A Randomized, Double Blind, Placebo-Controlled Trial to investigate the effects of Vitamin C, Hydrocortisone and Thiamine on the outcome of patients with Severe Sepsis and Septic Shock

Background and Significance:

The global burden of sepsis is substantial with an estimated 15 to 19 million cases per year; the vast majority of these cases occur in low income countries.[1] With more timely diagnosis and improvement in supportive care the 28-day mortality from sepsis in high income countries has declined to about 25%, however, the mortality from septic shock remains as high as 45%. [2,3] Moreover, the mortality from sepsis and septic shock in low income countries is reported to be as high as 60%.[4-6] In addition to short term mortality, septic patients suffer from a numerous short- and long-term complications and are at an increased risk of death for up to five years following the acute event. [7,8] Over the last 3 decades over 100 phase II and phase III clinical trial have been performed testing various novel pharmacologic agents and therapeutic intervention in an attempt to improve the outcome of patients with sepsis and septic shock; all of these studies have failed to show an improvement in patient outcomes. [9] New therapeutic approaches to sepsis are desperately required; considering the global burden of sepsis these interventions should be effective, cheap, safe and readily available.

A large body of experimental data has demonstrated that both corticosteroids and intravenous vitamin C reduce activation of nuclear factor KB (NFKB) attenuating the release of pro-inflammatory mediators, reduce the endothelial injury characteristic of sepsis thereby reducing endothelial permeability and improving macrocirculatory flow, augment the release of endogenous catecholamines and enhance vasopressor responsiveness.[10-17] In animal models these effects have resulted in reduced organ injury and increased survival. Corticosteroids have been evaluated in several clinical trials, with meta-analysis of these trials demonstrating somewhat conflicting outcomes. [18,19] Low-dose stress corticosteroids have proven to be safe with no increased risk of clinically important complications. While corticosteroids decrease vasopressor dependency the effect on survival is less clear. Several studies have investigated the use of intravenous vitamin C in critically ill patients. Nathens et al randomized 595 surgical ICU patients (91% trauma patients) to receive intravenous vitamin C and vitamin E for up to 28 days. [20] The vitamin combination was associated with a significant reduction in the incidence of multiple system organ failure (p=0.04) with a trend to reduced mortality and length of ICU stay. No adverse effects were noted with the vitamin combination. Fowler et al performed a pilot study in 24 patients with severe sepsis and septic shock. [21] In this study patients were randomized to placebo (n=8), low dose intravenous vitamin C (50 mg/kg) (n=8) or high dose vitamin C (200mg/kg). Vitamin C attenuated the inflammatory response with both doses of the vitamin being devoid of any side effects. Although the Sequential Sepsis Related Organ Failure Score (SOFA) fell significantly in both treatment arms the study was underpowered to determine any outcome benefit. Zabet and colleagues performed a RCT in which they evaluated the role of intravenous vitamin C in a dose of 100 mg/kg/day (about 7g/day) in 28 surgical ICU patients with septic shock. [22] In this study the mean

dose of norepinephrine and duration of norepinephrine administration were significantly lower in the ascorbic acid than the placebo group. The 28-day mortality was significantly lower in the ascorbic acid than the placebo group (14% vs. 64%, p = 0.009). No side effects related to the vitamin C infusion were reported. Du et al randomized 84 patients with severe pancreatitis to receive intravenous Vitamin C (10 g/day) for 5 days; the control group received 1g Vitamin C per day. [23] Symptoms resolved significantly quicker in the treatment group who had a significantly greater cure rate (75% vs 41%, p< 0.05). Tanaka et al randomized 37 patients with severe burn to very high dose vitamin C (about 110g/day) or placebo.[24] Patients who received vitamin C required less fluid resuscitation with a trend towards reduced length of stay and mortality. No adverse effects were noted with the very high dosages of vitamin C. Several studies have administered vitamin C in doses exceeding 100g/day as adjuvant therapy in patients with cancer with no discernable side effects. [25-32] Vitamin C appears to be toxic to normal human cells (not cancer cells) at a concentration on greater than 25 mM. [32] A dose of 6g/day will achieve a steady state serum concentration of about 240uM [33-35] which is about 100 times less than the dose required to cause cellular toxicity. The package insert for vitamin C [36] lists no contraindications or adverse effects of the drug and states that as much as "6 grams has been administered without evidence of toxicity". The only reported restriction to the use of high dose intravenous vitamin C is in patients with known glucose-6-phospahte deficiency (G6PD) in whom hemolysis has been reported. [37,38]

