ROZPRAWA DOKTORSKA

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Znaczenie bariery jelitowej w sepsie – ocena niedokrwienia jelit u pacjentów septycznych na podstawie wybranych biomarkerów.

Importance of intestinal barrier in sepsis – evaluation of intestinal ischemia in septic patients based on selected biomarkers.

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Pracę doktorską stanowi opublikowany cykl prac poświęcony analizie zmian stężeń biomarkerów uszkodzenia jelita u pacjentów przyjmowanych na Oddział Intensywnej Terapii z sepsą lub wstrząsem septycznym.

1. Tyszko M, Lipińska-Gediga M, Lemańska-Perek A, Kobylińska K, Gozdzik W, Adamik B. Intestinal Fatty Acid Binding Protein (I-FABP) as a Prognostic Marker in Critically Ill COVID-19 Patients. *Pathogens*. 2022; 11(12):1526. https://doi.org/10.3390/pathogens11121526

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2. Tyszko M, Lemańska-Perek A, Śmiechowicz J, Tomaszewska P, Biecek P, Gozdzik W, Adamik B. Citrulline, Intestinal Fatty Acid-Binding Protein and the Acute Gastrointestinal Injury Score as Predictors of Gastrointestinal Failure in Patients with Sepsis and Septic Shock. *Nutrients*. 2023; 15(9):2100. https://doi.org/10.3390/nu15092100

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Spis treści **strona**

Wykaz skrótów użytych w pracy:

AGI	Acute Gastrointestinal Injury
APACHE II	Acute physiology and Chronic Health Evaluation II
ARDS	Acute Respiratory Distress Syndrome
AUC	Are under the ROC curve
GI	Gastrointestinal
GIDS	Gastrointestinal Dysfunction Score
I-FABP	Intestinal fatty-acid binding protein
OIT	Oddział Intensywnej Terapii
ROC	Receiver operating characteristic
SAPS	Simplified Acute Physiology Score
SOFA	Sequential Organ Failure Assessment
USK	Uniwersytecki Szpital Kliniczny

1. WSTĘP

Sepsa jest definiowana jako zagrażająca życiu dysfunkcja wielonarządowa spowodowana nieprawidłową odpowiedzią organizmu na zakażenie.[1]Zaburzenia żołądkowo-jelitowe są stosunkowo częste u pacjentów septycznych i powiązane ze zwiększoną śmiertelnością. [2] Zmiany zachodzące w obrębie bariery jelitowej w trakcie sepsy są złożone i dotyczą między innymi zaburzonego przepływu trzewnego, hipoperfuzji, zwiększonej przepuszczalności ściany jelita, lokalnych zaburzeń ukrwienia tkanek w związku z uogólnioną koagulopatią oraz zmian w mikrobiomie jelitowym. [3–11] Innym wyjaśnieniem dużej częstości występowania zaburzeń żołądkowo-jelitowych u tych chorych może być upośledzona motoryka jelit spowodowana stosowaniem leków sedujących oraz przedłużoną wentylacją mechaniczną. [12] Zaburzenia żołądkowo-jelitowe w sepsie mogą dawać wielorakie objawy, takie jak niedożywienie, nietolerancja żywienia enteralnego, owrzodzenia i krwawienia, translokacja bakteryjna, a każdy z nich może przyczyniać się do pogorszenia niewydolności wielonarządowej i w efekcie do gorszych wyników leczenia sepsy i niepomyślnego rokowania. [13, 14]

Diagnostyka i monitorowanie funkcji układu pokarmowego na oddziale intensywnej terapii (OIT) pozostaje wyzwaniem dla klinicystów. Powszechnie stosuje się kilka skal służących do monitorowania stanu pacjentów przyjmowanych na OIT. Najczęściej stosowane są skale Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) i Simplified Acute Physiology Score (SAPS), niestety w żadnej z nich zaburzenia żołądkowo-jelitowe nie stanowią składowej oceny. Poszukiwania dobrego narzędzia do oceny zaburzeń układu pokarmowego u pacjentów OIT zaowocowały wprowadzeniem skali AGI (Acute Gastrointestinal Injury) oraz niedawno zaproponowanym I wciąż walidowanym GIDS (Gastrointestinal dysfunction score). Ich zadaniem jest określanie stopnia ciężkości zaburzeń żołądkowo – jelitowych u krytycznie chorych pacjentów. [2, 5, 15]

W związku z tym powstaje pytanie, czy pomiar biomarkerów może być przydatny do oceny uszkodzenia bariery jelitowej u pacjentów z sepsą. **Cytrulina, potencjalny wskaźnik do oceny funkcji układu pokarmowego u pacjentów OIT** jest aminokwasem głównie produkowanym u ludzi przez enterocyty w jelicie cienkim i następnie wydzielanym do krwiobiegu. Dlatego poziom stężenia cytruliny we krwi pacjentów może odzwierciedlać funkcjonalną masę enterocytów - jelitowych komórek nabłonka. [16] Wykonane do tej pory badania wykazały, że u krytycznie chorych pacjentów poziom cytruliny był zazwyczaj poniżej normy (30 do 50 mmol/mL); co więcej, niski poziom cytruliny w tej populacji korelował z objawami klinicznymi dysfunkcji żołądkowo – jelitowej, jak również ze zwiększoną śmiertelnością. [17–

[20] Większość badan oceniających przydatność cytruliny jako biomarkera funkcji komórek jelita było przeprowadzonych na populacji ogólnej pacjentów OIT, nie wykonywano jednak do tej pory dokładnych badań z uwzględniających specyfikę grupy pacjentów z sepsą/wstrząsem septycznym, szczególnie z sepsą o etiologii wirusowej. [19, 21]

Drugim białkiem poddanym analizie było I-FABP (Intestinal fatty acid binding protein), białko wiążące kwasy tłuszczowe specyficzne dla jelita. Jest to cytozolowe białko, znajdujące się w enterocytach jelita cienkiego i grubego. Jest uwalniane podczas uszkodzenia jelita, zatem może być uznane za marker funkcji enterocytów oraz uszkodzenia śluzówki jelita [22]. Wyniki badań oceniających przydatność I-FABP i cytruliny jako markerów prognostycznych w sepsie są niejednoznaczne [23–25].

