Probiotics in septic acute kidney injury, a double blind, randomized control trial

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Abstract

**Background:** During acute kidney injury (AKI) due to sepsis, the intestinal microbiota changes to dysbiosis, which affects the kidney function recovery (KFR) and amplifies the injury. Therefore, the administration of probiotics could improve dysbiosis and thereby increase the probability of KFR.

**Methods:** In this double-blind clinical trial, patients with AKI associated with sepsis were randomized (1:1) to receive probiotics or placebo for 7 consecutive days, with the objectives of evaluate the effect on KFR, mortality, kidney replacement therapy (KRT), urea, urine volume, serum electrolytes and adverse events at day 7.

**Results:** From February 2019 to March 2022, a total of 92 patients were randomized, 48 to the Probiotic and 48 to Placebo group. When comparing with placebo, those in the Probiotics did not observe a higher KFR (HR 0.93, 0.52-1.68, p = 0.81), nor was there a benefit in mortality at 6 months (95% CI 0.32-1.04, p = 0.06). With probiotics, urea values decreased significantly, an event not observed with placebo (from 154 to 80 mg/dl, p = 0.04 and from 130 to 109 mg/dl, p=0.09, respectively). Urinary volume, need for KRT, electrolyte abnormalities, and adverse events were similar between groups. (ClinicalTrial.gov NCT03877081) (registered 03/15/2019).

**Conclusion:** In AKI related to sepsis, probiotics for 7 consecutive days did not increase the probability of KFR, nor did other variables related to clinical improvement, although they were safe.

**Background**

The intestinal microbiota is made up of 100 trillion microorganisms, such as bacteria, viruses, fungi, and protozoa, that interact in health and disease processes (1, 2) and have been fundamental in the evolution of humans (3, 4). The great majority of intestinal bacteria (∼90%) are cataloged into three groups: Bacteroidetes, Firmicutes, and Actinobacteria. During physiological states, they contribute to the generation of short-chain fatty acids (SCFA) that have a profound interaction with the immune system and affect all the organ's function. In the kidney, for example, they interact four SCFA receptors (Gpr41, Gpr43, Gpr109a, and Olfr78) located on different sides of the nephron (5), promoting the physiological functioning of the kidney, maintaining the glomerular filtration rate (GFR) and tubular capacity to reabsorb and secrete solutes, a process called symbiosis (6).

In the face of disease and inflammation, the intestinal microbiome undergoes changes in its composition, causing the proliferation of pathogenic bacteria, which in turn promotes more local and systemic inflammation, elevated concentrations of uremic toxins, increased intestinal permeability, endotoxemia, and immunodeficiency (7, 8), affecting homeostasis through different pathways. This phenomenon is called intestinal dysbiosis (6) and has been associated with adverse clinical outcomes in experimental models and humans in many different clinical scenarios, such as the systemic inflammatory response syndrome (9), sepsis (10), chronic kidney disease (CKD) and acute kidney injury (AKI) (11, 12, 13, 14).
AKI occurs in one out of every four hospitalized patients, and 22.8% of these cases die during hospitalization (15, 16). The main cause of hospital acquired AKI (HA-AKI) is sepsis which accounts for 70% of cases in our community. Various efforts to find specific treatments to attenuate sepsis-induced AKI like antifibrotics, antiflammatory and immunomodulators have been unsuccessful (17, 18) and therefore management of sepsis-induced AKI is currently limited to treating the main etiology (19) and in severe cases, correcting its complications through kidney replacement therapies (KRT) (20). There is an urgent need to explore alternatives for the treatment of AKI (21).

Since AKI is a syndrome that generates intense systemic inflammation (22), attenuation of this phenomenon has been shown to improve renal function and parenchymal damage (23, 24). And since AKI and intestinal dysbiosis coexist amplifying local and systemic inflammation, facilitating the proliferation of harmful intestinal bacteria which generates a vicious circle that worsens clinical status, cause kidney injury and subsequent systemic failure, (14, 25). It seems reasonable that modulating the microbiota and improving intestinal dysbiosis during AKI by administrating probiotics could improve outcomes in these syndromes. We have conducted the first clinical trial of probiotic treatment for patients with sepsis-induced AKI (ClinicalTrial.gov NCT03877081) (registered 03/15/2019) with the hypothesis that by modulating intestinal dysbiosis with probiotics AKI recovery will improve.

