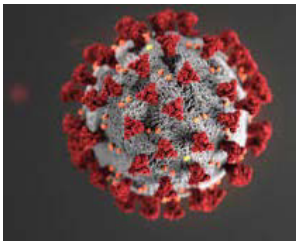

# Pop-Up COVID-19 Grant Application

## West Virginia Clinical and Translational Science Institute

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**TITLE OF APPLICATION**: Coronavirus Induced Acute Kidney Injury: Prevention using Urine Alkalinization

**PROJECT ABSTRACT** *(30 Lines or Less)*

Emerging evidence suggests that acute kidney injury (AKI) secondary to COVID-19 (COV-AKI) might result from direct infection of renal tubule epithelial cells (RTEC). A variety of epithelial cells express the ACE2 receptor which contains the receptor-binding domain (RBD) used by SARS-CoV-1 and SARS-CoV-2 to enter the cells. While direct infection of RTEC has not yet been proven data from multiple laboratories show virus in the kidney. It is this direct viral involvement of the RTEC that this proposal seeks to address.

One relatively simple approach would be to perturb the ability of the RBD to bind to its cellular (hACE2) receptor. Changes in pH may cause each amino acid residue, in the RBD, to assume a slightly different 'microscopic' conformation-dependent pKa value. Urine pH is normally 5.5-6.5 (not too dissimilar to alveolar fluid—6.4-6.86) and can be easily and safely manipulated. In fact, urine alkalinization protocols have been used for decades to reduce renal toxicity from various compounds (especially chemotherapy) and are recommended by US and European toxicology societies. Here, the strategy will be deployed not for ion trapping but to inhibit the virus from infecting RTEC. Alkalinizing the urine using IV sodium-bicarbonate solution to pH of 7.5 or more can be easily and safely achieved.

While severe AKI does not appear to be a major part of the SARS-CoV-2 syndrome for most patients, when severe AKI does occur, mortality is very high and preventing early AKI may reduce AKI severity as the disease progresses.

## *A.* SPECIFIC AIMS *(1 page max)*

COronaVirus Infectious Disease 2019 (COVID-19), since its initial discovery in December 2019 has affected millions of lives across the world. As of this writing it has infected over 3 million people across the world with almost a third of those in the United States (US). It has so far claimed over 200,000 lives worldwide with over a quarter of those in the US.

COVID-19 is caused by Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV2), a β-coronavirus. It most commonly affects lungs leading to acute respiratory distress syndrome, but involvement of other organs leading to cardiomyoapathies, acute kidney injury (AKI) and gastro-intestinal systems has been frequently described. AKI, a sudden dysfunction of kidneys, is common among critically ill patients and is associated with high mortality, morbidity and increase in healthcare costs. It is seen in 15-25% patients with COVID-19. The treatment relies on prevention of progression of AKI and management of its complications. Initiation of dialysis therapy is needed in 6-13% of critically ill patients with AKI and is associated with very high mortality. As such there is a ***critical need*** to identify and develop therapies to delay the progression of AKI in these patients.

Our ***overarching goal*** is to improve the outcomes of critically ill patients with or at risk for development of AKI. The ***objective*** of this applications is to determine the role of urine alkalization in prevention of development and progression of AKI in critically ill patients with COVID-19. A recent study has shown invasion of renal tubular epithelial cells by SARS-CoV2 virus. Urinary alkalinization, that has been used in millions of patients to reduce renal toxicity from various nephrotoxins including chemotherapeutic agents and drug overdoses (e.g. aspirin), will lead to a conformational change in the amino acids of the receptor binding domain of coronavirus leading to decreased binding affinity of the virus to its receptor on renal tubular epithelial cells. We hypothesize that alkalization of urine is safe and effective to prevent development of AKI in patients with COVID-19. To achieve our objective we are proposing to conduct a pragmatic randomized clinical trial of 40 patients testing positive for COVID-19 with the objective of preventing the development of moderate-severe AKI. We have two specific aims.

**Aim 1: To determine the feasibility and safety of a pragmatic urine alkalinization protocol for the prevention of AKI in patients testing positive for COVID-19.** Primary outcome will be the proportion of patients treated who achieve >50% of urine measurements above pH 7.0 over the duration of intervention. Other outcomes will include protocol adherence and provider acceptance.

