**SUPPLEMENTAL DIGITAL CONTENT**

**Title:**

Limiting Acute kidney injury Progression In Sepsis (LAPIS): Study Protocol and Trial Simulation

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**INDEX OF CONTENTS:**

[SUPPLEMENTAL TABLES 3](#_Toc59643457)

[**Supplemental Table S1.** List of inclusion and exclusion criteria for LAPIS trial. 3](#_Toc59643458)

[**Supplemental Table S2.** List of Potentially Nephrotoxic Agents. 4](#_Toc59643459)

[**Supplemental Table S3.** General characteristics of the cohort from Sapphire study and comparison between patients with and without the primary endpoint. 5](#_Toc59643460)

[**Supplemental Table S4.** General characteristics of the cohort from ProCESS trial and comparison between patients with and without the primary endpoint. 6](#_Toc59643461)

[**Supplemental Table S5.** Logistic regression in Sapphire cohort for the primary endpoint using predictors from LASSO regularization. 7](#_Toc59643462)

[**Supplemental Table S6.** Logistic regression in ProCESS cohort for the primary endpoint using predictors from LASSO regularization. 8](#_Toc59643463)

[**Supplemental Table S7.** Summary of protocol simulation in ProCESS trial for patients from the primary and sensitivity analysis cohort. 9](#_Toc59643464)

[SUPPLEMENTAL FIGURES 10](#_Toc59643465)

[**Supplemental Figure S1.** Experimental plan. 10](#_Toc59643466)

[**Supplemental Figure S2.** Study Design. 11](#_Toc59643467)

[**Supplemental Figure S3.** Protocol simulation in ProCESS trial in sensitivity analysis cohort. 12](#_Toc59643468)

[SAPPHIRE PARTICIPATING SITES AND INVESTIGATORS 13](#_Toc59643469)

[PROCESS PARTICIPATING SITES AND INVESTIGATORS 15](#_Toc59643470)

[LAPIS SIMULATION 16](#_Toc59643471)

[**Methods** 16](#_Toc59643472)

[**Sensitivity analysis for ProCESS trial** 17](#_Toc59643473)

[LAPIS TRIAL PROTOCOL 18](#_Toc59643474)

[**Objectives** 18](#_Toc59643475)

[**Endpoints** 18](#_Toc59643476)

[**Study Population** 20](#_Toc59643477)

[**Study Treatments** 21](#_Toc59643478)

[**Determination of Study Reference SCr and Staging of AKI** 21](#_Toc59643479)

[**Samples Collection** 22](#_Toc59643480)

[**Study Interventions** 23](#_Toc59643481)

[**Safety Assessment** 24](#_Toc59643482)

[**Sample size calculation** 25](#_Toc59643483)

[**Adaptive Changes** 25](#_Toc59643484)

[**Analysis** 26](#_Toc59643485)

[**Process Evaluation** 27](#_Toc59643486)

**SUPPLEMENTAL TABLES**

**Supplemental Table S1.** List of inclusion and exclusion criteria for LAPIS trial.

|  |  |
| --- | --- |
| **INCLUSION CRITERIA** | |
| 1 | Male or female, 21 years of age or older |
| 2 | Clinical diagnosis of sepsis or septic shock per the Sepsis-3 definitions |
| 3 | Admission to the ICU or planned admission to the ICU with an expected stay in the hospital of more than 48 hours |
| 4 | Expected to have an indwelling urinary catheter placed and kept until at least 48 hours after enrollment |
| 5 | Patient (or legal representative, or independent physician in accordance with national law) is able and willing to provide written informed consent |
| **EXCLUSION CRITERIA** | |
| 1 | Special populations, including women with known pregnancy, prisoners, or institutionalized individuals |
| 2 | Previous renal transplant |
| 3 | Stage 2 or 3 AKI at the time of screening; defined as the enrollment SCr to Study Reference SCr ratio ≥ 2.0 or enrollment SCr ≥ 4.0 mg/dL (≥ 353.6 µmol/L) |
| 4 | Receiving dialysis (either acute or chronic) or in imminent need of dialysis (defined as within 6 hours per attending physician judgment) at the time of enrollment |
| 5 | eGFR < 45 mL/min/1.73 m2, calculated using the modification of diet in renal disease study formula based on the SCr measurement obtained at the time closest to enrollment |
| 6 | Known ESRD or history of active nephrotic syndrome within the last 3 months |
| 7 | Known Stage 2-3 AKI within the last 2 weeks |
| 8 | Terminally ill (defined as an expectation of death within 6 months), has a do not resuscitate order that would restrict protocol required procedures, or is being admitted only for palliative care |
| 9 | History of organ transplant and receiving calcineurin inhibitors |
| 10 | Documented serious allergy (i.e., anaphylaxis) to vancomycin, aminoglycosides, penicillins, or cephalosporins (intravenous or oral) |
| 11 | Known current serum total bilirubin > 4 mg/dL |
| 12 | Patients already included in an observational study can be co-enrolled in LAPIS.  Patients already included in an interventional study may be enrolled with pre-approval of the sponsor according to the following rules:   * Co-enrollment in LAPIS will not be allowed with investigational drug and devices studies * Studies may be allowed if AKI or kidney function is not an endpoint with pre-approval of the LAPIS sponsor * Co-enrollment in LAPIS will not be allowed if co-enrollment is an exclusion criterion in the other study |
| 13 | Subjects with laboratory confirmed COVID-19 infection as the primary reason for hospital admission |

AKI = acute kidney injury; COVID-19 = Coronavirus disease 2019; eGFR = estimated glomerular filtration rate; ESDR = end-stage renal disease; ICU = intensive care unit; SCr = serum creatinine.