**Methods**

**Study participants**

This was a single-center double-blind randomized clinical trial that screened all consecutive patients admitted for AKI who met the diagnostic criteria for sepsis and had been evaluated by the Nephrology Department at the Hospital Civil de Guadalajara Fray Antonio Alcalde, a large referral center that cares patients without health care insurance and with low socioeconomic resources in Jalisco, México.

Patients were enrolled from February 2019 to March 2022. Due to the COVID-19 pandemic, we enrolled patients at slower rate and over a relatively long period (2019 through 2021). The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients gave their written informed consent before any study-related procedure. The study, which was approved by the local ethics committee (HCG/CEI-1342/18), was prospectively registered in clinicaltrials.gov (NCT03877081) on 03/15/2019. No funding was received to conduct this study.

**Definitions**

AKI was defined as an increase in serum creatinine (sCr) according to KDIGO (26), and CKD was defined according to the KDIGO guidelines (27). The estimated glomerular filtration rate (eGFR) in ml/min/1.73 m² was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Eq. (28). Baseline eGFR was considered according to the more recent sCr measured within the previous 3
months. In those patients without baseline sCr, we estimated it by back-calculating the MDRD equation, assuming eGFR 100 ml/min/1.73m; this surrogate was preferred because it has been shown to be more accurate than assuming 75 ml/min/1.73 m² (29). Sepsis was defined according to the Sepsis-3 criteria (30). Kidney function recovery (KFR) was defined as the return of sCr to < 0.30 mg/dL from the baseline value up to 7 days of follow-up. Seven days of was chosen to define renal recovery since after day 7 if patients do not recover from AKI it is consider that they have developed acute kidney disease (AKD) and they have increase risk of adverse outcomes (31, 32)

All co-morbidities and clinical data were prospectively collected during the first evaluation.

Adverse events were pre-specified according to those most frequently reported with the use of probiotics, such as abdominal distension, nausea, rash, vomiting and diarrhea (33), and they were prospectively recorded on a daily basis by the nephrology staff. Additional data were collected from the medical records and hospital electronic database. Appropriate adherence to treatment was defined as > 80% of the administered capsules being consumed.

**Study Outcomes**

The primary outcome was to assess KFR at day 7 (sCr return to < 0.30 mg/dL from the baseline value). Secondary outcomes included variables related to KFR during the treatment and the follow-up period, namely, the change in urinary volume, percentage of decrease in sCr, in-hospital mortality, mortality during follow-up, KRT requirement, electrolytes and acid-base abnormalities. Pre-specified adverse events mentioned above were prospectively reported.

**Randomization and treatment assignments**

Randomization was carried out by a computer-generated stratified sequence with a 1:1 allocation ratio in blocks of 5, with the strata defined by sex. The researchers with a concealed opaque envelope system performed group assignment after informed consent was obtained. A double blind, double dummy design was used. The nephrology staff in white bottles that were only marked with the patient’s assignment number administered the treatment.

Given the lack of previous clinical trials on this topic, a formal sample size calculation was not performed, and we chose a convenience sample size of 92 patients. All patients received the personalized management suggested by the KDIGO guidelines AKI bundle of care (26). The study design is described in **Supplemental Fig. 1**. Inclusion criteria were age > 18 years, the presence of sepsis-induced AKI, who were willing to participate and had signed informed consent. Patients with CKD KDIGO stages 4–5 or on chronic dialysis, kidney transplant, pregnancy, or who had not signed the informed consent form were excluded.

Patients who met the inclusion criteria and signed informed consent were randomized to the intervention group (probiotics) or the control group (placebo), and 2 capsules *per os* (or through an enteral tube) were
administered every 24 hours. Blood and urine tests were taken to measure the variables of interest every 24 hours and were processed in a certified central laboratory.

The results are reported following the CONSORT statement for clinical trials.