**Aim 2: To determine the effectiveness of urine alkalinization for prevention of AKI in patients testing positive for COVID-19.** Primary outcome will be the number of days alive and free of stage 2-3 AKI (up to 28) in each group. Secondary endpoints will include the proportion of patients developing stage 2-3 AKI (or stage 3 if already at stage 2 at enrollment); ventilator-free days to 28 and hospital-free days to 60.

Completion of this study will provide clinically relevant information regarding the role of urine alkalinization in prevention of AKI associated with COVID-19. This will have wide ranging implications for patients with COVID-19. Dr. John Kellum, Professor and Vice Chair for Research in the Department of Critical Care Medicine at the University of Pittsburgh, a co-I on this application and my co-mentor for the WVCTSI research scholar application is also in the process of conducting this study at the University of Pittsburgh. At the completion of this study, the results from both centers (WVU and UPitt) will then be pooled and changes in AKI rates, and survival will be examined among patients with COVID-19.

## B. RESEARCH STRATEGY *(5 pages max)*

### 1. Background

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is rapidly spreading around the world. The current outbreak of infections with SARS-CoV-2 is termed Coronavirus Disease 2019 (COVID-19). Two other coronavirus infections, SARS in 2002-2003 and Middle East Respiratory Syndrome (MERS) in 2012, both caused severe respiratory syndrome in humans. All 3 of these emerging infectious diseases are caused by β-coronaviruses. COVID-19, declared a pandemic by the World Health Organization on Mar 11, 2020, is associated with high mortality in patients admitted to intensive care units (ICUs) [1]. Though COVID-19 primarily affects lungs leading to Acute Respiratory Distress Syndrome (ARDS), other life-threatening acute organ dysfunctions such as acute kidney injury (AKI) and cardiomyopathies are also commonly described.

AKI, defined as a sudden decrease in function and damage to kidneys is common among critically ill patients. The creatinine based Kidney Disease Improving Global Outcomes (KDIGO) criteria define AKI as an increase in serum creatinine by 1.5 times the baseline creatinine of by $\geq$ 0.3mg/dL[2]. The incidence of AKI at 2.1 per 1000 population is similar to that of heart attacks [3]. It is seen in 10-15% of all hospitalized patients but has been described in over 50% of critically ill patients [4, 5]. It has been described to be present among 15-25% patients with COVID-19 [6, 7].

AKI is not only common but is associated with 3-4 times higher mortality [8, 9], development of chronic kidney disease [10], end stage renal disease [11] and increase in healthcare expenditure [12]. There are no definitive therapies for AKI and treatment primarily rests on prevention of progression of AKI and management of its complications such as hyperkalemia, acidosis and volume overload. Approximately 6-13% of critically ill patients develop severe AKI that requires life-saving dialysis therapy [5, 13] but dialysis itself is associated with high mortality[13] and life-threatening complications such as infections. Therefore there is a great interest in developing therapies and treatment approaches that can prevent AKI in critically ill patients.

### 2. Hypothesis

We hypothesize that alkalization of urine is safe and effective to prevent development of AKI in patients with COVID-19

### 3. Significance

Although COVID-19 primarily affects the lungs and may cause severe hypoxemia, other organs including the GI tract, heart and kidney are affected. Acute kidney injury secondary to COVID-19 (COV-AKI) is reported to occur in about 15-25% of patients hospitalized with COVID-19 infection.[6, 7] However, AKI was much more common in non-survivors (>50%). The majority of AKI cases are mild to moderate with renal replacement requirement in about 25% of AKI cases. But with COVID-19 taking the form of a pandemic, this has translated into thousands of patients needing dialysis. There are already reports of hospitals running out of dialysis machines to provide life-saving therapy critical for COVID-19 patients with AKI. As such there is a critical need to identify therapies that can delay the development or progression of AKI in these patients. Although kidney failure appears to occur late in the course, patients may begin to

develop AKI within the first 3 days of hospitalization.[6] Thus, there may be a long window for treatment and prevention of early AKI that may decrease renal failure.