**Supplemental Table S2.** List of Potentially Nephrotoxic Agents.

|  |  |  |
| --- | --- | --- |
| Acyclovir | Enalapril | Lithium |
| Ambisome | Enalaprilat | Mesalamine |
| Amikacin | Foscarnet | Methotrexate |
| Amphotericin B | Gadopentetate dimeglumine | Nafcillin |
| Captopril | Gadoextate disodium | Piperacillin/tazobactam |
| Carboplatin | Ganciclovir | Piperacillin |
| Cefotaxime | Ibuprofen | Sirolimus |
| Ceftazidime | Ifosfamide | Tacrolimus |
| Cefuroxime | Iodixanol | Ticarcillin/clavulanic acid |
| Cidofovir | Iohexol | Tobramycin |
| Cisplatin | Iopamidol | Valacyclovir |
| Colistimethate | Ioversol | Valganciclovir |
| Cyclosporine | Ketorolac | Vancomycin |
| Dapsone | Lisinopril | Zonisamide |

**Supplemental Table S3.** General characteristics of the cohort from Sapphire study and comparison between patients with and without the primary endpoint.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sapphire - Patient characteristics** | **Total**  **(N = 203)** | **Endpoint –**  **(N = 158)** | **Endpoint +**  **(N = 45)** | ***P*** |
| **Age** | 66 (54 - 74) | 65 (53 - 74) | 70 (58 - 75) | 0.142 |
| **Sex,**  **Male**  **Female** | 107 (52.7%)  96 (47.3%) | 85 (53.8%)  73 (46.2%) | 22 (48.9%)  23 (51.1%) | 0.680 |
| **Race,**  **White**  **Black**  **Other** | 156 (76.8%)  18 (8.9%)  29 (14.3%) | 117 (74.1%)  15 (9.5%)  26 (16.5%) | 39 (86.7%)  3 (6.7%)  3 (6.7%) | 0.184 |
| **Hypertension** | 125 (61.6%) | 93 (58.9%) | 32 (71.1%) | 0.188 |
| **Diabetes** | 58 (28.6%) | 44 (27.8%) | 14 (31.1%) | 0.810 |
| **Coronary artery disease** | 43 (21.2%) | 32 (20.3%) | 11 (24.4%) | 0.689 |
| **Chronic heart failure** | 30 (14.8%) | 22 (13.9%) | 8 (17.8%) | 0.686 |
| **COPD** | 37 (18.2%) | 25 (15.8%) | 12 (26.7%) | 0.149 |
| **History of cancer** | 63 (31.0%) | 46 (29.1%) | 17 (37.8%) | 0.355 |
| **History of CKD** | 14 (6.9%) | 8 (5.1%) | 6 (13.3%) | 0.110 |
| **Myocardial infarction** | 2 (1.0%) | 1 (0.6%) | 1 (2.2%) | 0.923 |
| **Cerebrovascular accident** | 8 (3.9%) | 6 (3.8%) | 2 (4.4%) | 1.000 |
| **Peripheral vascular disease** | 23 (11.3%) | 15 (9.5%) | 8 (17.8%) | 0.200 |
| **Liver cirrhosis** | 4 (2.0%) | 4 (2.5%) | 0 (0.0%) | 0.638 |
| **Peptic ulcer disease** | 8 (3.9%) | 8 (5.1%) | 0 (0.0%) | 0.269 |
| **Hyperlactatemia at baselinea** | 47 (23.2%) | 41 (25.9%) | 6 (13.3%) | 0.116 |
| **APACHE III score at baseline** | 75 (55 - 97) | 73 (53 - 93) | 93 (68 - 114) | 0.002 |
| **SOFA score at baseline** | 8 (5 - 10) | 8 (5 - 10) | 8 (6 - 11) | 0.263 |
| **[TIMP-2]•[IGFBP7], first testb** | 0.47 (0.18 - 1.18) | 0.41 (0.14 - 0.93) | 0.80 (0.35 - 1.72) | <0.001 |
| **[TIMP-2]•[IGFBP7], second testc** | 0.49 (0.21 - 0.95) | 0.41 (0.14 - 0.82) | 0.91 (0.56 - 2.66) | <0.001 |
| **[TIMP-2]•[IGFBP7], third testd** | 0.33 (0.15 - 0.74) | 0.27 (0.11 - 0.62) | 0.86 (0.38 - 3.22) | <0.001 |

The primary endpoint is a composite of progression of 2 or more stages of AKI, death, or dialysis within 72 hours from enrollment.

Categorical variables are presented as numbers (%), continuous variables as medians (interquartile range).

aCategorical variable defined as value above the upper limit of laboratory normality reference.

bAt approximately 6 from sepsis diagnosis and start of the treatment. No missing values.

cAt approximately 18 from sepsis diagnosis and start of the treatment. 8 missing values (4%).

dAt approximately 30 from sepsis diagnosis and start of the treatment. 16 missing values (8%), 5 of these have missing value also at the second test.

APACHE = acute physiology and chronic health evaluation III; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; IGFBP7 = insulin-like growth factor binding protein 7; SOFA = sequential organ failure assessment; TIMP-2 = tissue inhibitor of metalloproteinases-2.

**Supplemental Table S4.** General characteristics of the cohort from ProCESS trial and comparison between patients with and without the primary endpoint.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ProCESS - Patient characteristics** | **Total**  **(N. 607)** | **Endpoint –**  **(N. 463)** | **Endpoint +**  **(N. 144)** | ***p*** |
| **Age** | 61 (49 - 73) | 60 (48 - 72) | 66 (55 - 78) | <0.001 |
| **Sex,**  **Male**  **Female** | 329 (54.2%)  278 (45.8%) | 260 (56.2%)  203 (43.8%) | 69 (47.9%)  75 (52.1%) | 0.083 |
| **Race,**  **White**  **Black**  **Other** | 432 (71.2%)  127 (20.9%)  48 (7.9%) | 326 (70.4%)  97 (21%)  40 (8.6%) | 106 (73.6%)  30 (20.8%)  8 (5.6%) | 0.477 |
| **Hypertension** | 343 (56.5%) | 252 (54.4%) | 91 (63.2%) | 0.064 |
| **Diabetes** | 195 (32.1%) | 142 (30.7%) | 53 (36.8%) | 0.168 |
| **Chronic heart failure** | 63 (10.4%) | 41 (8.9%) | 22 (15.3%) | 0.027 |
| **Chronic respiratory disease** | 129 (21.3%) | 97 (21.0%) | 32 (22.2%) | 0.745 |
| **Active cancer** | 117 (19.3%) | 86 (18.6%) | 31 (21.5%) | 0.433 |
| **Renal disease history** | 37 (6.1%) | 23 (5.0%) | 14 (9.7%) | 0.037 |
| **Myocardial infarction** | 65 (10.7%) | 49 (10.6%) | 16 (11.1%) | 0.858 |
| **Cerebral vascular disease** | 59 (9.7%) | 37 (8.0%) | 22 (15.3%) | 0.010 |
| **Peripheral vascular disease** | 47 (7.7%) | 32 (6.9%) | 15 (10.4%) | 0.169 |
| **Dementia** | 52 (8.6%) | 33 (7.1%) | 19 (13.2%) | 0.023 |
| **Liver cirrhosis** | 35 (5.8%) | 25 (5.4%) | 10 (6.9%) | 0.474 |
| **Peptic ulcer disease** | 30 (4.9%) | 25 (5.4%) | 5 (3.5%) | 0.351 |
| **HIV infection** | 15 (2.5%) | 12 (2.6%) | 3 (2.1%) | 0.731 |
| **Lactate at baseline, mmol/L** | 4.23 (2.3 - 5.4) | 4.2 (2.3 - 5.3) | 4.4 (2.3 - 5.6) | 0.358 |
| **APACHE II score at baseline** | 17 (14 - 22) | 17 (13 - 21) | 20 (16 - 25) | <0.001 |
| **SOFA score at baseline** | 6 (3 - 8) | 5 (3 - 8) | 7 (5 - 9) | <0.001 |
| **[TIMP-2]•[IGFBP7], first testa** | 0.27 (0.10 - 0.81) | 0.23 (0.09 - 0.62) | 0.57 (0.16 - 1.97) | <0.001 |
| **[TIMP-2]•[IGFBP7], second testb** | 0.26 (0.10 - 0.62) | 0.22 (0.09 - 0.50) | 0.5 (0.16 - 1.24) | <0.001 |