**Interventions**

Patients in the intervention group received 2 capsules of Simbin-RNL® or 2 capsules of placebo (maltodextrin) every 24 hours for 7 consecutive days. The gastro-resistant Simbin-RNL® capsule contained 540 mg of a mixture of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium longum* (90 billion Colony Forming Units (CFU) in the 2-capsule serving (4.5 x10e10 CFU per capsule), agave inulin (the contribution of prebiotic fiber per serving is 600 mg per 2 capsules), magnesium stearate and silicon dioxide. The Simbin-RNL® formula is composed of a mixture of a food supplement of probiotic strains and agave inulin (a prebiotic) that acts as soluble fiber, which arrives intact in the intestine to be used as food for the anaerobic intestinal microbiota, promoting the growth of saccharolytic bacteria and increasing the concentration of SCFA, alterations in intestinal pH, inhibition of pathogens via the generation of antibacterial compounds, competitive elimination of pathogens in receiver binding sites and contention for available nutrients (7). This formula was chosen for this trial since it has shown kidney function benefits in experimental models of AKI, improving kidney function measured by sCr, urea and attenuating histological injury (10, 34).

**Statistical analysis**

Categorical variables are presented as numbers and percentages, and comparisons between groups were performed with the chi-square or Fisher exact test as appropriate. According to the Shapiro–Wilk test for data distribution, continuous variables are summarized as the means ± standard deviations (SD) if normally distributed or medians and interquartile ranges (25–75th) if non-normally distributed and were compared using Student’s t-test or the Mann–Whitney U test, respectively. For variables measured at multiple time points, repeated measures analysis of variance tests were used for the comparisons between groups. Time to renal recovery and time to death were both plotted on Kaplan–Meier curves, and the groups were compared with the log-rank test. A multiple regression analysis was performed with the enter method, introducing into the model all of the baseline variables with a p value ≤ 0.20 at bivariate analysis. All tests were two-tailed, and a p value less than 0.05 were considered significant. Statistical analysis and graphics were performed with MedCalc Statistical Software (Ostend, Belgium. Ver 19.1.3) and GraphPad Prism (California, USA. Ver 9.2.0) respectively.

**RESULTS**

From February 2019 to March 2022, 621 patients with AKI were consulted by nephrology, 123 did not have sepsis or they lacked variables of interest for the analysis, so 498 were assessed for eligibility, among which 372 did not meet inclusion criteria, and 34 did not signed the consent form; therefore, 92 patients were randomized, 48 to the intervention group (probiotics) and 44 to the placebo group. (Fig. 1)
The baseline demographic characteristics of the randomized study participants are described in Table 1. The mean age was 56.9 ± 16.3 years, 51% (47) were female, almost half of them had diabetes (46%) and a quarter had CKD (27%). We did not observe any severe electrolyte alterations, and all had mild metabolic acidosis (pH and HCO$_3^-$, 7.33 ± 0.06 and 19.1 ± 3.9, respectively). Most of the patients (92%) had severe AKI (KDIGO stages 2 and 3, 15% and 77%, respectively) with a mean SOFA score of 6 (4–8), and 9.3% had septic shock.
Table 1
Study baseline characteristics according to the probiotics or placebo groups.