Ascorbate donates a single electron in all its redox reactions, generating the ascorbate radical. This radical is not very reactive with anything but itself. Dismutation of two ascorbate radicals forms a molecule each of ascorbate and dehydroascorbate. Hydrolysis of the lactone ring of dehydroascorbate irreversibly converts it to 2,3-diketo-1-gulonic acid which is then converted to oxalate. Oxalate is normally excreted by the kidney and serum levels will increase with renal impairment. In patients with renal impairment receiving mega-dose vitamin C, supersaturation of serum with oxalate may result in tissue deposition as well as crystallization in the kidney. [39,40] Glyoxylate, a byproduct of intermediary metabolism, is either reduced to oxalate or oxidized to CO_2 by the enzyme glyoxylate aminotransferase; thiamine pyrophosphate is a co-enzyme required for this reaction. [41] Thiamine deficiency increases the conversion of glyoxylate to oxalate resulting in hyper-oxalosis. [42,43] Thiamine deficiency is common in septic patients and is associated with an increased risk of death.[44]

Based on experimental data we believe that hydrocortisone and vitamin C act synergistically in patients with sepsis. This hypothesis is supported by in-vitro research performed by Marik et al who have demonstrated that hydrocortisone together with vitamin C protects the vascular endothelium from damage by endotoxin while neither agent alone had this effect. [45] Previous research has demonstrated that vitamin C reverses oxidation of the glucocorticoid receptor (GR) a likely manifestation of sepsis.[46] Oxidation of the GR limits binding of the GR to both ligand and DNA responsive units decreasing the activity of glucocorticoids. [46] Furthermore, glucocorticoids increase the expression of the sodium vitamin C transporter-2 (SVCT-2) which is an essential transport protein necessary for vitamin C to be transported intracellularly.[47] Based on these data we suggest that the combination of hydrocortisone and vitamin C will act synergistically to

limit the pro-inflammatory response, limit the endothelial injury and improve microcirculatory function and vasopressor responsiveness in patients with sepsis and septic shock. The addition of thiamine, may limit the develop of hyper-oxalosis. Furthermore, thiamine has been demonstrated to reduce the developed of acute kidney injury in patients with sepsis and to reduce mortality in thiamine deficient septic patients. We therefore propose that a metabolic resuscitation protocol including vitamin C, corticosteroids and thiamine and thiamine will limit the develop or organ failure and reduce mortality in patients with severe sepsis and septic shock.

We performed a retrospective before-after clinical study, in which we compared the outcome and clinical course of consecutive septic patients treated with intravenous vitamin C, hydrocortisone and thiamine during a 7-month period (treatment group) compared to a control group treated in our ICU during the preceding 7 months. [48]

Patents with sepsis predictably have very low serum vitamin C levels, which can only be corrected with intravenous vitamin C in a dose of more than 3gm per day. [21,33,35] Based on published clinical data, vitamin C pharmacokinetic modeling as well as the package insert, we decided to administer 6gm vitamin C per day divided in 4 equal doses. [20-22,33-36] This dosage is devoid of any reported complications or side effects. Hydrocortisone was dosed according to the consensus guidelines of the American College of Critical Care Medicine.[49] Thiamine was included in the Vitamin C protocol at a dose of 200mg q 12 hourly.

The primary outcome was hospital survival. A propensity score was generated to adjust the primary outcome. There were 47 patients in both treatment and control groups with no significant differences in baseline characteristics between the two groups. The hospital mortality was 8.5% (4 of 47) in the treatment group compared to 40.4% (19 of 47) in the control group ($p < 0.001$). The propensity adjusted odds of mortality in the patients treated with the vitamin C protocol was 0.13 (95% CI 0.04-0.48, p=002). The SOFA score decreased in all patients in the treatment group with none developing progressive organ failure. Vasopressors were weaned off all patients in the treatment group, a mean of 18.3 ± 9.8 hours after starting treatment with vitamin C protocol. The mean duration of vasopressor use was 54.9 ± 28.4 hours in the control group (p<0.001).