Wyznaczenie odpowiednich markerów do prognozowania przebiegu choroby u pacjentów z sepsą i wstrząsem septycznym dałoby narzędzie, z którego można korzystać w codziennej praktyce OIT. Wczesne identyfikowanie pacjentów o wysokim ryzyku rozwinięcia niewydolności żołądkowo-jelitowej pozwoliłoby na odpowiednią modyfikację leczenia żywieniowego, zmianę farmakoterapii lub interwencję chirurgiczną wcześniej, niż widoczne będą jednoznaczne objawy. Dodatkowo biomarkery służyć mogą do monitorowania stanu pacjenta podczas pobytu na OIT oraz do przewidywania wyników leczenia.

2. CELE PRACY

Celem przedstawionego cyklu prac była:

1. Analiza zmian stężeń wybranych biomarkerów uszkodzenia bariery jelitowej w grupie pacjentów septycznych leczonych na oddziale intensywnej terapii.
2. Zbadanie zależności między oznaczonymi stężeniami biomarkerów, a ciężkością stanu klinicznego pacjenta ocenianego za pomocą skal klinicznych, w tym skali AGI (Acute Gastrointestinal Injury).
3. Ocena przydatności oznaczonych biomarkerów uszkodzenia bariery jelitowej do przewidywania ryzyka zgonu w analizowanej kohorcie.

3. PUBLIKACJE STANOWIĄCE PRACĘ DOKTORSKĄ

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Article

Intestinal Fatty Acid Binding Protein (I-FABP) as a Prognostic Marker in Critically Ill COVID-19 Patients

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Abstract: Gastrointestinal symptoms are common in critically ill COVID-19 patients. There is currently no generally recognized method of assessing gastrointestinal injury in unconscious or sedated intensive care unit (ICU) patients. I-FABP (intestinal fatty acid binding protein) and citrulline have previously been studied as potential biomarkers of enterocyte damage in various gastrointestinal tract diseases, and changes in the levels of these markers may reflect intestinal wall damage in COVID-19. Patients with critical COVID-19, with diagnosed sepsis, or septic shock requiring ICU treatment were included in the study. Blood samples for citrulline and I-FABP were taken daily from day 1 to 5. I-FABP levels were significantly higher in patients who eventually died from COVID-19 than in survivors, and the optimal I-FABP cut-off point for predicting 28-day mortality was 668.57 pg/mL (sensitivity 0.739, specificity 0.765). Plasma levels of I-FABP, but not citrulline, were associated with significantly higher mortality and appeared to be a predictor of poor outcome in multivariate logistic regression analysis. In conclusion, I-FABP seems to be an effective prognostic marker in critically ill COVID-19 patients. Assessing mortality risk based on intestinal markers may be helpful in making clinical decisions regarding the management of intestinal injury, imaging diagnostics, and potential surgical interventions.

Keywords: COVID-19; I-FABP; citrulline; biomarker; intestinal injury; sepsis; intensive care

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1. Introduction

The severe acute respiratory syndrome caused by the severe acute respiratory coronavirus 2 (SARS-CoV-2) virus was a serious challenge for healthcare systems during the COVID-19 pandemic, especially for already overcrowded intensive care units (ICU). In some patients hospitalized due to a COVID-19 infection, rapid deterioration of the respiratory system function and the development of sepsis were observed, both conditions requiring immediate ICU treatment [1,2]. The current definition defines sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection [2]. Sepsis can be the final pathway of infection caused by a variety of pathogens, including bacteria, fungi, viruses, and parasites. According to the World Health Organization, viral pathogens such as seasonal influenza viruses, dengue viruses, and highly contagious pathogens of public health concern such as avian and swine flu viruses, Middle East respiratory syndrome coronavirus, Ebola, yellow fever viruses, and, most recently, severe severe acute respiratory coronavirus 2 can lead to the development of sepsis [3]. Prior to the COVID-19 pandemic, viral sepsis was rare among ICU patients with occurrence rates ranging from 1.0% to 6.3% in different regions [4–6]. Unfortunately, sepsis of viral origin has become a daily survival struggle in the ICU, as COVID-19 can develop from a mild infection to septic shock within hours. The results of a recent meta-analysis showed that

the prevalence of sepsis associated with the coronavirus disease in the ICU was 77.9% [7]. The lungs are the main target for the SARS-CoV-2 virus, and respiratory failure remains the most important problem on ICU admission; however, later some patients develop failure of other vital organs leading to the development of multiple organ failure and often death [8–11].