The primary endpoint is a composite of progression of 2 or more stages of AKI or death within 72 hours, or dialysis within 48 hours from enrollment.

Categorical variables are presented as numbers (%), continuous variables as medians (interquartile range).

aAt 6 hours from enrollment/sepsis diagnosis. No missing values.

bAt 24 hours from enrollment/sepsis diagnosis. No missing values.

APACHE = acute physiology and chronic health evaluation II; HIV = human immunodeficiency virus; IGFBP7 = insulin-like growth factor binding protein 7; SOFA = sequential organ failure assessment; TIMP-2 = tissue inhibitor of metalloproteinases-2.

**Supplemental Table S5.** Logistic regression in Sapphire cohort for the primary endpoint using predictors from LASSO regularization.

|  |  |  |
| --- | --- | --- |
| **Predictors - Sapphire** | **OR (95% CI)** | ***p*** |
| **[TIMP-2]•[IGFBP7], first testa** | 1.03 (0.63 - 1.72) | 0.914 |
| **[TIMP-2]•[IGFBP7], second testb** | 1.44 (0.78 - 2.73) | 0.253 |
| **[TIMP-2]•[IGFBP7], third testc** | 2.29 (1.37 - 4.08) | 0.003 |

[TIMP-2]•[IGFBP7] values were log transformed.

aAt approximately 6 from sepsis diagnosis and start of the treatment.

bAt approximately 18 from sepsis diagnosis and start of the treatment.

cAt approximately 30 from sepsis diagnosis and start of the treatment.

AKI = acute kidney injury; CI = confidence interval; IGFBP7 = insulin-like growth factor binding protein 7; OR = odds ratio; TIMP-2 = tissue inhibitor of metalloproteinases-2.

**Supplemental Table S6.** Logistic regression in ProCESS cohort for the primary endpoint using predictors from LASSO regularization.

|  |  |  |
| --- | --- | --- |
| **Predictors - ProCESS** | **OR (95% CI)** | **p** |
| **[TIMP-2]•[IGFBP7], first testa** | 1.43 (1.15 – 1.77) | 0.001 |
| **[TIMP-2]•[IGFBP7], second testb** | 1.58 (1.27 – 1.98) | <0.001 |

[TIMP-2]•[IGFBP7] values were log transformed.

aAt 6 hours from enrolment/sepsis diagnosis.

bAt 24 hours from enrollment/sepsis diagnosis.

AKI = acute kidney injury; CI = confidence interval; IGFBP7 = insulin-like growth factor binding protein 7; OR = odds ratio; TIMP-2 = tissue inhibitor of metalloproteinases-2.

**Supplemental Table S7.** Summary of protocol simulation in ProCESS trial for patients from the primary and sensitivity analysis cohort.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment levels after [TIMP-2]•[IGFBP7] testsa** | **N**  **(column %)** | **Progression of 2 AKI stages b** | **Death b** | **Dialysis c** | **Primary endpoint d** |
| **ProCESS trial-** both [TIMP-2]•[IGFBP7] available (primary analysis cohort) | | | | | |
| **SOC** | 221 (36%) | 30/221 (14%) | 0/221 (0%) | 0/221 (0%) | 30/221 (14%) |
| **L1 KSSB** | 177 (29%) | 32/177 (18%) | 3/177 (2%) | 2/177 (1%) | 37/177 (21%) |
| **L2 KSSB** | 119 (20%) | 34/119 (29%) | 3/119 (3%) | 1/119 (1%) | 36/119 (30%) |
| **L3 KSSB** | 90 (15%) | 37/90 (41%) | 11/90 (12%) | 0/90 (0%) | 41/90 (46%) |
| **TOTAL** | **607 (100%)** | **133/607 (22%)** | **17/607 (3%)** | **3/607 (0.5%)** | **144/607 (24%)** |
| **ProCESS trial**– at least first [TIMP-2]•[IGFBP7] available (sensitivity analysis cohort) e | | | | | |
| **SOC** | 260 (38%) | 37/260 (14%) | 1/260 (0.4%) | 0/260 (0%) | 37/260 (14%) |
| **L1 KSSB** | 193 (28%) | 37/193 (19%) | 4/193 (2%) | 2/193 (1%) | 43/193 (22%) |
| **L2 KSSB** | 148 (21%) | 48/148 (32%) | 14/148 (9%) | 3/148 (2%) | 55/148 (37%) |
| **L3 KSSB** | 90 (13%) | 37/90 (41%) | 11/90 (12%) | 0/90 (0%) | 41/90 (46%) |
| **TOTAL** | **691 (100%)** | **159/691 (23%)** | **30/691 (4%)** | **5/691 (0.7%)** | **176/691 (25%)** |

aPatients did not receive these interventions but these interventions would have been recommended based on the biomarker results.

bWithin 72 hours from enrollment.

cWithin 48 hours from enrollment.

dpresence of at least 1 between progression of 2 or more stages of AKI, death, or dialysis.

e84 out of 691 (12%) patients had the second [TIMP-2]•[IGFBP7] missing.