<table>
<thead>
<tr>
<th></th>
<th>All (n = 92)</th>
<th>Probiotics (n = 48)</th>
<th>Control (n = 44)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)—mean (SD)</td>
<td>56.9 ± 16.3</td>
<td>55.5 ± 16.7</td>
<td>58.4 ± 15.9</td>
<td>0.39</td>
</tr>
<tr>
<td>Female (%)</td>
<td>47 (51)</td>
<td>25 (52)</td>
<td>22 (50)</td>
<td>0.84</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>42 (46)</td>
<td>27 (56)</td>
<td>23 (52)</td>
<td>0.70</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>38 (41)</td>
<td>20 (42)</td>
<td>18 (41)</td>
<td>0.94</td>
</tr>
<tr>
<td>Chronic kidney disease (%)</td>
<td>25 (27)</td>
<td>11 (23)</td>
<td>14 (32)</td>
<td>0.34</td>
</tr>
<tr>
<td>Chronic heart failure (%)</td>
<td>5 (5)</td>
<td>2 (4)</td>
<td>3 (7)</td>
<td>0.57</td>
</tr>
<tr>
<td>Baseline sCr (mg/dL)</td>
<td>1.1 (0.8–1.6)</td>
<td>1.1 (0.8–1.6)</td>
<td>1.1 (0.8–1.8)</td>
<td>0.82</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCr, mg/dL—mean (IQR)</td>
<td>3.4 (2.3–5.0)</td>
<td>3.7 (2.3–5.5)</td>
<td>2.9 (2.2–4.5)</td>
<td>0.26</td>
</tr>
<tr>
<td>Urea, mg/dL—mean (IQR)</td>
<td>137 (81–187)</td>
<td>154 (83–189)</td>
<td>130 (70–173)</td>
<td>0.26</td>
</tr>
<tr>
<td>Urinary volume, ml/day—mean (IQR)</td>
<td>1090 (500–1470)</td>
<td>1000 (500–1500)</td>
<td>1095 (600–1400)</td>
<td>0.87</td>
</tr>
<tr>
<td>Sodium, mEq/L—mean (SD)</td>
<td>135 ± 8.6</td>
<td>133 ± 9</td>
<td>136 ± 8</td>
<td>0.09</td>
</tr>
<tr>
<td>Potassium, mEq/L—mean (IQR)</td>
<td>4.4 (3.7–5.1)</td>
<td>4.5 (3.9–5.2)</td>
<td>4.1 (3.6–4.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Chloride, mEq/L—mean (SD)</td>
<td>102 ± 9.2</td>
<td>101 ± 9.4</td>
<td>103 ± 9.1</td>
<td>0.37</td>
</tr>
<tr>
<td>Calcium, mg/dL—mean (IQR)</td>
<td>7.8 (7.1–8.3)</td>
<td>7.7 (7.0–8.1)</td>
<td>7.9 (7.4–8.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>pH—mean (DE)</td>
<td>7.33 ± 0.06</td>
<td>7.33 ± 0.06</td>
<td>7.33 ± 0.07</td>
<td>0.81</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L—mean (SD)</td>
<td>19.1 ± 3.9</td>
<td>18.8 ± 3.9</td>
<td>19.6 ± 3.8</td>
<td>0.49</td>
</tr>
<tr>
<td>PCO₂, mmHg—mean (IQR)</td>
<td>35 (29–40)</td>
<td>33 (30–40)</td>
<td>36 (29–44)</td>
<td>0.53</td>
</tr>
<tr>
<td>Lactate, mmol/L—mean (IQR)</td>
<td>1.2 (0.9–1.6)</td>
<td>1.3 (0.9–1.6)</td>
<td>1.1 (1.0–1.4)</td>
<td>0.94</td>
</tr>
<tr>
<td>Hemoglobin, g/dL—mean (RIQ)</td>
<td>10.2 (8.6–12.0)</td>
<td>10.2 (8.4–11.7)</td>
<td>10.2 (8.9–12.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Leucocytes, 10³ cél/µL—mean (IQR)</td>
<td>13.2 (9.3–17.9)</td>
<td>13.4 (9.9–18.4)</td>
<td>12.6 (9.1–17.6)</td>
<td>0.52</td>
</tr>
<tr>
<td>Platelets, 10³ cél/µL—mean (IQR)</td>
<td>212 (132–305)</td>
<td>212 (130–321)</td>
<td>194 (136–289)</td>
<td>0.82</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110 ± 19</td>
<td>111 ± 19</td>
<td>109 ± 20</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Regarding adherence, 81% in the probiotic group and 77% in the control group consumed at least 80% of the doses (p = 0.63).

**Primary outcome**

The KFR by day 7 is presented in Table 2 and Fig. 2. A total of 40 (43%) patients had KFR, 25 (50%) in the probiotic group and 21 (48%) in the control group, without significant difference (p = 0.82) between groups. The relative risk for recovery at 7 days in intervention group was 0.93 (95%CI 0.52–1.68, p = 0.81). Thus, no benefit was observed in patients who received probiotics in terms of improvements in kidney function after an episode of AKI.
Table 2