Activation of the systemic inflammatory reaction caused by severe lung infection can lead to a disruption of the integrity of the intestinal barrier (the gut–lung axis,) increasing its permeability to intestinal microorganisms and endotoxins. It was shown by Dickson et al., some intestinal bacteria (e.g., *E. faecalis*) can be isolated from post-sepsis lungs, which seems to prove the role of intestinal translocation in the development of ARDS and sepsis [12,13]. Gastrointestinal symptoms may occur with respiratory symptoms of COVID-19 [14]. In a study by Kang et al. nearly half of COVID-19 patients were shown to have severe gastrointestinal problems in addition to respiratory [15]. In a population of COVID-19 patients treated at the ICU, a common problem was dysfunction of the gastrointestinal tract and feeding intolerance (56%) manifested by large gastric residual volume, abdominal distension, and vomiting [16]. In addition, gastrointestinal problems in these patients were associated with poor treatment outcomes, including development of cardiac, renal, hepatic, and hematologic complications, longer hospital stay, and an increased risk of death. The mechanisms contributing to the deterioration of the intestinal barrier in the course of COVID-19 are not fully understood and have been the subject of many studies. The SARS-CoV-2 virus can directly infect the epithelial cells of the intestinal tract, causing changes in tissue structure and breakdown of the epithelial barrier [17] It was previously confirmed that the metallopeptidase, angiotensin-converting enzyme 2 (ACE2) is a cell receptor for SARS-CoV viruses including SARS-CoV-2 [18,19]. The ACE 2 receptor shows a high level of expression in the gastrointestinal system and it has been shown that receptor ACE2 and the SARS-CoV-2 viral protein nucleocapsid stained positive mainly in the cytoplasm of gastrointestinal epithelial [20,21].

That is why it is so important to monitor the function of the gastrointestinal tract in ICU patients. Monitoring methods are limited and there is currently no generally recognized method of assessing gastrointestinal injury in unconscious or sedated ICU patients [22]. Clinical evaluation (e.g., an assessment of the presence of abdominal pain, distention, bowel sounds, diarrhea, bleeding) is commonly carried out but does not provide complete information on intestinal function. There is currently no validated score to assess gastrointestinal dysfunction in ICU patients, but two scores, the AGI (Acute Gastrointestinal Injury) score and the GIDS (Gastrointestinal Dysfunction) score, have been developed and await validation [23–25].The AGI score, first introduced a few years ago, aims to assess gastrointestinal malfunction in ICU patients based on gastrointestinal symptoms and their severity. However, it is a subjective descriptive assessment, and it may not be clear to the attending physician in the ICU how to grade an individual patient; for example, a patient with a rapidly deteriorating clinical condition may have a higher AGI score, despite having the gastrointestinal symptoms of a less severely ill patient. Therefore, a modified version of the AGI score, the GIDS score, has been recently proposed [24]. The clinical utility of this tool must be validated prospectively in future studies before it can be recommended for clinical use in ICU patients [23,24].

In addition to clinical evaluation, numerous endogenous proteins have been proposed as biomarkers of intestinal injury, but so far none have been routinely used in ICU patients. In this study, citrulline and I-FABP (intestinal fatty acid binding protein) were of interest as potential prognostic factors for treatment outcomes in ICU patients diagnosed with a COVID-19 infection. I-FABP is a cytosolic protein which plays a key role in the cellular uptake and metabolism of fatty acids in enterocytes and is released from cells in cases of injury [26]. Under physiological conditions, only small amounts of I-FABP are found in the bloodstream (57–310 pg/mL); therefore, an elevated level of circulating I-FABP may reflect intestinal wall damage [27]. The results of a previously published meta-analysis indicate that serum I-FABP measurements may be useful in the diagnosis of acute intestinal

ischemia, with an AUC of 0.86, a sensitivity of 0.80, and a specificity of 0.85 [28]. I-FABP measurements can be especially useful in emergency situations as a quick and less invasive assessment; however, biomarker results should always be evaluated in parallel with histopathological and clinical findings. The diagnostic and prognostic potential of early necrotizing enterocolitis (NEC) markers, including markers of intestinal epithelial damage (intestinal fatty acid binding protein and liver fatty acid binding protein) and markers of excessive inflammatory response such as serum amyloid A, has been confirmed in neonates with suspected NEC [29]. Both epithelial damage and inflammation markers were significantly higher in those infants who later developed NEC, as early as 6 h after suspected NEC, suggesting that intestinal mucosal damage and a strong inflammatory response are detectable even before the onset of NEC symptoms. In the onset of heart failure, low cardiac output, splanchnic circulation congestion, and reduced intestinal blood perfusion can lead to disruption of the mucosal barrier and increased intestinal permeability [30]. In patients with acute heart failure, elevated I-FABP was associated with significantly worse clinical outcomes, including increased incidence of death, left ventricular assist device placement, or heart transplantation [31]. In another study, concomitant increases in serum I-FABP and endotoxin levels were observed in patients undergoing cardiac surgery with cardiopulmonary bypass, implying ischemia-reperfusion injury of the intestinal mucosa; the magnitude of the changes in the intestines depended on the duration of the cardiac bypass time [32].

The second protein of interest to us as a potential biomarker of intestinal damage was citrulline. It is an amino acid produced almost entirely by enterocytes from glutamine; therefore, its concentration may reflect the function and mass of the epithelial cells in the lining of the small intestine [33]. The importance of citrulline as a marker of intestinal wall damage was previously demonstrated in animal models, including models of sepsis, viral enteritis, and intestinal damage from chemotherapy [34–36]. In a rat model of sepsis, blood levels of citrulline were significantly reduced, with clear damage to the intestinal mucosa confirmed by histology [36].

Only few studies have focused on the usefulness of measuring intestinal injury biomarkers in intensive care patients, and the role of I-FABP and citrulline as potential biomarkers of intestinal cell damage in the most severe COVID-19 infections remains to be established [37–40].

In this study, we analyzed changes in the level of biomarkers of gastrointestinal damage in COVID-19 patients with the most severe symptoms who required immediate ICU treatment. We aimed to define a model utilizing the new biomarkers along with routinely monitored clinical and laboratory parameters to predict the risk of mortality in the analyzed cohort. Being able to detect an intestinal injury early can facilitate diagnosis, treatment, and decision making, particularly in deciding to initiate enteral nutrition in ICU patients.