The results of our before-after retrospective study suggest that the early use of intravenous vitamin C, together with corticosteroids and thiamine prevented progressive organ dysfunction and reduced the mortality of patients with severe sepsis and septic shock. However, our study has several important limitations, namely the small sample size, single center design, and the use of non-concurrent controls. We believe that the results of our study provide sufficient information for the design of an adequately powered, pragmatic randomized controlled trial to confirm the findings of our retrospective study. We believe that is essential to perform such a trial as vitamin C, hydrocortisone and thiamine are extremely safe, cheap and widely available and this therapeutic approach has the potential to save the lives of millions of patients who die from sepsis globally each year.

The Steps to the Cure .. for sepsis.

It is important to emphasize that the 'metabolic resuscitation protocol" is an adjustive therapy for the treatment of patients with severe sepsis and septic shock. All components must be addressed to ensure the optimal outcome for the patient (this is not a bundle but a common sense approach to patient care supported by the best available medical literature. Early administration of the correct antibiotics, adequate source control and a common sense approach to fluid management are essential for a good outcome. The steps to a cure are outlined below:

- Early Diagnosis
    - Clinical suspicion, CBC + diff, PCT
- Early administration of the correct antibiotics, in the correct dose
- Source Control
- Conservative, physiologic approach to fluid resuscitation
- Early use of Norepinephrine
- The "Metabolic Resuscitation Protocol"
    - Steroids, Vitamin C and Thiamine
- Multidisciplinary, team approach to patient care
- State-of-the-art evidence based supportive care
    - Lung protective ventilatory strategy
    - Bolus feeding with Whey based feeding formula
    - Early mobility
    - Limited use of sedation,
    - etc, etc

Physiologic guided conservative fluid strategy

It is highly likely that our approach to fluid resuscitation, vasopressors and state-of-the-art supportive measures played a major role in the remarkable reduction of mortality and organ failure that was observed in our study. We believe that large fluid boluses (30ml/kg) and the aggressive use of intravenous fluids as promoted by the Surviving Sepsis Campaign is devoid of scientific support, is illogical and unphysiologic and leads to severe fluid overload which has been shown in multiple studies to be an independent predictor of death. We recommend the early use of norepinephrine in patients who remain hypotensive after small volume resuscitation. The use of our "metabolic resuscitation protocol" (Vitamin C, corticosteroids and thiamine) restores vascular tone by multiple mechanism allowing the withdrawal of vasopressors within 24 hours (average duration pressors was 18 hrs). Consequently at 24 and 72 hours patients have a small positive fluid balance and are protected from developing progressive organ failure that characterizes salt-water drowning.

Sepsis, Delirium and long-term Cognitive Dysfunction: Prevention Using Vitamin C.

Sepsis survivors are at an increased risk death, major adverse cardiovascular events, ischemic stroke, hemorrhagic stroke, myocardial infarction, recurrent sepsis and heart failure compared to population controls. [7,8] In addition, sepsis survivors incur long-term consequences, including developing new physical, psychiatric, and cognitive deficits. These deficits often limit their mobility and ability to perform day-to-day activities and impair quality of life.[8,50] Yende et al demonstrated that among patients who lived independently prior to severe sepsis, at six months' post hospital discharge one third had died and of those who survived, a further one third had not returned to independent living.[51]

Survivors of critical illness suffer from marked muscle wasting known as Critical Illness Myopathy (CIM), which may take years (if ever) to recover. he clinical significance CIM in survivors of critical illness was first highlighted by the studies of Herrdige et al in survivors of ARDS. Herridge and coworkers evaluated 109 survivors of ARDS for up to 5 years after discharge from the ICU. At 1 year the distance walked in 6 minutes was 66% of predicted which increased to 76% of predicted at five years.[52] The mean score for the physical component of the SF-36 Heath Survey was 25 at one year and 41 at 5 years (mean norm score matched for age and sex is 50). At one year 48% of patients had returned to work which increased to 77% at 5 years. Similarly, Needham and colleagues evaluated the physical performance of patients with ARDS who were enrolled in the EDEN study at 12 months following hospital discharge.[53,54] At 12 months, the mean 6-minute walk test distance was 66% of predicted while the SF-36 physical health summary score was 39 (mean norm score of 50). Cuthbertson et al demonstrated that sepsis survivors have a significantly reduced physical quality of life scores when compared to population norms.[55]