Primary and secondary objectives

<table>
<thead>
<tr>
<th></th>
<th>All (n = 92)</th>
<th>Probiotics (n = 48)</th>
<th>Control (n = 44)</th>
<th>p</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Objective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney function recovery— n (%)*</td>
<td>40 (43)</td>
<td>24 (50)</td>
<td>21 (48)</td>
<td>0.82</td>
<td>1.04</td>
</tr>
<tr>
<td><strong>Secondary objective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead— n (%)</td>
<td>45 (49)</td>
<td>18 (37)</td>
<td>27 (61)</td>
<td>0.02</td>
<td>0.61</td>
</tr>
<tr>
<td>Kidney replacement therapy— n (%)</td>
<td>17 (19)</td>
<td>12 (26)</td>
<td>5 (12)</td>
<td>0.11</td>
<td>2.19</td>
</tr>
<tr>
<td>Urea, mg/dL—mean (RIQ)</td>
<td>108 (148–232)</td>
<td>80 (31–148)</td>
<td>109 (53–160)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>sCr—mean (IQR)</td>
<td>2.0 (0.8–2.8)</td>
<td>1.7 (0.7–3.2)</td>
<td>2.2 (1.2–2.7)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Urinary volume, ml/día—mean (IQR)</td>
<td>1200 (900–1725)</td>
<td>1125 (750–1400)</td>
<td>1750 (1204–2375)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L—mean (IQR)</td>
<td>4.0 (3.5–4.5)</td>
<td>4.0 (3.6-5.0)</td>
<td>4.0 (3.5–4.5)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Sodium, mEq/L—mean (SD)</td>
<td>136 ± 6.1</td>
<td>134 ± 5.9</td>
<td>137 ± 5.8</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Chloride, mEq/L—mean (SD)</td>
<td>102 ± 7.8</td>
<td>101 ± 7.6</td>
<td>104 ± 7.7</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>pH—media (SD)</td>
<td>7.38 ± 0.11</td>
<td>7.35 ± 0.13</td>
<td>7.40 ± 0.07</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate, mmol/L—mean (SD)</td>
<td>22.2 ± 4.7</td>
<td>21.6 ± 4.6</td>
<td>22.9 ± 4.9</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Follow-up, days—mean (IQR)</td>
<td>382 (193–967)</td>
<td>642 (227–986)</td>
<td>370 (150–822)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Last eGFR/ml/min 1.73m2—mean CKD-EPI (IQR)</td>
<td>39 (23–94)</td>
<td>46 (23–99)</td>
<td>33 (24–59)</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

Secondary outcomes

Results of the secondary outcomes are shown in Table 2 and Fig. 3. Among all patients, 45 (49%) died during the study, 18 (37%) in the probiotic group and 27 (61%) in the placebo group, favoring probiotics with a relative risk (RR) of 0.61 (95%CI 0.39–0.94. p = 0.02). Kaplan-Meier survival analysis at day 180, showed a Hazard Ratio (HR) of 0.56, (95%CI 0.32–1.04 p = 0.06) (Fig. 3A). Causes of death were similar between the groups, being sepsis the most common (39%), followed by cancer/neoplasia (13%), cardiorespiratory (11%), hematological (9%), gastrointestinal (9%) and neurological (6%).

A total of 17 (19%) patients required KRT duration of the study follow-up period, mostly due to uremia, volume overload, and electrolyte abnormalities, 12 (26%) in the probiotic group and 5 (12) in the placebo
group, with a RR of 2.19 (95% CI 0.84–5.72, p = 0.11), as shown in Table 2.

Additionally, urea (mg/dL) decreased significantly in the probiotic group from 154 (70–173) to 80 (31–148), which was not observed in the placebo group, where it only decreased from 130 (70–173) to 109 (53–160), (p = 0.09), confirming that the decrease was greater with probiotics (between subjects p-value = 0.04) (Table 2, Fig. 3B).

Urinary volume (ml/day) increased from 1,000 (500-1,500) to 1,100 (750-1,400) in the intervention group (p = 0.65), and from 1,095 (600-1,400) to 1,750 (1,200-2,300) in the placebo group (p = 0.05), with a significant difference between the groups (Table 2, Fig. 3C).

The patients were followed for a median of 382 days (193–967), and it was observed that their renal function deteriorated, expressed by an overall eGFR of 39 (23–94 ml/min/1.73 m²), eGFR was 46 (23–99 ml/min/1.73m²) in the probiotic group, and 33 (24–59 ml/min/1.73m²) in the control group, meeting criteria for CKD grade 3a and 3b, respectively, without a significant difference between groups (Table 2).

In an exploratory multiple regression analysis, including baseline sodium and potassium, heart rate, diastolic blood pressure and stages KDIGO 3 in the model and weighted by allocation group, the results for renal recovery and mortality remained non-significant, with p = 0.51 and 0.19, respectively.