% = percentage; AKI = acute kidney injury; L = level of the kidney-sparing sepsis bundle; N = number of patients; KSSB = kidney-sparing sepsis bundle; SOC = standard of care.

**SUPPLEMENTAL FIGURES**

**Supplemental Figure S1.** Experimental plan.

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aSamples aliquoted, frozen, and sent to the Central laboratory.

bUrine output every 2 hours will be recorded each day a urinary catheter is in place, then every 6 hours once the urinary catheter is removed.

cSamples tested on site for [TIMP-2]•[IGFBP7] and aliquots frozen and sent to the Central laboratory.

IGFBP7 = insulin-like growth factor binding protein 7; hrs = hours; KSSB = kidney-sparing sepsis bundle; SOC = standard of care; TIMP-2 = tissue inhibitor of metalloproteinases-2; UO = urine output.

**Supplemental Figure S2.** Study Design.

****

aClinical diagnosis of sepsis or septic shock per Sepsis-3 definitions.

IGFBP7 = insulin-like growth factor binding protein 7; KSSB = kidney-sparing sepsis bundle; SOC = standard of care; TIMP-2 = tissue inhibitor of metalloproteinases-2.

**Supplemental Figure S3.** Protocol simulation in ProCESS trial in sensitivity analysis cohort.



The figure shows the protocol algorithm simulated in ProCESS. These patients did not receive these levels of kidney-sparing sepsis bundle, but these interventions would have been recommended based on the biomarker results. In each box it is shown the total number of patients in this level of treatment while in the round brackets it is shown the percentage of these patients that reach the primary endpoint.

The first [TIMP-2]•[IGFBP7] test was performed at 6 hours from enrollment/sepsis diagnosis while the second was at 24 hours from enrollment/sepsis diagnosis.

In case the second [TIMP-2]•[IGFBP7] was missing (for 84 patients), the patient remained at the current treatment level obtained after the first [TIMP-2]•[IGFBP7].

For the rules of the red circled numbers, see legend of Figure 1.

aIn ProCESS the dialysis endpoint was evaluated at 48 hours from enrollment instead of 72 hours.

AKI = acute kidney injury; ICU = intensive care unit; IGFBP7 = insulin-like growth factor binding protein 7; L = level of the kidney-sparing sepsis bundle; SOC = standard of care; TIMP-2 = tissue inhibitor of metalloproteinases-2.

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See also supplemental material (Additional file 1) from “*Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A, Birkhahn R, Cely CM, Chawla LS, Davison DL, Feldkamp T, Forni LG, Gong MN, Gunnerson KJ, Haase M, Hackett J, Honore PM, Hoste EA, Joannes-Boyau O, Joannidis M, Kim P, Koyner JL, Laskowitz DT, Lissauer ME, Marx G, McCullough PA, Mullaney S, Ostermann M, Rimmelé T, Shapiro NI, Shaw AD, Shi J, Sprague AM, Vincent JL, Vinsonneau C, Wagner L, Walker MG, Wilkerson RG, Zacharowski K, Kellum JA. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013 Feb 6;17(1):R25. doi: 10.1186/cc12503. PMID: 23388612; PMCID: PMC4057242*.”

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See also https://crisma.upmc.com/progressakistudy/processteam.asp?logged=0 or supplemental material (Supplement 1) from “*ProCESS Investigators, Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014 May 1;370(18):1683-93. doi: 10.1056/NEJMoa1401602. Epub 2014 Mar 18. PMID: 24635773; PMCID: PMC4101700.”*

**LAPIS SIMULATION**

**Methods**

*Sapphire study*

The detailed inclusion and exclusion criteria were reported in the Supplementary Appendix of “Kashani K et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013 Feb 6;17(1):R25.” Among them we report the following inclusion criteria:

* males and females 21 years of age or older;
* expected to remain in the ICU for at least 48 hours after enrollment;
* within 24 hours prior to enrollment at least one among respiratory sepsis-related organ failure assessment (SOFA) score of ≥ 2 (PaO2/FiO2 <300) or cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required).

In our analysis and simulation, from the original cohort of 723 patients, we included only patients with sepsis. Sepsis status in Sapphire was determined based on the clinical diagnosis assigned by the treating physicians at the study sites. If a patient had sepsis noted in the medical record within 5 days prior to study enrollment, or if sepsis was noted as a reason for hospital or ICU admission, then the patient was considered to have sepsis.

We then excluded patients who have KDIGO AKI stage 2 or 3 at enrollment.

Our final cohort for the purpose of our analysis and simulation consisted of 203 patients.

In the simulation, if any [TIMP-2]•[IGFBP7] result was missing or unavailable, we applied the last of the “general rules” valid for the LAPIS protocol, and the subject remained at the same treatment level and used the appropriate decision rules according to the next test in the algorithm (see Supplemental Digital Content – Study Interventions – General Rules).

*ProCESS trial*

The detailed inclusion and exclusion criteria were reported in the Supplementary Appendix of “ProCESS Investigators. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014 May 1;370(18):1683-93. doi: 10.1056/NEJMoa1401602”. Among them we report the following inclusion criteria:

* males and females 18 years of age or older;
* presence of ≥ 2 of the systemic inflammatory syndrome criteria (SIRS);
* refractory hypotension (defined as a systolic blood pressure that either was less than 90 mmHg or required vasopressor therapy to maintain 90 mmHg even after an intravenous fluid challenge) or serum lactate level ≥ 4 mmol/L;
* enrolled in the study within 2 hours after the earliest detection of shock and within 12 hours after arrival to the emergency department.

In our analysis and simulation, from the original cohort of 1341 patients, we excluded patients with missing admission serum creatinine (sCr), reference sCr ≥ 4 mg/dL (n. 8), under chronic dialysis for end-stage renal disease (n. 83), KDIGO AKI stage 2 or 3 at enrollment (n. 399), missing [TIMP-2]•[IGFBP7] value at the first (6 hours) and/or the second timepoint (24 hours) (total n. 237).

Our final cohort for the purpose of our analysis and simulation consisted of 607 patients.

**Sensitivity analysis for ProCESS trial**

Since for ProCESS we had only two comparable timepoints for [TIMP-2]•[IGFBP7] tests, we thought that having just one of them could have been too much unreliable for our protocol simulation and for this reason we exclude all patients with any missing value for [TIMP-2]•[IGFBP7] from the primary analysis cohort. We did not apply the rule for missing [TIMP-2]•[IGFBP7] values (differently to what we did for Sapphire and what is planned for LAPIS trial).