Similar to AKI in other settings,[14] COV-AKI is likely to be of variable etiology. Most patients present with single-organ failure (severe hypoxemia). Thus, remote organ injury, including AKI can simply be a consequence of critical hypoxia. Late cardiac involvement, reported in some series, may also cause cardio-renal syndrome. Furthermore, while typical cases do not appear to cause significant hyperinflammation, a few patients develop a microphage-activation syndrome-like phenotype with cytokine storm and high plasma ferritin. This may be the cause of severe AKI in this subgroup. **However, AKI might also result from direct infection of renal tubule epithelial cells (RTEC).** A variety of epithelial cells express the ACE2 receptor and this receptor is used by β-coronaviruses to enter the cells.[15, 16] An autopsy study of 26 COVID-19 patients recently confirmed the invasion of RTECs by SARS-CoV2 with COVID-19[17].

It is this direct viral involvement of the RTEC that this proposal seeks to address. One relatively obvious solution would be to block the ACE2 receptor pharmacologically and hence prevent the virus from entering the cells. While this could be an attractive therapy, ACE2 activity has been shown to be protective in lung injury[18] and no renal specific ACE2r antagonists are available to study. An alternative approach would be to perturb the ability of the receptor-binding domain (RBD) of β-coronaviruses[15] to bind to its cellular (hACE2) receptor. Changes in pH[19] may cause each amino acid residue in the RBD to assume a slightly different 'microscopic' conformation-dependent pKa value. Conformational change may alter the affinity of the RBD for its receptor and this may not jeopardize the function of the enzyme. The pH-induced potential for minor conformational change and/or steric hindrance[20] is possible due to the bulky phenylalanine residue in position 486 (F486 in h2019-nCoV RBD6) and in positions 442 and 472 (Figure 1).[21]

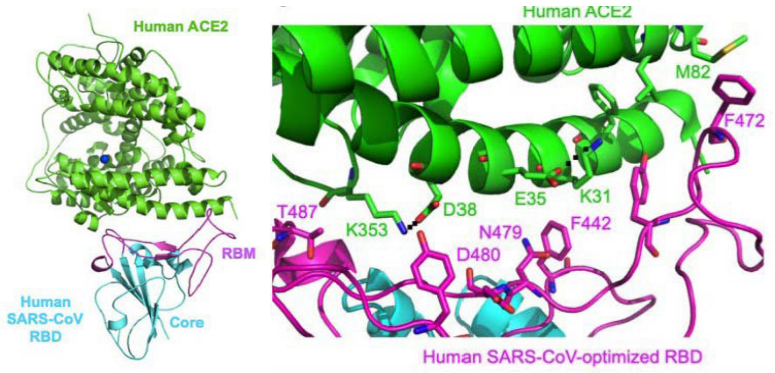


**Figure 1**. Structure of hACE2 receptor and the SARS-CoV RBD.[22]

4. Innovation

Thus, the innovative idea of simply drinking sodium bicarbonate solutions to induce minor changes in blood pH has been proposed.[21] This idea suffers from two major limitations. First, the pH change required to have significant effect on disrupting the RBD is likely to be too great to safely achieve in blood—a rule of thumb is at least 1 pH unit.[23] Second, even if this could be achieved the expression of ACEr is on the apical (lumen) surface of epithelial cells not the basolateral (plasma) side.[16] However, neither of these are problems for the kidney. Urine pH

is normally 5.5-6.5 (not too dissimilar to alveolar fluid—6.4-6.8[24]) and can be easily and safely manipulated. In fact, urine alkalization protocols have been used for decades to reduce renal toxicity from various compounds (especially chemotherapy) and are recommended by US and European toxicology societies.[25] Here, the strategy will be deployed not for ion trapping but to inhibit the virus from infecting RTEC. Alkalinizing the urine using IV sodium-bicarbonate solution to pH of 7.5 or more can be easily and safely achieved. Acetazolamide can also be added if systemic alkalemia is an issue.[25] An additional advantage of this approach is that it is unlikely to interfere with other interventions that can be studied in these patients (e.g. steroids) which are directed at the lungs.

5. Approach

We will conduct a pragmatic RCT using alkalinization of urine such that most ICU COVID-19 positive patients can be enrolled. Both specific aims will be achieved by the conduct of the trial yet both are independent. The statistical analysis plan for each aim will detail the specific endpoints and hypotheses being testing.