2. Materials and Methods

2.1. Patients

We conducted a single-center prospective observational study on consecutive adult patients with SARS-CoV 2-induced sepsis or septic shock admitted to the ICU of the University Hospital between January 2020 and January 2021. The inclusion criteria were as follows: (1) a SARS-CoV2 infection confirmed by a positive result of real-time reverse-transcriptase polymerase chain reaction assay of nasal or pharyngeal swab probes prior to ICU admission, (2) critical COVID-19 according to WHO interim guidance [41], and (3) a diagnosis of sepsis or septic shock as defined by the SEPSIS-3 definition on admission to the ICU [2]. The exclusion criteria were as follows: (1) age < 18 years old and (2) a history of previous abdominal surgery or previous severe gastrointestinal disabilities (chronic inflammatory diseases, such as *ulcerative colitis*, Lesniowski-Crohn disease, viral hepatitis). Severe gastrointestinal inflammatory diseases may alter the levels of intestinal biomarkers [42,43]. The study protocol was accepted by the local Bioethical Committee

(No. KB—822/2018). Written informed consent was obtained from the patient or a legally authorized representative. The study protocol complies with the 1975 Declaration of Helsinki as revised in 1983 [44].

All patients were treated according to the standard ICU procedures as described in the Surviving Sepsis Campaign guidelines [22,45]. Patients were transferred to the ICU from the emergency room or another hospital ward immediately after intubation. ARDS from SARS-CoV 2 infection was treated according to standard practice and an ECMO evaluation was made in the event of a rapid deterioration in respiratory function unresponsive to treatment. Empirical antibiotics were administered immediately after ICU admission and blood cultures and other relevant samples were taken regularly to detect any co-infections. Renal replacement therapy was implemented as needed.

2.2. Data Collection

Clinical data from each patient were collected daily from day 1 to 5. The clinical status of the patients was determined with the Acute Physiology and Chronic Health Evaluation (APACHE) II score on admission to the ICU and the Sequential Organ Failure Assessment (SOFA) score was used for daily monitoring of organ function. Both scores are standard prediction tools used at the ICU for septic patients. The APACHE II scale is based on the values of clinical and laboratory parameters (inhaled oxygen fraction, oxygen partial pressure, body temperature, mean arterial pressure, blood pH, heart rate, respiratory rate, serum sodium, serum potassium, serum creatinine, hematocrit, white blood cells, and Glasgow score), disease-related variables (history of severe organ failure or immunodeficiency), and it takes into account the type of ICU admission (elective/emergency). The SOFA scale enables the monitoring of the patient's clinical condition on the basis of indicators of the functioning of the following systems: respiratory (PaO_2/FiO_2 ratio), cardiovascular (mean arterial pressure and the dose of vasopressors), hepatic (bilirubin concentration in the blood), coagulation (platelet count), renal (creatinine concentration in the blood/urine output), and neurological (Glasgow coma scale). The prevalence of organ dysfunction in patients with COVID-19 on admission to the ICU was calculated based on the SOFA scores. Additionally, the gastrointestinal injury (AGI) score and feeding intolerance described as high gastric residual volumes (GRV > 500 mL) were evaluated daily. All laboratory parameters were measured at the certified hospital laboratory. Mortality at 28 days was recorded. Data were recorded on the length of stay in the ICU and in the hospital.

2.3. Biomarker Analysis

Blood samples for determining plasma citrulline and I-FABP (intestinal fatty acid binding protein) were collected (2.7 mL, tubes containing 0.109 M sodium citrate as an anticoagulant, BD Vacutainer, BD, NJ) via a routinely inserted arterial cannula on ICU admission and on days 2, 3, 4, and 5. Plasma was separated immediately after centrifugation at 2000× *g* for 10 min and stored at –70 °C. I-FABP protein was analyzed using a commercially available ELISA kit (Quantikine ELISA Human FABP2/I-FABP Immunoassay, R&D Systems, Minneapolis, MN, USA). Citrulline was analyzed using a commercial ELISA kit (Human citrulline ELISA Kit, Cusabio Biotech Co., LTD, Houston, TX, USA).

2.4. Statistical Analysis

We used Statistica 13 software (StatSoft, Inc., Tulsa, OK, USA) and the R 3.6.01: R Core Team (2013) to perform the analysis. Continuous variables are reported as mean values ± standard error and minimum–maximum and categorical as frequencies with percentages. The distribution was not normal based on the Shapiro–Wilk test and the analysis was performed with nonparametric tests. The Mann–Whitney U test was used to compare continuous variables between study groups at each time point. Categorical variables were analyzed using the Chi-square test or Fisher exact test. A comparison of the predictive accuracy of the biomarkers measured on admission to the ICU was made using receiver operating characteristic curve (ROC) analysis, by calculating the area under

the curve (AUC), including 95% confidence intervals (CI), to determine sensitivity and specificity. Youden's statistic was used to select the optimum cut-off point. Survival analysis of time to death was performed using the Kaplan–Meier curve and a Chi-square test. Univariate and multivariate logistic regression analysis was performed to evaluate the association between baseline values of the studied biomarkers of gastrointestinal damage, other parameters and covariates, and ICU outcome. Potential 28-day mortality prognostic variables were first selected based on their ease of measurement on ICU admission or their previously demonstrated role as a predictor of mortality. The results were reported as an odds ratio (OD) and 95% confidence intervals (CI). The first prepared model included biomarkers and all selected variables. The collinearity of the variables was tested and collinear features were excluded from the analysis. The choice of the best model was proposed based on the Akaike information criterion and the backward selection of the model. All the tests were conducted with a 5% significance level.