In ProCESS, a total of 59 patients were missing only the first [TIMP-2]•[IGFBP7] while 84 patients were missing only the second [TIMP-2]•[IGFBP7], 94 patients were missing both the first and second [TIMP-2]•[IGFBP7].

We then performed a sensitivity analysis on a cohort in which we excluded only patients who were missing the first [TIMP-2]•[IGFBP7] (59+94 patients).

This sensitivity analysis cohort consisted of 691 patients and it was used to perform a new protocol simulation whose results are reported in Supplemental Table S7 and Supplemental Figure S3.

The overall endpoint rate was almost the same between the two cohorts (24 vs. 25%), also the endpoint rate for SOC, Level 1 KSSB and Level 3 KSSB were very similar (14% vs. 14% for SOC; 21% vs. 22% for Level 1; 46% vs. 46% for Level 3). A slight increase was found in the positive endpoint rate for Level 2, from 30 to 37%, mostly due to an increase in mortality rate (from 3 to 9%). This may be explained by the fact that the reason for missing the second [TIMP-2]•[IGFBP7] value can be due to a lack of urine sample because the patients was dead or anuric in the first 24 hours from enrollment. Also, we must consider that not having for these patients a second test and, for all of patients in ProCESS, a third test could have prevented some patients from reaching Level 3 KSSB.

**LAPIS TRIAL PROTOCOL**

**Objectives**

*Primary objective*

The primary objective of the LAPIS study is to evaluate the effect of [tissue inhibitor of metalloproteinases-2]•[insulin-like growth factor-binding protein 7] ([TIMP-2]•[IGFBP7]) guided implementation of kidney-sparing care measures (intervention arm) in comparison with standard of care (SOC) assessment and treatment (control arm) on clinical outcomes in patients with a sepsis diagnosis.

*Secondary objective*

The secondary objective is to evaluate the effect of [TIMP-2]•[IGFBP7]-guided implementation of kidney-sparing care measures in comparison with SOC on economic outcomes (resource utilization, including intensive care unit (ICU) length of stay) in patients with a sepsis diagnosis.

*Exploratory objectives*

The exploratory objectives of this study are as follows:

* to evaluate whether measuring procalcitonin (PCT) levels in addition to [TIMP-2]•[IGFBP7] testing at the same time points improves acute kidney injury (AKI) risk assessment in subjects with a diagnosis of sepsis
* to evaluate the effect of [TIMP-2]•[IGFBP7]-guided escalation or de-escalation of kidney sparing care measures (intervention arm) in comparison with SOC assessment and treatment (control arm) on practice patterns in subjects with a diagnosis of sepsis.

**Endpoints**

*Primary Endpoint*

Composite endpoint is defined as death, dialysis (defined as any form of renal replacement therapy (RRT)), or progression of 2 or more stages of AKI (from Stage 0 to 2/3 or from Stage 1 to 3) in the intervention arm compared with the control arm within 72 hours after enrollment.

Enrollment is defined as the time when the informed consent form is signed.

For the purposes of the primary endpoint, dialysis is defined as any form of RRT, including:

* Continuous veno-venous hemofiltration
* Continuous veno-venous hemodialysis
* Continuous veno-venous hemodiafiltration
* Peritoneal dialysis
* Intermittent dialysis or sustained low efficiency dialysis/prolonged intermittent RRT

*Secondary Endpoints*

* Death, dialysis, or AKI Stage 2 or 3 within 48 and 72 hours of enrollment
* Stage 2 or 3 AKI within 72 hours of enrollment
* ICU length of stay

*Exploratory Endpoints*

Other clinical endpoints:

* Death within 72 hours of enrollment
* Progression of 2 or more stages of AKI (Stage 0 to 2/3 or Stage 1 to 3) within 48 and 72 hours of enrollment
* Dialysis (defined as any form of RRT) within 72 hours of enrollment
* Stage 2 or 3 AKI within 48 hours of enrollment
* Progression of Stage 1 to Stage 2 AKI within 24, 48, and 72 hours of enrollment
* Death, dialysis, or progression of 2 or more stages of AKI (Stage 0 to 2/3 or Stage 1 to 3) within 48 hours of enrollment
* Proportion of subjects with renal recovery (defined as < 120% Study Reference creatinine value) at the time of hospital discharge or Day 60 after enrollment, whichever is sooner
* All AKI stages (1 to 3) based on serum creatinine (SCr) or urine output (UO) within 72 hours after enrollment
* AKI Stage 2/3-free days at the time of hospital discharge or Day 60 after enrollment, whichever is sooner
* Time to death, dialysis, or progression of 2 or more stages of AKI (Stage 0 to 2/3 or Stage 1 to 3) within 72 hours of enrollment
* Time to renal recovery prior to hospital discharge or Day 60 after enrollment, whichever is sooner
* All-cause mortality (in hospital and at Day 30 from enrollment)
* Confirmed urinary and vascular catheter infections occurring within 30 days of enrollment
* Proportion of subjects with cardiac arrest within 72 hours of enrollment
* Use of any protocol-defined nephrotoxic drugs
* Duration (hours) of urinary catheter use within 72 hours after enrollment
* Total daily fluid given by type (crystalloids [unbalanced and balanced], albumin, hydroxyethyl starches, gelatin, dextran) within 72 hours of enrollment
* Relationship between protocol adherence and the primary and secondary endpoints

Other measures of resource utilization including:

* ICU-free days at Day 30
* RRT-free days at Day 30
* 30-day hospital re-admission (count/rate) assessed within 60 days from enrollment
* Stage 2/3 AKI based on SCr or UO within 72 hours after enrollment
* Any RRT therapy used; assessed within 72 hours and while hospitalized (within 30 days from enrollment)
* Consultations with nephrologists, infectious diseases, and available hospital resources (e.g., clinical pharmacists) within 72 hours of enrollment
* Invasive hemodynamic monitoring use-free days at Day 30
* Ventilator free days at Day 30

**Study Population**

Approximately 540 subjects will be enrolled at approximately 18 sites in Europe (Belgium, France, Germany) and in the United States. Subjects will be enrolled only if they meet all the inclusion criteria and none of the exclusion criteria reported in Supplemental Table S1.