Trial overview: Study inclusion and exclusion criteria are provided in Table 1.

Study design: Open-label randomized trial (1:1) comparing urine alkalinization to standard care. The study will test both feasibility and safety (aim 1) as well as efficacy (aim 2).

Study Management: Study personnel will identify patients and obtain video-enabled informed consent from patients or legally authorized representatives; collect study data and ensure sample collection, processing and storage. The patient's bedside nurse will perform urine pH measurement (dipstick) and deliver the study drug. The study is open-label to facilitate safety and allow for any management of complications (e.g. calcium supplementation).

**Table 1. Inclusion/exclusion Criteria**

| *Inclusion Criteria* | *Exclusion Criteria* |
|---|---|
| Confirmed COVID-19 + | Stage 3 AKI by KDIGO criteria |
| Admission to ICU or step-down units at WVU | CKD stage 4-5 |
| Foley catheter* and central line (or PICC) in place | Contraindications to Na bicarbonate therapy (e.g. met. alkalosis, severe heart failure) |
| Age ≥18y | Urine pH > 7.0 |
| | ECMO Patients |

*Foley Catheter can be removed 72 hours after enrollment if appropriate documentation of urine output can be maintained

Urine alkalinization may also increase the elimination of certain drugs including hydroxychloroquine. We do not intend to exclude patients taking these drugs but it will be discussed in the consent form and with the treating physicians.

Intervention: The intervention is a modification/simplification of a standard protocol used for decades for the treatment of various drug toxicities (e.g. salicylate) or to mitigate kidney damage from chemotherapy.[25] Simplifications are intended to tailor the intervention to the management of COVID-19 patients and reduce or eliminate any additional staff exposure. We will continue urine alkalinization for 10 days or until patient is discharged from ICU or step-down unit or until the stage 3 AKI or a safety endpoint is reached. Urine pH will be measured at POC by dipstick. The major elements of the interventional protocol are shown in Table 2. Note, much of the monitoring (e.g. blood gases, calcium) is already performed for routine care. The protocol simply ensures that these measurements are timed to maximize patient safety and minimize burden on bedside nurses. The protocol has been developed with input from Dr. John Kellum, Professor and Vice Chair for Research in the Department of Critical Care Medicine at the University of Pittsburgh.

| **Table 2. Protocol highlights** |
|---|
| Sodium bicarbonate 225 mmol (225 mL of an 8.4% solution) intravenously over 1h |
| Recheck urine pH (dipstick) in 2-4 hours (from start of infusion) |
| Repeat boluses of intravenous sodium bicarbonate to achieve urine pH ≥7.2 |
| Monitor urine pH every 2-4 hours until target is reach and then q6-8 hours |
| Sodium bicarbonate 8.4% solution should not exceed 900 ml (4 boluses) in 24h |
| Monitor plasma sodium, potassium, calcium every 12 hours and Acid-base status at least every 24 hours (Note: Arterial pH should not be > 7.50, or venous >7.55) |
| Acetazolamide (Diamox) can be administered at physicians' discretion to control systemic alkalinization and to help avoid fluid accumulation. |
| Avoid fluid accumulation. If urine output does not increase to compensate for the increased fluid (it will in most cases, especially if kidney is protected), Lasix or Diamox can be added. |
| *If fluid balance cannot be maintained within +1L in 24 hours – protocol will be halted and resumed only if either the fluid balance can be correct to within +500mL in 24 hours or if the primary team decides that the patient has clinically appropriate fluid balance. |

Data collection: Information will be collected about patient demographics – date of birth, age, sex, race, weight, height, co-morbidities including but not limited to diabetes, hypertension, heart failure, coronary artery disease, previous cardiac surgeries, chronic kidney disease, cancers. Given the long history and familiarity with urine alkalinization, potential adverse effects (e.g. metabolic alkalosis, hypocalcemia) are well known. Data about renal function (creatinine, urine output, daily ins and outs), basic metabolic panel, blood gas, home medications, medications given during hospital stay and chart review to identify any relevant complications will also be performed. The provider survey will be performed using an online survey instrument and a link will be emailed to the provider to complete.