**Additional outcomes of interest**

Potassium values decreased and chloride increased during the study in both groups but they did not change significantly when comparing interventions. Only sodium decreased in the probiotic group (134 ± 5.9) and it increased in the placebo group (137 ± 5.8) (p = 0.01). Calcium values remained stable during the intervention, with no differences between groups (Table 2, Supplemental Fig. 2). Acid-base status, assessed by serum pH and bicarbonate levels, improved with increasing pH and bicarbonate during the study, with no difference between groups (Table 2, Supplemental Fig. 2).

**Adverse events**

The pre-specified adverse events during the study period are presented in Table 3. A total number of 53 were documented, and gastrointestinal symptoms predominated. All were considered mild, and they were similar between both groups of the study; none warranted suspending the interventions. The probiotic group presented 34 adverse events; of these, 7 patients presented > 2 events, and 27 presented only one event. Abdominal distention was the most common with 8 reported cases, followed by nausea and diarrhea, 6 cases each. In the placebo group, 31 presented an adverse event, 6 patients had > 2, and the most common was diarrhea with 9 reported cases, abdominal distention with 7, and vomiting with 6 cases.
Table 3
Adverse events during the 7 days of study period

<table>
<thead>
<tr>
<th></th>
<th>Probiotics (34)</th>
<th>Placebo (31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Abdominal distention</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Nausea</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Rash</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>other</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Discussion

In this double-blind randomized clinical trial carried out in patients with AKI secondary to sepsis, we found for the first time that the administration of probiotics for 7 days, compared to placebo, did not improve KFR but it had a trend to decrease mortality rate, in addition to having an acceptable safety profile.

KFR was observed in half of the patients in this study during the 7-day period, similar to what was previously reported for patients with sepsis-induced AKI (35, 36). In this clinical scenario, it is important to implement measures focus in improving kidney recovery within 7 days, since if it is not done there is an increased risk of progression to AKD, which increases the risk of developing de novo CKD or CKD progression, increased the risk of cardiovascular complications and death (36). Recovery of kidney function has been an unresolved issue for many years, which has been recognized as a priority (37), and so far, there is no available treatment that has consistently achieved this objective. In this study, the lack of efficacy of probiotics in promoting KFR is difficult to contrast with other results since there are no other similar trials. However, previous experimental models have been encouraging. In mice induced to pyelonephritis with E. coli injection, it was shown that the administration of Lactobacillus acidophilus and Bifidobacterium before and after sepsis significantly improved renal function and attenuated inflammation and renal fibrosis (38). Similarly, administration of SCFA improved renal function after AKI, an effect mediated by the decrease in sCr and urea values, and it also improved the percentage of necrosis seen in kidney biopsies. These improvements were associated with an attenuation of inflammation and significantly lower levels of IL-1β, IL-6, TNFα and MCP-1 (39). The administration of Lactobacillus salivarius following cisplatin-induced AKI decreased the markers of inflammation and severity scores on kidney histology (40) and, interestingly, maintained intestinal wall permeability, suggesting that it would prevent bacterial translocation to the portal circulation and thereby modulate systemic inflammation (41). SCFA involvement has been implicated in AKI in humans as well; it was observed that after AKI, the levels of D-amino acids increase, especially D-serine, which are produced from SCFA, suggesting a physiological mechanism of protection against kidney insult (42).
We showed that the administration of probiotics show a trend to decreased in the mortality rat. AKI related to sepsis has a poor prognosis. A meta-analysis reported that 45% of affected patients die during their stay in intensive care units and up to 49% during hospitalization (43), so it is extremely important to try to reduce these numbers. Probiotics have been shown to decrease mortality in experimental models as well. In rats, induced abdominal sepsis by cecal ligation, it was shown that the administration of the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium longum* for 7 days decreased the risk of dying by 40% (44). The involvement of intestinal dysbiosis and colon-associated uremic toxin production in AKI patients was related to AKI severity and an increased likelihood of dying (11). Even in patients with hospital-acquired AKI, it has been seen that the highest concentrations of uremic toxins generated from intestinal dysbiosis, such as indoxyl sulfate, were associated with an almost 3-fold increase in the risk of dying (45), and their attenuation improved AKI, evaluated by the RIFLE classification (46). Our results might show that a significant difference in mortality might be observable with an increase in the number of cases and an extension of the observation period. In other words, it may be a hasty conclusion to conclude that the administration of probiotics is not effective at this point.

