any study subject enrollment. Identification of study subjects will be determined based on clinical and laboratory tests and without regard to gender or racial/ethnical origin. Subjects will be initially selected for the study based upon their medical histories and laboratory studies

obtained prior to the study, according to the inclusion and exclusion criteria enumerated in Section E of the protocol.

During the subject recruitment, the investigator will explain the study in detail to the patient or their representative (e.g.. power of attorney), and provide a copy of the informed consent to the subject for review. If the patient agrees to participate, the IRB approved consent form will be signed. Each patient will receive a copy of the signed consent form. The original consent forms will be stored in the research office at the ICU for record review from authorized parties if needed.

When consent is obtained from a legally authorized representative, a subject's incapacity to consent will first be verified and documented by two physicians, one of whom will be independent of the study team for patients who are unconscious or have documented diagnosis of delirium. In the case of conscious, non-delirious patients, documentation of incapacity to consent will include evaluation by a psychiatrist or psychologist. When subjects who were decisionally impaired at the time of enrollment are informed of study participation the subject will be consented is he/she wishes to continue on the study. The PI, research nurse or member of the research team will consent the subject.

There are no direct benefits for study participation.

G. Risks and Side Effects:

During this study, patients will be given a single dose of furosemide. Furosemide is a diuretic commonly given to patients with AKI. Furosemide has a favorable safety profile. The risks of furosemide administration are as follows: hypersensitivity reactions, volume depletion, hypokalemia, hypomagnesemia, and ototoxicity. In order to mitigate any risk of hypovolemia, patients will be given isotonic saline milliliter for milliliter after the furosemide bolus.

The patient may be exposed to some risks related to blood drawing. We will collect only five (pre-furosemide, 2 hours post-furosemide, 24 hours post-furosemide, ICU discharge and hospital discharge, . Most of the blood samples will be taken by an intravenous catheter which is commonly used in the ICU for treatment purposes. This method of blood drawing will minimize the risks for any possible side-effects. In case the blood is drawn by a needle procedure, there are possibilities of a bruise and slight pain. Rarely, fainting or infection may occur. In addition to blood samples, we will collect two samples of urine samples for all patients in the study. Each urine sample will be 50 milliliter (10 teaspoons of urine over 24 hours). Collection of urine samples does not carry additional risks for the patient because all patients will have an indwelling bladder catheter as part of their study eligibility.

In addition to specimen samples, clinical and laboratory data will be collected from the medical records. . Patients will sign a release of information form at the time of consent that will permit the study team to contact their outside physicians for any / all relevant lab work collected (comprehensive metabolic panel and any urine studies) – this will be done

to allow for determination of post-discharge kidney function to determine if there was any loss of kidney function following the episode of AKI. All data will be held confidential to the investigator in the study. All names and individual identifiers will be separated from the collected data. If and when the data is published, it will be presented in an anonymous fashion to avoid any violation of patient's confidentiality.

Adverse Events
Adverse Events are negative reactions associated with furosemide not present at baseline: hyper-sensitivity reactions (allergic reactions, e.g. rash), dehydration, electrolyte depletion and tinnitus occurring within 24 hours post dose administration. Non-serious AEs will be reported at the time of continuing review.

A study-treatment related adverse event which fits any of the criteria below, is considered a serious adverse event (SAE):

- Results in death
- Is life-threatening (meaning that the patient 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 in-patient 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.)

SAEs will be reported to the IRB within 24 hours of awareness and followed until resolved.

H. Benefits:

There are no direct benefits to the patient in the study. The patient will receive an assessment of renal function that might not otherwise occur but for this study (urine sediment, and renal history and physical assessment). The risks for this study are slightly more than minimal risk.

I. Multi-Institutional Collaboration

The following sites are conducting this study in parallel, with the University of Chicago as the coordinating site:

- George Washington University (PI: Dr. Lakhmir Chawla)
- University of California-San Francisco (PI: Dr. Kathleen D. Liu)

Each site is responsible for study conduct and monitoring independently.

Samples and data from both of all sites will be shared and sent to the University of Chicago. All exchanged samples and data will be de-identified. We will not send or receive data

and/or samples until IRB approval has been granted and subcontracts have been fully executed at respective sites.

J. Outside Consultants/Collaborators (for future research)

Samples of plasma and urine will be stored at UCMC laboratory after initial analysis has been completed per protocol and could be shared in the future with other researchers after they have gained approval by proper authorities to conduct research. These samples will be de-identified prior to sharing for the purposes of future research.

K. Costs To Subjects:

The Section of Nephrology will provide the medicine used in this study free of charge to the patient. The patient will not be charged for anything that is part of the study.

L. Conflicts Of Interest:

There is no conflict of interest.

M. Confidentiality:

In addition to specimen samples, clinical and laboratory data will be collected from the medical records. All data will be held confidential to the investigator in the study. All names and individual identifiers will be separated from the collected data. If and when the data is published, it will be presented in an anonymous fashion to avoid any violation of patient's confidentiality.

N. Subject Compensation:

There is no compensation for being in the study. The medicine and the diagnostic evaluation that are part of the study will be conducted at no charge to the subject.

O. Facilities and Equipment:

All procedures will take place in the UCMC hospital.

P. Monitoring Plan:

The data collected will be monitored by the PI.

Q. References

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