The study population will include adult subjects who present with a diagnosis of sepsis, including septic shock (according to study inclusion/exclusion criteria).

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety.

The choice to include patients with ≥ 21 year of age was based on the approved indication from the Food and Drug Administration for NEPHROCHECK® Test. Since a consistent amount of study sites will be in the United Stated, we decided to apply the same age criterion also for the study sites in Europe.

Regarding exclusion criteria, the rationale for many of them is to try to avoid conditions in which AKI is no longer a modifiable condition and our interventions will not have any margin to show a potential benefit. This matter regards the choice to exclude patients who already have AKI stage 2-3 at the moment of screening, AKI stage 2-3 episode within the last 2 weeks or who are already receiving (acute or chronic) dialysis. Another exclusion criterion that shared a similar rationale is the presence of eGFR < 45 mL/min/1.73 m2, picturing a possible condition of pre-existing moderate-severe chronic kidney disease in which superimposed AKI is usually less modifiable.

The reason to exclude patients with previous renal transplant or other organ transplant receiving calcineurin inhibitors is related to avoiding possible confounders related to their need to take immunosuppressive and nephrotoxic drugs that may affect the endpoints.

We excluded patients with documented allergy to potentially vancomycin, aminoglycoside, penicillin, or cephalosporins, because these patients could have a biased selection of their antibiotics, regardless of whether assigned to intervention or control group.

Total bilirubin value > 4 mg/dL in sepsis is usually associated with severe liver dysfunction, hemolysis, or even disseminate intravascular coagulation. All these conditions may be present in the context of multiple organ failure, picturing a much too severe condition in which the risk of death or AKI is less modifiable. Moreover, it has been reported that total bilirubin values above 7.2 mg/dL interfere with [TIMP-2]•[IGFBP7] test and reduce its levels by 10-20% (Uettwiller-Geiger DL, Vijayendran R, Kellum JA, Fitzgerald RL. Analytical characteristics of a biomarker-based risk assessment test for acute kidney injury (AKI). *Clin Chim Acta*. 2016 Apr 1;455:93-8). To avoid any possible confounder with stated this exclusion criteria at a stricter level.

We decided to exclude patients with coronavirus disease 2019 (COVID-19) as the primary reason for hospital admission since the preliminary data regarding AKI in these patients suggest some particular, and still not completely understood, feature and we do not want to add potential additional confounders in the response to the interventions. Patients admitted to the hospital for any other reason and diagnosed with sepsis, once enrolled in LAPIS trial, will not be excluded if subsequent testing for COVID-19 will result positive.

**Study Treatments**

*Randomization and assignment to treatment groups*

Subjects will be enrolled and randomly assigned during the screening period to either the control arm or intervention arm using a 1:1 allocation ratio. An interactive web response system will be used to administer the randomization schedule.

*Treatments Administered*

The study does not include any investigational treatment or device to be administered. The study will compare SOC subject management with management guided by the use of the NEPHROCHECK® Test (Astute Medical, San Diego, CA). From now on NEPHROCHECK® Test will be referred only as the risk score [TIMP-2]•[IGFBP7].

*Blinding*

A study of this nature cannot be blinded from healthcare providers who will need to enact protocol-required clinical interventions. Data grouped by treatment arm allocation will not be provided to any party, except the unblinded statisticians and the data and safety monitoring board (DSMB). The DSMB will receive data from the unblinded statistician in aggregate and presented by treatment arm.

*Treatment Adherence*

Interventions suggested for SOC and kidney-sparing sepsis bundle (KSSB) algorithms will be recorded on the electronic case report form (eCRF) both for patients in the intervention and control arm. This will allow us to compare the frequency of use, the timing of use and the extent to which use in the control arm follows expectations in the treatment algorithm. For only the intervention arm, the extent to which KSSB interventions were followed according to the [TIMP-2]•[IGFBP7]-guided treatment algorithm will be assessed with a protocol Adherence Score system (see below “Adherence Analysis and Score”).

**Determination of Study Reference SCr and Staging of AKI**

At enrollment, diagnosis and stage of AKI will be based upon the Kidney disease: Improving global outcomes (KDIGO) guidelines using SCr values. A *Study Reference SCr* will be established for AKI staging to determine subject eligibility as well as for endpoint determination.

*Index admission* is the current admission to the study center hospital (i.e. in case of subject transfer, does not include the admission to a hospital that the subject is transferring from).

*Enrollment SCr* is the SCr measurement closest to the time of enrollment.

*Admission SCr* is the first SCr measurement during the index admission.

If there is only one SCr available during the index admission, then the enrollment SCr and the admission SCr will be the same.

*Historical SCr* is(are) SCr measurement(s) during the 365 days prior to enrollment, excluding the index admission. If there are more than 10 values of historical SCr only the 10 most recent will be considered.

The *Study Reference SCr* will be determined as follows:

* If available, take the median of *historical SCr* values
* Compare the median of the *historical SCr* values and the *admission SCr* value
* The Study Reference SCr is the lower of the two value of this comparison
* If a *historical SCr* value is not available, the *Study Reference SCr* value is equal to the *admission SCr* value

Staging of AKI:

* Stage 1: 1.5-1.9 times *Study Reference* OR an increase of *enrollment SCr* ≥ 0.3 mg/dL (≥ 26.5 µmol/L) above *Admission SCr* if within 48 hours (if enrollment occurs after 48 hours from admission, this last criterion cannot be used)
* Stage 2: 2.0-2.9 times *Study Reference*
* Stage 3: 3.0 times Study Reference OR increase in SCr values to ≥ 4.0 mg/dL (≥ 353.6 µmol/L)

**Samples Collection**

*Standard of Care (Control Arm)*

Subjects have their urine collected for serial [TIMP-2]•[IGFBP7] testing at the following time points:

* Between 6 and 9 hours of the subject being clinically diagnosed as having sepsis (preferably at the end of the first 6-hour resuscitation bundle)
* Between 6 and 9 hours after the first urine sample is collected
* Between 12 and 15 hours after the second urine sample is collected

In the control arm samples will be shipped to a central laboratory for processing and will not be available for clinical decision making. In the intervention arm samples will be processed locally and reported to the investigator.

For all subjects, whole blood for a PCT level will be drawn each time a urine sample is collected. In addition, whole blood for a SCr level will be drawn each time a PCT test sample is collected and will then be collected approximately every 12 hours after the third sample until 72 hours after enrollment. Blood samples will be shipped to the central laboratory for SCr measurement.