In addition to physical impairments, cognitive and neuropsychiatric complications are common following critical illness, particularly sepsis. The post sepsis-syndrome is similar in many respects to the post-traumatic stress disorder; patients suffer memory impairment, abnormalities of higher executive function, nightmares, anxiety disorders and depression. Semmler et al demonstrated that sepsis survivors showed cognitive deficits in verbal learning and memory and had a significant reduction of left hippocampal volume compared to healthy controls.[56] Iwashyna et al demonstrated that the prevalence of moderate to severe cognitive impairment increased 11% among patients who survived severe sepsis (OR of 3.34; 95% CI 1.53-7.25).[57] In addition, these authors demonstrated that sepsis survivors were at greater risk of development of functional limitations. Furthermore, the reported changes in physical and cognitive functioning noted after severe sepsis were worse than those seen after non-sepsis general hospital admissions.

Delirium is very common in critically ill patients particularly those with sepsis. The risk of developing delirium is dependent on a complex interplay between predisposing and precipitating risk factors. Delirium is associated with negative outcomes including greater cognitive impairment after ICU discharge.[58,59] It is postulated that the neuro-psychiatric complications

that occur in sepsis survivor may represent persistent neurological dysfunction initiated and or propagated by acute ICU delirium.

Vitamin C is concentrated almost 100-fold in neurons.[60-62] Vitamin C is an essential co-factor for the synthesis of dopamine, serotonin and norepinephrine. It is therefore not surprising that altered neuropsychiatric function has been reported in both the acute and chronic forms of scurvy.[63,64] Indeed, in the historical descriptions of scurvy, patients were described as having "gone insane".[63] Vitamin C levels are undetectable or very low in critically ill patients, particularly those with sepsis. Anecdotally, we have noted that none of our septic patients treated with our vitamin C protocol required treatment for delirium. Furthermore, a patient transferred to our facility for severe cognitive dysfunction following septic shock, was noted to have acute vitamin C deficiency (acute scurvy) with a serum vitamin C level of 11.4 umol/l (Normal 40-60). This patient was treated with the intravenous vitamin C protocol and demonstrated a dramatic improvement in neurological function within days of treatment.

In an animal models, administration of ascorbic acid has been demonstrated to produce an antidepressant-like effect mediated by interaction with the monoaminergic and GABAa systems.[65,66] Gariballa evaluated the clinical significance of vitamin C deficiency among hospitalized acutely-ill older patients.[67] In this study patients with vitamin C biochemical depletion had significantly increased symptoms of depression compared with those with higher concentrations at baseline and at 6 weeks. In a randomized controlled trial, Zhang et al demonstrated that 500mg vitamin C PO BID improved the mood and decreased depression symptoms in a cohort of acutely hospitalized patients.[68] In a randomized double-blind, placebo-controlled 14 day trial, Brody demonstrated that oral vitamin C resulted in a decrease in the Beck Depression scores with improved sexual intercourse frequency.[69] A randomized, double-blind, placebo-controlled study in pediatric patients demonstrated that ascorbic acid was an effect adjunct to fluoxetine in the treatment of major depression.[70]

We therefore propose that treatment with vitamin C during the acute phase of illness may limit the incidence and or severity/duration of delirium which may likely limit or prevent the long-term neuro-psychiatric complications of sepsis. Furthermore, as treatment with the vitamin C cocktail reduces the duration of mechanical ventilation, duration of vasopressor support and duration of ICU stay we postulate that this intervention may promote early mobility and limit muscle loss thereby limiting CIM characteristic of critical illness and sepsis.

Specific Aims of the Study:

The aim of this study is to determine the effect of the combination of intravenous vitamin C, hydrocortisone and thiamine on the clinical course and outcome of patients with severe sepsis and septic shock.

Study Design:

This study will be performed at ****   All patients admitted to the ICU of participating hospitals with the primary diagnosis of severe sepsis or septic shock will be screened for inclusion into this study. The diagnosis of severe sepsis and septic shock will be based on the 1992 American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions.[71]

Inclusion Criteria:

   i.   Diagnosis of severe sepsis or septic shock within 12 hours of admission to the ICU.