3. Results

3.1. Study Population

During the 12-month study period, 69 consecutive COVID-19 patients admitted to the intensive care unit were screened for inclusion criteria; of these, 42 met the criteria and 27 were excluded due to prior abdominal surgery or chronic intestinal disease. Out of 42 patients, in 2 the level of I-FABP and citrulline was not measurable due to high hemolysis of the sample and ultimately 40 patients were included in the analysis. The study group consisted of 15 adult females and 25 adult males; the mean age of the patients was 60.12 $\pm$ 15.5 years (28–90). Most of the patients (72%) were admitted to the ICU directly from the Emergency Department, and 28% were transferred from another hospital ward. The SARS-CoV2 virus was detected in all patients prior to admission to the ICU, and the diagnosis was confirmed in all patients upon admission to the ICU. All patients had severe respiratory failure secondary to COVID-19; ARDS was diagnosed in 85% of patients and pneumonia in 15%. Septic shock was the second-most-common dysfunction (40%) diagnosed on ICU admission, renal dysfunction requiring renal replacement therapy in 25% of patients, liver dysfunction in 13%, coagulopathy in 15%, and CNS dysfunction in 25%. The APACHE II score used to characterize patient morbidity on admission to the ICU was 17.2 $\pm$ 1.0 pts. (6.0–33.0), and the SOFA score calculated to assess the clinical status of patients was 8.0 $\pm$ 0.4 pts (3.0–13.0). The mean length of ICU stay was 19 days (2–106) and the 28-day mortality rate was 57%. The baseline characteristics of the study group divided into Nonsurvivors and Survivors based on 28-day mortality are presented in Table 1.

At ICU admission, all patients in our study required immediate support of the respiratory system, including 29 patients (73%) who were treated with mechanical ventilation and 11 (27%) with mechanical ventilation and V-V ECMO. Ten patients (25%) had renal replacement therapy administered at ICU admission. According to the Sepsis-3 criteria, sepsis on admission to the ICU was diagnosed in all patients, and 40% of patients presented with septic shock. The results of laboratory tests on admission to the ICU are presented in Table 2. Higher creatinine and lactate levels were found in Nonsurvivors compared with Survivors and other parameters were similar in both groups.

3.2. Acute Gastointestinal Injury Score and Feeding Intolerance

The Acute Gastrointestinal Injury (AGI) score was used to monitor gastrointestinal dysfunction in ICU patients. On ICU admission, the overall incidence of AGI grade I/II was 100%. On subsequent days, most patients (>90%) had an AGI grade I/II and less than 10% had AGI III/IV. The incidence of feeding intolerance (gastric residual volumes > 500 mL) was 8% on ICU admission and increased on subsequent days. Differences in the distribution of the AGI scores and feeding intolerance rates between Survivors and Nonsurvivors were not significant ($p < 0.05$). The distribution of AGI scores and the incidence of food intolerances assessed on days 1–5 are presented in Table 3.

Table 1. Characteristics of the study population.

Parameter	Nonsurvivors, N = 23	Survivors, N = 17	*p*
Age (years)	65 ± 3 (39–90)	53 ± 3 (28–75)	0.025
Sex, male N(%)	16 (69)	9 (53)	0.283
APACHE II score	19 ± 1 (9–33)	14 ± 1 (6–24)	0.018
SOFA score	9 ± 1 (3–13)	7 ± 1 (4–12)	0.126
Comorbidities *n* (%)			
Hypertension	3 (13)	6 (35)	0.100
Coronary heart disease	6 (26)	2 (12)	0.239
Chronic kidney disease	6 (26)	0	0.026
Diabetes	3 (13)	2 (12)	0.645
Obesity	1 (4)	3 (17)	0.197
COPD	1 (4)	0	0.575
BMI	31 ± 2 (20–42)	29 ± 1 (25–37)	0.395
Length of stay, ICU (day)	13 ± 2 (3–44)	27 ± 6 (2–106)	0.049
Length of stay, hospital (day)	16 ± 2 (3–47)	29 ± 4 (10–61)	0.004

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; COPD, Chronic Obstructive Pulmonary Disease; BMI, Body Mass Index; ICU, Intensive Care Unit. Mean ± SE (min-max) and percentage of the total.

Table 2. Laboratory findings and treatments at ICU admission.

Parameter	Nonsurvivors, N = 23	Survivors, N = 17	*p*
PCT (ng/mL)	1.05 ± 0.24 (0.03–3.89)	0.43 ± 0.11 (0.05–1.58)	0.156
CRP (mg/L)	159 ± 24 (17–412)	174 ± 23 (11–342)	0.603
WBC (10^3/uL)	13.2 ± 1.0 (7.1–24.0)	14.6 ± 1.8 (5.7–34.5)	0.681
Platelets (10^3/uL)	237 ± 21 (60–505)	293 ± 35 (111–553)	0.373
D-dimer (mg/L)	15.27 ± 4.97 (0.54–87.15)	14.33 ± 6.7 (0.47–98.12)	0.198
Fibrinogen (g/L)	5.46 ± 0.46 (2.23–9.04)	6.03 ± 0.5 (2.54–10)	0.493
Creatinine (mg/dL)	1.29 ± 0.13 (0.47–2.66)	0.86 ± 0.10 (0.46–1.87)	0.015
PaO_2/FiO_2	150 ± 12 (52–282)	145 ± 14 (61–252)	0.902
Bilirubin (mg/dL)	0.9 ± 0.1 (0.2–3.9)	1.0 ± 0.2 (0.4–2.8)	0.758
Ferritine (ng/mL)	1368 ± 392 (443–3474)	714 ± 237 (71–1725)	0.201
Lactate (mmol/L)	2.21 ± 0.21 (0.70–5.20)	1.51 ± 0.15 (0.70–0.15)	0.024

PCT, Procalcitonin; CRP, C-Reactive Protein; WBC, White Blood Cell; PaO_2/FiO_2, arterial oxygen partial pressure (PaO_2 in mmHg) to fractional inspired oxygen (fraction). Data are presented as Mean ± SE (min-max).