UO will be assessed and recorded at least every 2 hours (hourly if possible) for all subjects who have a urinary catheter in place from the time of enrollment until 72 hours after enrollment. For subjects whose urinary catheter is removed prior to the 72 hours time point, all urine volumes will be recorded at 6 hours intervals (at least) from the time the catheter is removed until 72 hours after enrollment. The average hourly UO during each interval will be calculated from data entered into the eCRF.

**Study Interventions**

*Control arm - Standard of care (SOC)*

Subjects randomly assigned to the control arm will be treated according to the attending clinician’s clinical judgment and the site’s SOC for treating sepsis subjects.

*Intervention arm - Standard of Care (SOC)*

As it is SOC to promote the de-escalation of care in low‑risk patients, when subjects have 3 negative [TIMP-2]•[IGFBP7] values and, if medically appropriate according to their judgment, the treating clinician may consider de-escalation of care.

*Intervention arm - Kidney-sparing Sepsis Bundle (KSSB)*

Subjects with any [TIMP-2]•[IGFBP7] test result > 0.3 will be offered KSSBs with 3 levels of care possible depending on the quantitative value of the test results and test result trends over time (see Figure 1 for protocol treatment algorithm).

All KSSB interventions are derived from the internationally recognized KDIGO guidelines for the prevention of AKI. Many of these interventions are performed routinely in ICUs in Europe and the US. These interventions include acceptable alternatives for sepsis care that pose a reduced risk to the kidney and/or interventions that are appropriate for some patients with sepsis but for which there is no universal standard as to when they should be applied. The institution of any KSSB intervention must be medically appropriate according to the clinical judgment of the treating clinician. The treating clinician will always have the option to decline the use of any KSSB intervention if they feel it is not in the best interest of the subject.

**Level 1** kidney-sparing interventions may include the following:

* Strong recommendation to discontinue the potentially nephrotoxic agents listed below (Supplemental Table S2) until at least 72 hours after enrollment
* Recommendation to discontinue drugs in the following classes until at least 72 hours after enrollment:):
  + Nonsteroidal anti-inflammatory drugs
  + Angiotensin-converting enzyme inhibitors
  + Angiotensin receptor blockers
  + Aminoglycosides
  + Radiocontrast agents
* When vancomycin or aminoglycosides are required, dosing will be based upon therapeutic drug level monitoring until at least 72 hours after enrollment
* Review all medications as soon as possible in consultation with available hospital resources (e.g., clinical pharmacists) until at least 72 hours after enrollment
* Use of only balanced fluids (crystalloid) for fluid boluses until at least 72 hours after enrollment
* Strict daily measurement of total fluids in and out until at least 72 hours after enrollment
* Use of diuretics and fluids only after determining fluid status and need. Do not use diuretics except in the management of fluid overload until at least 72 hours after enrollment
* Provision of alternative options to radiocontrast procedures (consideration of alternative imaging methods, use of the lowest possible dose of contrast medium, avoidance of all unnecessary IV iodinated contrast dyes, etc.) until at least 72 hours after enrollment.

**Level 2** kidney-sparing interventions may include Level 1 interventions plus the following:

* Institution of functional hemodynamic monitoring (e.g., with FloTrac™, PiCCO®, ultrasound) to optimize the volume status and hemodynamic parameters and to assess fluid responsiveness. To be kept in place for at least 72 hours after enrollment.

**Level 3** kidney-sparing interventions may include Level 1 and Level 2 interventions plus the following:

* Review the study subject’s kidney status with available hospital resources (e.g., consultation with nephrologist)
* Review the study subject’s infectious disease management with available hospital resources (e.g., infectious disease specialist).
* Consideration of seeking other sources of infection (interventions could include imaging procedures, skin examination, etc.).

**General Rules:**

* Once the KSSB is started, the physician will not de-escalate levels within 72 hours of enrollment
* If the first [TIMP-2]•[IGFBP7] value is between 0.3 and 1, Level 1 KSSB is started
* If the first [TIMP-2]•[IGFBP7] value is 1 or higher, Level 2 KSSB is started
* In the event that any [TIMP-2]•[IGFBP7] result is missing or unavailable, the subject will remain at the current KSSB treatment level and use the appropriate decision rules according to the next test in the algorithm. For example, if a subject is at KSSB Level 1 after the first test, and the second test result is unavailable for any reason, the subject will remain at KSSB Level 1, and the decision tree for the third test will be used when the third [TIMP-2]•[IGFBP7] result is available.

**Safety Assessment**

As this protocol does not involve the use of an interventional medicine or device, only serious adverse events (SAEs), serious adverse device effects (SADEs) and non-serious adverse events that are definitely, probably or possibly related to the device or a protocol related procedure will be collected.

*Definitions of Adverse Events (AE)*

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study treatment.

*Serious Adverse Events (SAEs)*

An SAE is defined as any event that:

* Results in death
* Is immediately life-threatening
* Requires inpatient hospitalization or prolongation of existing hospitalization
* Results in persistent or significant disability/incapacity
* Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

For this protocol, a SADE will be any SAE that is assigned as definitely, probably, or possibly related to the device or strategies recommended in the treatment bundles specified by this protocol.

A serious unanticipated adverse device effect is any SADE impacting health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

**Sample size calculation**

We assumed that the rate of the primary endpoint for the control arm is 35%, and we wish to detect a 30% reduction in this rate with biomarker-guided therapy, and we planned to test the null hypothesis of no difference in endpoint rate between groups. To ensure alpha of 0.05 and power of 80%, we need 258 subjects with full data to analyze in each arm of the study (total study sample size of 516 subjects). To ensure that enough subjects are available for per-protocol after accounting for unevaluable subjects, we planned a final enrollment increase of 5% to a total of 540 subjects. We will perform an intent-to-treat analysis.

**Adaptive Changes**

This study will use an adaptive design and pre-specify possible adaptive changes to the protocol. These changes to the protocol can be enacted at any time but will most likely come after the 50% enrollment interim analysis.