Sample collection: We will collect urine samples (1 before, 1 at 24 hours and 1 at 48 hours) from initiation of the protocol. Urine samples will be collected, processed and stored in accordance

with EH&S requirements for COVID-19 samples. At a later date and using alternative funding, we plan to analyze these samples for additional biomarkers either at WVU or at partner institution(s) in that study.[26] These biomarkers measure the cellular stress in the kidney[27] and will be examined in an ancillary study. The samples will be stored initially at the laboratories in WVU and then shipped to partner institution(s) for further testing of urine biomarkers as detailed above.

Statistical analysis: Descriptive statistics will be used for reporting: continuous variables expressed as median and interquartile range while categorical variables as frequency and percentages. No imputation will be performed and missing data will be treated as missing.

*Aim 1: To determine feasibility and safety of a pragmatic urine alkalinization protocol for the prevention of AKI*

Primary feasibility outcome will be the proportion of patients treated who achieve >50% of urine measurements pH $\geq$ 7.2 over the duration of treatment. Following specific adverse events will be monitored – hypokalemia (K<3.0meq/L), hypocalcemia (Ca<7.6mg/dL when corrected for albumin), Hypernatremia (Na>155meql/L), Volume overload (gaining weight >10% from admission weight during the protocol time-period, need for positive pressure ventilation or intubation due to volume overload), arrhythmias due to hypokalemia or hypocalcemia. To assess safety and feasibility, summary statistics including frequency, mean, standard deviation, median and interquartile range will be provided as applicable. Baseline data will be summarized comparing treatment groups over time. Chi-square/t-test will be used to compare the difference between treatment groups. Mixed model /ANCOVA will be used for repeated measurements over time.

As per the requirements of COE, a Data Safety Monitoring Board (DSMB) has been established that includes Dr. Awori J Hayanga, Dr. Chris Cook, Dr. Matthew Ellison and Dr. Lisa Giblin Sutton. The DSMB will meet to review any adverse events after 5 and 25 patients have been recruited. In addition, as per the requirements of COE, the DSMB will meet to review any adverse events after the first 5 patients randomized to the interventional drug (open label arm) have been recruited and completed the study.

*Aim 2: To determine the efficacy of urine alkalinization for prevention of AKI*

Primary efficacy outcome will be the number of days alive and free of stage 2-3 AKI (up to 28) in each group. Secondary endpoints will include the proportion of patients developing stage 2-3 AKI (or stage 3 if already at stage 2 at enrollment); ventilator-free days to 28 and hospital-free days to 60. For AKI-free, ventilator-free, and hospital-free days, zero truncated negative binomial models will be implemented.

Severe AKI will be considered as binary outcome. Multivariable logistic regression will be used to assess the overall odds associated with treatment adjusted by baseline covariates (age, demographics, baseline renal function). Random forest decision tree regression method will be implemented to assess patient variable importance towards the outcome. Hazard will be assessed using cox proportional hazard model.

Sample size determination: The study will be powered to achieve a 50% increase in the number of days alive and free of stage 2-3 AKI to day 28. We hypothesis an increase from 10 +/- 5 days to 15 +/- 5 days. The sample size needed to achieve this difference is 16 patients per group. We plan to enroll 20 patients per group for a total sample size of 40 patients. Dr. John Kellum, Professor of Critical Care Medicine at the University of Pittsburgh and a co-investigator on this application is working towards a similar trial at the University of Pittsburgh (see attached letter of

support). The results from both these studies will then be pooled and changes in AKI rates, and survival will be examined.

*Relevance to the current COVID-19 pandemic*

There are at least four reasons why this study will have an important impact. First, when severe AKI does occur, mortality is very high.[6, 7] Preventing early AKI may reduce severe AKI as the disease progresses. Second, AKI has considerable effects on the immune system reducing a variety of immune functions[28] and hence might contribute to reduced host response. Third, this is unlikely to be the last β-coronavirus outbreak and different strains may cause even more AKI. Finally, the results of this study would provide critical proof of concept data that could be used to design other therapies to reduce viral RBD affinity. For example, alveolar pH could also be manipulated. Showing feasibility with this safe and available strategy could be the first step toward other related efforts.

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