Table 3. Incidence of acute gastrointestinal injury based on the AGI score (AGI grade I/II and AGI grade III/IV) and incidence of feeding intolerance assessed on days 1–5.

Parameter	Day 1	Day 2	Day 3	Day 4	Day 5
AGI score (%)					
grade I/II	100	93	90	97	92
grade III/IV	0	7	10	3	8
Feeding intolerance (%)	8	10	10	19	9

3.3. Differences in I-FABPand Citrulline Levels between Survivors and Nonsurvivors

Blood samples taken every morning from the first to the fifth day of ICU treatment were used to analyze changes in I-FABP and citrulline levels in the course of the COVID-19 infection. I-FABP levels were higher in Nonsurvivors (1217.6 ± 282.1, 980.5 ± 155.3, 1350.8 ± 275.2, 1008.7 ± 172.2, 1198.1 ± 273 pg/mL, respectively on days 1–5) than in Survivors (560.0 ± 86.3, 465.2 ± 93.5, 550.1 ± 114.3, 88.7 ± 207.7, 860.0 ± 209.1 pg/mL, respectively on days 1–5), with statistically significant differences on days 1–3 and insignificant differences on days 4–5 (Figure 1, left panel). This pattern was not observed with citrulline, where similar levels were noted in Nonsurvivors (24.9 ± 2.6, 23.0 ± 2.79, 22.7 ± 2.5, 22.2 ± 2.8, 22.2 ± 2.5 nmol/mL, respectively on days 1–5), and Survivors (25.0 ± 3.5, 25.4 ± 3.9, 22.1 ± 3.9, 22.6 ± 4.3, 23.5 ± 4.7 nmol/mL, respectively on days 1–5) throughout the observation period (Figure 1, right panel).

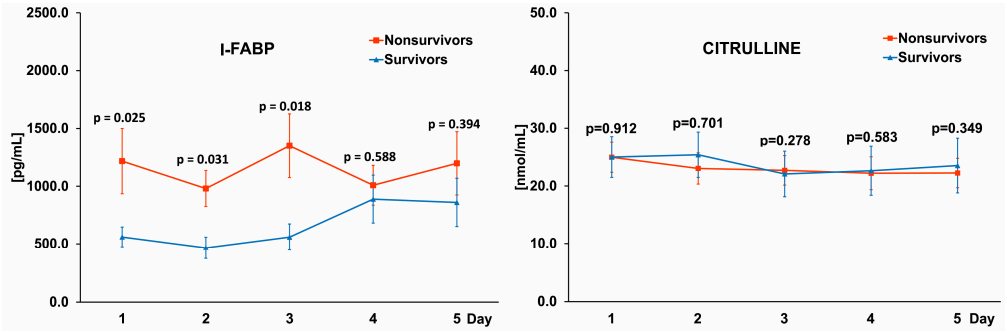

Figure 1. Graphs comparing the levels of I-FABP (intestinal fatty acid binding protein) (**left** panel) and citrulline (**right** panel) in the blood of Nonsurvivors and Survivors. The differences in the levels of biomarkers between study groups on corresponding days were marked with a *p*-value.

3.4. Prediction of Mortality

In the ROC curve analysis, the baseline I-FABP showed an ability to predict 28-day mortality with an AUC of 0.710 (95% CI 0.547–0.873, $p = 0.011$). The optimal threshold value for the baseline I-FABP was 668.57 pg/mL (sensitivity 0.739, 95% CI 0.376–0.740; specificity 0.765, 95% CI 0.274–0.863). The calculated threshold was used for the Kaplan–Meier analysis, which confirmed a significantly lower 28-day survival in the group with a baseline I-FABP above 668.57 pg/mL (log-rank test $p = 0.007$) (Figure 2). The ROC curve of the baseline citrulline concentration did not discriminate between Survivors and Nonsurvivors ($p > 0.05$).

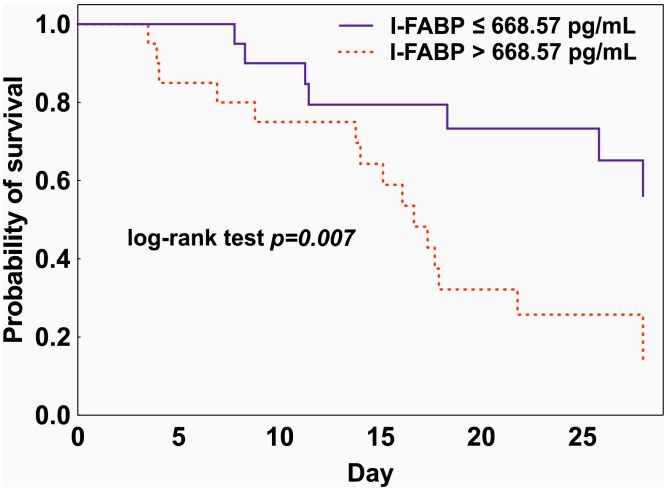

Figure 2. Kaplan–Meier curve showing 28-day survival stratified for patients with a baseline level of I-FABP ≤ 668.57 pg/mL (blue line) and >668.57 pg/mL (red line).