The changes will be based on the relative risk reduction (RRR) observed between the control and [TIMP-2]•[IGFBP7] test arms. The following two changes will be pre-specified:

1. Sample size modification

Data for the use of [TIMP-2]•[IGFBP7] test in sepsis are limited, and the sample size is based on the best available data assuming a baseline rate of 35% and an RRR of 30%. If the RRR is different from the assumed 30%, the sample size will be modified as follows:

* RRR < 7%, then discontinue study for futility
* (7% ≤ RRR ≤ 17%), then increase sample size by 20%
* (17% < RRR < 37%), then continue study with original sample size
* (37% ≤ RRR < 43%), then decrease sample size by 20%
* RRR ≥ 43%, then discontinue study for success

1. Septic shock subjects

Since it is known that septic shock subjects have a higher risk of mortality, it is possible that outcomes in this group of subjects may be different and mask the effect of [TIMP-2]•[IGFBP7] test-guided therapy in the remaining subjects. If the RRR is less than 17%, the effect of excluding septic shock subjects will be evaluated. If the RRR after excluding septic shock subjects increases sufficiently to reduce the sample size required or avoid a stop for futility, then septic shock subjects will be excluded for the remainder of the study.

**Analysis**

*Economic Analyses*

The economic impact of [TIMP-2]•[IGFBP7] test may be assessed with an evaluation of healthcare utilization based on use of the test. These healthcare services and interventions will include the following:

* Days in hospital, including re-admissions up to 30 days
* Days in ICU
* Days of RRT
* Days of ventilator support
* Days of hemodynamic monitoring
* Number of consultations
* Number of [TIMP-2]•[IGFBP7] test measurements

*Procalcitonin Analysis*

The observed values for PCT at each time point and/or the pattern of PCT values will be used as independent variables in a logistic regression analysis after adjustment for study arm. Both the primary endpoint and the development of Stage 2/3 AKI will be considered as dependent variables. If appropriate, this analysis will adjust for other variables as well. A significant value for PCT will indicate an additional benefit from knowledge of PCT values or patterns.

*Adherence Analysis and Score*

Adherence to KSSB Levels 1 to 3 will be defined by a score and evaluated within the [TIMP-2]•[IGFBP7] test-guided therapy arm. The number of subjects experiencing the primary endpoint will be compared among those with varying levels of adherence as well as between those who have varying levels of adherence and all 3 [TIMP-2]•[IGFBP7] values compared with other subjects.

Below is reported the Adherence Score and its items:

* KSSB Level 1:
  1. Any documented use of protocol-defined nephrotoxic drugs results in 1 point
  2. If vancomycin/aminoglycosides are deemed required and ordered, lack of documented order for therapeutic drug levels for vancomycin/aminoglycosides results in 1 point
  3. Lack of medication review for nephrotoxicity with hospital resources (e.g., with pharmacy consultation) results in 1 point
  4. Documented use of any non-crystalloids for fluid boluses results in 1 point
  5. Lack of documented order for protocol-defined Intake/Output measurement results in 1 point
  6. Unless fluid overload is denoted in eCRF, any documented diuretic use results in 1 point
  7. Any documented use of protocol-defined nephrotoxic radiocontrast media results in 1 point
* KSSB Level 2:

1. Lack of a documented order for a form of functional hemodynamic monitoring (as defined in the protocol) results in 1 point

* KSSB Level 3:

1. Lack of review of the study subject’s kidney status with available hospital resources (e.g., consultation with nephrologist) results in 1 point
2. Lack of review of the study subject’s infectious disease management with available hospital resources (e.g., infectious disease specialist) results in 1 point

*Interim Analysis of Safety and Efficacy*

Interim safety reviews after 25%, 50%, and 75% of total enrollment will be performed by the DSMB. The DSMB may review, as necessary, enrollment and demographic information, medical history, SAEs/SADEs, and deaths in order to assess risk and benefit. Additional data may be requested by the DSMB in consultation with the unblinded statistician. The DSMB may receive data in aggregate and presented by treatment arm.

If there are no safety issues at the evaluations after 25% and 75% of total enrollment, the study will continue, and there will be no formal comparison of the primary endpoint to a boundary value.

There will be one formal interim analysis for efficacy after 50% of the subjects (129 per arm) have been enrolled. The interim analysis will assess the assumptions used for sample size calculations, including rate of occurrence of the primary endpoint and frequency of enrolled subjects experiencing the primary endpoint.

**Process Evaluation**

Different steps have been considered to allow a proper process evaluation in the LAPIS trial. Using these steps, we will evaluate both the extent to which the process was followed and the impact of the complex intervention.

*Planning*

In the planning phase of the trial, a Science Committee (SC) composed by members from the sponsor company (bioMérieux SA, Marcy l’Etoile, France) and international experts in the field of critical care nephrology was established to design the trial and help during all the trial processes. A principal investigator was designated to oversight the trial.

*Design and conduct*

Regarding the design, previous scientific evidence and unmet clinical needs were assessed by the SC to better define the aim and the design of the study. Then a protocol clearly describing the rationale, the intervention and the methodology of the trial was prepared.

The trial will be multicenter, with multiple study sites across different countries both in the United States and Europe, to allow an international validation.

The study will be conducted and data generated, documented, and reported in compliance with the protocol that follows Good Clinical Practice (GCP), and applicable regulatory requirements. This study will be conducted in accordance with the provisions of the Declaration of Helsinki (October 2008) and the ICH guidelines on GCP. Approval of the protocol was obtained from institutional review boards/research ethics boards/independent ethics committees according to country regulations. The trial was also registered on clinicaltrials.gov (#NCT04434209).

*Data collection and analysis*

Data will be collected similarly for intervention and control arms including demographic, medical history, interventions (also in the control arm, if activities similar to the interventions are performed, they will be reported in the eCRF). Collection of samples and data will be performed at different timepoints, according to the protocol and trial design. A central laboratory will be used to evaluate in a homogeneous way serum creatinine values for outcome assessment. [TIMP-2]•[IGFBP7] values will be collected on the control arm (results masked to investigators) as well as intervention arm so that we will be able to evaluate the extent to which knowledge of the result drove implementation of interventions. Protocol adherence will be assessed through a score allowing determination of overall as well as individual intervention adherence. A detailed analysis plan was prepared in advance describing the intention-to-treat analysis, the sample size calculation, the analysis of the endpoints, the planned sub-group analysis, as well as the interim safety and efficacy analysis at pre-specified enrollment rates.

*Reporting*

After completion of the study, the data will be considered for reporting at a scientific meeting and/or for publication in an international peer-reviewed scientific journal. We will report the results of our trial following the international guidelines for publication of randomized controlled trial (e.g., CONSORT 2010 statement).