Considering other parameters that have been used to evaluate renal function in patients with AKI, such as the need for KRT and sCr concentrations, we did not observe a positive effect of the administration of probiotics. However, serum urea concentrations only improved significantly in the probiotic group, not in the placebo group, although no significant differences were found between them. This effect could be explained by the modulation of intestinal dysbiosis with probiotics and thus the attenuation of urea generation by intestinal bacteria (8), especially in the context of AKI associated with sepsis (47). Uremia and other colon-derived toxins have an impact on the KFR (46) and mortality (45). The decrease in urea in AKI has been the subject of debate for decades, but recent clinical trials have taken into account urea values > 240 mg/dL to decide when to start KRT in AKI patients (ELAIN and AKIKI2 trials) (48, 49), so its decrease could be relevant by delaying the start of KRT in a certain scenario.

The finding of increased urinary volume and serum sodium in the placebo group compared to probiotics could be explained by the excretion of free water and thus vascular decongestion. We believe this difference does not profoundly impact the clinical course of these patients since urinary volume and sodium remained within ranges considered safe (50, 51).

It is important to comment on the values of eGFR observed during the long-term follow-up of these patients (~1 year), which was 39 ml/min/1.73 m², with no differences between the study groups, which means that they would be classified as having CKD G3a, which implies a deterioration to almost half their baseline eGFR, which was ~74 ml/min/1.73 m². The devastating sequelae in renal function after an episodse of sepsis-induced AKI have been previously demonstrated and having one of the worst adverse long-term prognosis (52, 53, 54).

Considering the reported adverse events, we believe that the administration of probiotics to patients with sepsis-induced AKI was well tolerated and has an acceptable safety level. No adverse events were considered serious, and none of the patients stopped treatment due to any of adverse events reported.
For decades, different therapeutic agents have been investigated for the management of AKI associated with sepsis with disappointing results; examples such as statins (55), erythropoietin (56), steroids (57), alkaline phosphatase (58) and pirfenidone (17) are important justified efforts, and the search for a drug that consistently improves kidney function and potentially decreases the probability of dying continues.

**Limitations and strengths**

Our results must be interpreted with caution, as this was a single-center study without an a priori calculation of sample size due to the lack of literature to estimate an expected minimal clinically important difference between groups, so a type II error cannot be ruled out; for instance, according to the observed difference in the primary outcome between groups, the post hoc calculated power was 50% in our sample, maintaining an $\alpha$-error probability of 5%. There was also a lack of measurements of the intestinal microbiota in feces, as well as systemic inflammation parameters, and a lack of biomarkers of renal tubular damage that reflect the true kidney injury. All patients were receiving antibiotics, which may impact the probiotics administered.

The strengths of the study lie in its design and the adequate adherence of the allocation groups; to our knowledge, this is the first randomized control trial of AKI septic patients treated with probiotics (10, 34).

**Conclusion**

In AKI associated with sepsis, the administration of probiotics for 7 days was safe, and compared with placebo, it did not improve renal function but there was a trend to decreased mortality.

**Abbreviations**


**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Institutional Review Board HCG/CEI-1342/18, was registered with the Clinical Trials identifier NCT03877081, 03/15/2019

Informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Institutional Review Board "Comité de ética en Investigación del OPD Hospital Civil de Guadalajara Fray Antonio Alcalde".
Consent for Publication

Not applicable.

Availability of data and materials

Are available in the historical archive of the Hospital Civil Fray Antonio Alcalde.

If any information is requested, please contact Principal Investigator Dr. Jonathan Chávez-Iñiguez (jonarchi_10@hotmail.com).

Competing interest

Conflicts of interest/Competing interests: the authors declare no conflict of interest.

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Authors’ contributions

JSCI, MIE, AMG-G, ACH, RCD, GCA, were responsible for the design, analysis and interpretation of the data. EMHB, ACRM, FRA, MLPM, MPC, JATG, CPH, GNB, RMG, LAV, KRL and GGG were responsible for data collection. All authors have read and approved the manuscript.

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References


Figures
Figure 1

CONSORT Flow Diagram
Figure 2

Primary objective, kidney function recovery during the 7 days of the study trial.
Figure 3

Secondary outcomes, A) Survival, B) Serum urea, and C) Urinary output.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
- SupplementalFigure1.pdf
- SupplementalFigure2.pdf