Exclusion criteria:

    i.   Age < 18 years
   ii.   Pregnant
  iii.   DNR/DNI with limitations of care
  iv.   Patients with an fatal underlying disease who are unlikely to survive to hospital discharge
   v.   Patients with a primary admitting diagnosis of an acute cerebral vascular event, acute coronary syndrome, active gastrointestinal bleeding, burn or trauma [72-74]
  vi.   Requirement for immediate surgery [72-74]
 vii.   Patients with HIV and a CD4 < 50 mm^2 [72-74]
viii.   Patients with known glucose-6 phosphate dehydrogenase (G-6PD) deficiency.[38]
  ix.   Patients with severe sepsis/septic shock transferred from another hospital
   x.   Patients with features of sepsis/septic shock > 24 hours
  xi.   Patients who require treatment with corticosteroids for an indication other than sepsis (chronic corticosteroid use, known Addison's Disease, etc)

Study end-points:

Primary end-point
i. Hospital Mortality

Secondary end-points
i. 60-day mortality
ii. 28-day mortality
iii. Time to vasopressor independence. Defined as the time from starting the active treatment/placebo to discontinuation of all pressors
iv. PCT clearance (PCT-c) calculated using the following formula: initial PCT minus PCT at 96 hours, divided by the initial PCT multiplied by 100. [75,76]
v. Delta SOFA score, defined as the initial SOFA score minus the day 4 SOFA score
vi. ICU mortality
vii. ICU length of stay (LOS) and ICU free days. ICU free days is calculated as the number of days alive and out of the ICU to day 28
viii. Hospital LOS
ix. Incidence of delirium and duration of delirium

Optional Secondary end-points

i. Quality of life measures at 6 months, including physical and cognitive function, level of independence, etc.

Vitamin C, Hydrocortisone and Thiamine dosing protocol and randomization

This is a double-blind placebo controlled study. Only the pharmacist will be aware of the treatment allocation. Patients will be randomized to receive either vitamin C/hydrocortisone/thiamine or triple placebo using a random number table provided to the dispensing pharmacists. Each patient will be allocated a unique subject ID which will be linked to the randomization sequence. Only the dispensing pharmacists will have a record of the subject ID and randomization sequence. The vitamin C/placebo, hydrocortisone/placebo and thiamine/placebo will be formulated as follows:

Vitamin C: Vitamin C is provided by the manufacturer as a 50 ml vial at a concentration of 500mg/ml. Three (3) ml of vitamin C will be placed in a 50 ml bag of Normal Saline (1500mg vitamin C in 50ml bag) which will then be infused over 1 hour. The bag will be labeled by the pharmacy as Vitamin C. The dosing schedule is 1500mg every 6 hours for 4 days or until discharge from the ICU.

Vitamin C placebo will consist of an identical bag of 50cc normal saline (but with no vitamin C) and will be labelled vitamin C. Placebo will be infused over 30-60 minutes as per the infusion instructions of the active vitamin.

Hydrocortisone: Patients will be treated with hydrocortisone 50mg IV q 6 hourly for 4 days or until ICU discharge.
Optional dosing strategy: Hydrocortisone 50 mg bolus, followed by a 24-hour continuous infusion of 200 mg for 4 days.
Hydrocortisone placebo will be provided as an identical syringe/50 ml bag of normal saline.

Off label treatment with hydrocortisone or another corticosteroid will not be allowed.

Thiamine: As a high percentage of septic patients have been shown to be thiamine deficient patients will receive intravenous thiamine 200mg q 12 hourly for 4 days or until ICU discharge. [44] Thiamine is also a cofactor for the metabolism of oxalate (a byproduct of vitamin C metabolism), with thiamine deficiency increasing oxalate levels. [42] To simplify the study both the intervention and control group will receive thiamine.

Power Calculation:

The published hospital mortality for patients with severe sepsis and septic shock in the USA approximates 40%. Based on our previous study and patient modeling we project that the combination of hydrocortisone, vitamin C and thiamine could reduce the mortality to 15%. Assuming a type 1 error of 5% (alpha of 0.05) and a power of 80% (the ability to detect a difference between two groups when a difference exists) would require a sample size of 100 patients. To account for dropouts and other exclusory factors we will therefore aim for a sample size of 140 patients.