In addition, a univariate and multivariate logistic regression analysis was performed to create a model predicting 28-day mortality in the analyzed cohort. The choice of the variables from the set of biomarkers measured on admission to the ICU (I-FABP, citrulline, WBC, procalcitonin, CRP, creatinine, and lactate), and covariates (age, sex, APACHEII and SOFA scores, shock on admission) was determined by the minimizing of the Akaike information criterion and the backward selection of the model. Collinear covariates were excluded from the analysis. Therefore, the only variables that were included in the final model were the initial APACHEII, I-FABP, PCT, creatinine and the presence of septic shock on admission to the ICU. An initial high level of I-FABP (>668.57 pg/mL), a high APACHEII score, and the presence of shock were significant predictors of bad outcome. The initial PCT and creatinine had no statistical significance in the model. The results of the univariate and multivariate logistic regression analysis are presented in Table 4.

Table 4. Univariate and multivariate logistic regression analysis of the predictors of 28-day mortality.

	Univariate Analysis		Multivariate Analysis	
	Odds Ratio	***p***	**Odds Ratio**	***p***
APACHE II	1.165730×10^{0}	0.018	1.583274×10^{0}	0.018
shock	1.166667×10^{1}	0.004	8.958130×10^{1}	0.020
I-FABP	7.428571×10^{0}	0.005	5.114207×10^{1}	0.030
age	1.062695×10^{0}	0.018		
sex	4.921875×10^{-1}	0.286		
BMI	1.066471×10^{0}	0.422		
SOFA	1.234788×10^{0}	0.125		
PCT	2.708447×10^{0}	0.006	3.078094×10^{1}	0.187
CRP	9.986929×10^{-1}	0.669		
WBC	1.002122×10^{0}	0.971		
Creatinine	5.259518×10^{0}	0.024	2.216123×10^{2}	0.685
Citrulline	9.998672×10^{-1}	0.995		
Lactate	2.901363×10^{0}	0.057		
PaO_2/FiO_2	1.001565×10^{0}	0.078		

APACHE II, Acute Physiology and Chronic Health Evaluation II; I-FABP, Intestinal Fatty Acid Binding Protein; PCT, Procalcitonin; CRP, C-Reactive Protein; WBC, White Blood Cell; PaO_2/FiO_2, arterial oxygen partial pressure (PaO_2 in mmHg) to fractional inspired oxygen (fraction).

3.5. Differences in I-FABP Levels between Patients with Sepsis and Shock

Impaired blood flow in the gastrointestinal mucosa in septic shock can lead to damage to intestinal cells and the release of cytosolic proteins into the bloodstream. All the studied patients had sepsis on admission to the ICU and 40% were diagnosed with shock. Moreover, a diagnosis of shock was a significant predictor of mortality in a multivariate logistic regression analysis with an odds ratio of 89.58. We tested whether the concentrations of I-FABP and citrulline were different between COVID-19 patients with and without shock. Indeed, I-FABP levels were higher in the group of patients presenting with shock compared with the non-shock patients throughout the study period and citrulline levels were similar (Figure 3).

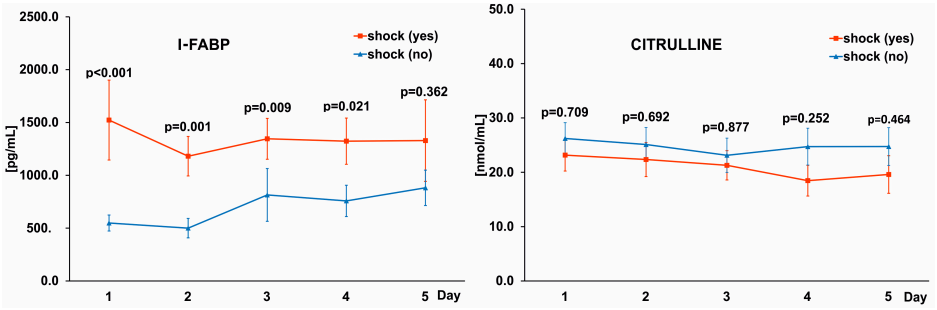

Figure 3. Graphs comparing the levels of I-FABP (**left** panel) and citrulline (**right** panel) in the blood of patients with and without septic shock. The differences in the levels of biomarkers between study groups on corresponding days were marked with a *p*-value.

4. Discussion

Previously, it was shown that the SARS-CoV-2 virus can directly infect intestinal cells through the angiotensin-converting enzyme 2 to gain access to enterocytes [17]. As a consequence, some COVID-19 patients may have mild to severe symptoms of intestinal dysfunction. We focused on two issues: (1) monitoring intestinal cell damage with specific biomarkers I-FABP and citrulline, and (2) the relationship between the level of these biomarkers and mortality. The study included patients with critical COVID-19 with the most severe symptoms requiring ICU treatment. All patients required mechanical ventilation; ARDS was diagnosed in 85% of patients and pneumonia in 15% on admission to the ICU. Our results showed that damage to the intestinal cells is a potential factor that could contribute to the outcome of treatment in COVID-19 patients. Plasma levels of I-FABP, but not citrulline, were associated with significantly higher mortality and appeared to be a predictor of poor outcome in multivariate logistic regression analysis. I-FABP levels were significantly higher in patients who eventually died from COVID-19 than in survivors, and the optimal I-FABP cut-off point for predicting death was 668.57 pg/mL.