Data Collection:

The following deidentified demographic and clinical data will be collected.

i.   Age (18-90); patients over age 90 will be recorded as 90 years
ii.   Sex
iii. Weight
iv.   Admitting diagnosis and site of infection
v.   Culture results
vi.   Co-morbidities, including diabetes, hypertension, cardiac failure, COPD, malignancy
vii. Requirement for mechanical ventilation (Y/N).
viii. Use of vasopressor agents (Y/N). The hourly dosage of vasopressors will be recorded as the norepinephrine equivalent dosage. [77,78]
ix.   Duration of vasopressor use
x.   Time to vasopressor independence form start of active drug/placebo
xi.   Daily urine output (for first 4 days)
xii. Fluid balance after 24 and 72 hours
xiii. Presence of acute kidney injury (AKI). Presence of AKI: Acute kidney injury (AKI) will be defined using the KDIGO criteria; namely, an increase of the s-creatinine > 0.3 mg/dl or a level > 1.5 times the baseline value. [27] If the baseline s-creatinine is not known a value > 1.5 mg/dl will be regarded as diagnostic of AKI.
xiv. Length of ICU and hospital stay
xv.   ICU, hospital, 28-day and 60-day survival
xvi. Routine laboratory data for 4 days including:
    a. serum creatinine
    b. white cell count (WBC)
    c. platelet count
    d. total bilirubin
    e. PaO_2/FiO_2 ratio
    f. Procalcitonin (PCT)
    g. lactate level
xvii. The patients' admission APACHE II and APACHE IV scores will be recorded. The APACHE IV score allows calculation of the predicted hospital mortality and predicted ICU length of stay (LOS).
xviii. The daily SOFA (Sepsis-related Organ Failure Assessment) score will be recorded for the first 4 treatment days.

xix.   CAM +ve Delirium (y/n) and days with delirium [79,80]

The APAPCHE II score (incrementing score of 0-71) and APACHE IV score (incrementing score 0-286) are standardized measures of disease severity that are used to predict hospital mortality and ICU LOS. [81,82] The SOFA score was designed to sequentially assess the severity of organ dysfunction in patients who are critically ill from sepsis (incrementing score 0-24). [83]. The SOFA scores is calculated 24 hours after admission to the ICU and daily thereafter. SOFA scores that increase by about 30 percent are associated with a mortality of at least 50 percent.[84]

Data will be collected in the attached Excell spreadsheet (Appendix A – Sepsis Data spreadsheet). No personal identifiers will be recorded on the spreadsheet. We will not be recording names, dates of birth, social security or EMR numbers or any other HPI data in the data collection spreadsheet. Each record will be assigned a unique study ID number (subject ID key). A separate password protected spreadsheet will have a list of subject ID number and the corresponding medical record numbers (Key data sheet). This spreadsheet will only be accessible to the principle investor and sub-PI. The pharmacist will have a copy of the "Key data sheet-Code" with an additional field which will include the randomization code to active vitamin protocol or placebo. Only the pharmacist will have access to the "Key data sheet-Code" until after the study is completed, when it will be made available to the principle investor and sub-PI.

Data analysis:

Summary statistics will be used to describe the clinical data and presented as mean ± SD, median with interquartile range (IQR) or percentages as appropriate. Chi squared analysis with Fisher's exact test (when appropriate) and Student's t test (Mann Whiney U test for non-normal distributions) were used to compare data between the active treatment group and the placebo group with statistical significance declared for probability values of 0.05 or less.

Data Safety & Storage (dependent on requirement of IRB and local standards):

The main risk to subjects is the accidental release of PHI. Careful record management methods will be in place to ensure this type of privacy breach does not occur.

Subject ID Key: Each subject will be given a unique subject ID number. A subject ID key will be used to match the subjects Medical Record Number (MRN). The subject ID key, linking the subject ID numbers to the MRN will be kept in a password-protected file and stored separately from the data set in the locked office of the principal investigator and the pharmacist in charge of randomization. Only the research team (PI, sub PI and research pharmacist) will have access to this information, and they will not disclose this information to any other person or entity. The subject ID key will be destroyed as soon as possible after the data set has been completely abstracted and validated for accuracy and completeness.

Data set: Similarly, the data set will be kept in a password-protected file and stored separately from the subject ID key in the locked office of the principal investigator. Only the research team will have access to this information, and they will not disclose this information to any other person or entity. Three years after the completion of the study, all collected data will be destroyed by permanently deleting electronic copies.

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