SARS-CoV-2 RNA mainly infects lung cells; however, fecal swabs testing positive for SARS-CoV-2 RNA occurred in 30–50% of patients [46]. The virus can cause intestinal inflammation and changes the permeability of the intestinal wall, making absorption by enterocytes difficult [47]. The results of gastrointestinal imaging in COVID-19 infections showed changes of varying severity—thickening of the intestinal wall, possible hyperemia and mesenteric thickening, and intestinal ischemia [48]. There are several methods for assessing intestinal permeability. Oral administration of molecules labeled with radioisotopes or indigestible sugars such as lactulose and mannitol have been used to determine intestinal absorption. In some studies, gastric tonometry has been used to measure gastric intramucosal pH and gastric partial pressure of carbon [49]. There is currently no standard procedure for assessing intestinal permeability in patients treated in the intensive care unit. Most of them are unconscious or sedated, and kidney failure is common; therefore, methods for determining the urinary excretion of orally administered tracer molecules cannot be used.

Various endogenous proteins have been proposed as biomarkers of intestinal permeability, but it has not yet been established which indicators are most reliable in clinical practice. I-FABP and citrulline have previously been studied as potential biomarkers in various gastrointestinal tract diseases. In Crohn's disease, serum levels of I-FABP in patients with active disease were significantly higher than in patients in remission and in the control group, indicating the potential utility of I-FABP as a marker of disease severity [50]. Elevated levels of I-FABP associated with villous atrophy were recorded in patients with untreated coeliac disease and a significant improvement of enterocyte function was seen in a majority of patients several months after the initiation of appropriate nutritional treatment [51]. The usefulness of I-FABP as an early biomarker in detecting intestinal damage has been assessed in patients with multiple trauma, where delayed diagnosis of intestinal injury might increase the risk of complications, including sepsis [27]. I-FABP levels were significantly higher in trauma patients with intestinal injury compared with the non-injured group (2101.0 pg/mL vs. 351.4 pg/mL, $p < 0.05$), indicating that I-FABP could be used as an early biomarker for the detection of intestinal injuries. Citrulline, another potential marker of the function of small intestinal epithelial cells, is produced almost entirely by enterocytes from glutamine. According to the previously published report, healthy subjects have plasma citrulline concentrations between 20 and 60 mmol/L, with a mean of 40 mmol/L [52]. The results of a meta-analysis indicate that low citrulline levels can be a diagnostic indicator of acute and chronic intestinal failure, reflecting a reduced enterocyte mass and, to a lesser extent, impaired intestinal absorption [53]. In a pediatric population, citrulline was established as an objective, easily measurable indicator for mucosal barrier injury in patients with chemotherapy induced mucosal barrier injury [54]. In our study, the mean citrulline concentration measured at ICU admission was lower than previously reported values in healthy subjects (25.0 nmol/mL vs. 40 nmol/mL,

respectively), indicating enterocyte damage [52]. The lower citrulline values observed in patients with sepsis in our study are consistent with previous reports. Ware and colleagues showed that citrulline levels decreased in sepsis patients, especially those who developed acute respiratory distress syndrome [55]. In another study, similarly low levels of citrulline were reported in the general population of ICU patients; interestingly, using a threshold of 20 nmol/mL (which is the lower level of the normal range for plasma citrulline concentration), citrulline levels were not associated with 28-day mortality [38]. In our study, citrulline was not statistically significant in predicting 28-day mortality and was not included in the multivariate regression model. This can be explained by the fact that COVID-19 patients admitted to the ICU had lower levels of citrulline already on admission compared with the values measured in healthy people (40 nmol/mL); therefore, we were unable to observe a decrease in concentration during the 5-day ICU observation. In addition, a recent study of metabolic profiles in the serum of COVID-19 patients showed already significant changes in amino-acid metabolism at the time of admission to the hospital [56]. The authors found that l-glutamine, the main precursor of citrulline produced in the intestines, is the most altered amino-acid with reduced concentration in the acute phase of COVID-19. Thus, citrulline production may be limited by glutamine availability, potentially leading to lower baseline citrulline levels in critically ill COVID-19 patients. The beneficial effects of glutamine supplementation in COVID-19 have been evaluated in several studies where adding enteral L-glutamine to regular nutrition reduced hospital stay and improved outcomes [57].

The usefulness of markers of intestinal injury was also evaluated in viral infections. HIV infects and destroys the immune system of the gastrointestinal tract and causes structural abnormalities in the intestinal mucosa [58,59]. Changes in I-FABP levels as a biomarker of intestinal barrier dysfunction in acute and chronic HIV infections were investigated. Skowyra et al. measured I-FABP serum levels in HIV-infected patients and confirmed that HIV caused damage to the intestinal mucosa when compared with healthy volunteers (2100 vs. 1260 pg/mL) [60]. In another study it was found that high I-FABP was associated with worse immune function, increased inflammation, and viremia in chronically untreated HIV infections [61]. There are only a few published studies with findings on biomarkers of intestinal barrier dysfunction in COVID-19 patients, especially among ICU patients who are at the highest risk of death, and the results of these have been inconsistent. Saia et al. observed significantly increased levels of I-FABP indicating damage to enterocytes in SARS-CoV-2 infections [62]. In addition, critically ill and non-survivors showed higher levels of I-FABP compared with patients with a less severe clinical course. Giron et al. investigated markers of intestinal wall damage in COVID-19 patients using a multiplex assay [37]. They found that COVID-19 infection was associated with elevated levels of markers of tight junction permeability in the digestive tract, including zonulin and hREG3A (regenerating family member 3 alpha) protein, a marker of intestinal stress. In addition, the markers of disrupted intestinal barrier integrity correlated with markers of systemic inflammation. Giron et al. did not show significant increases in the levels of I-FABP, which may suggest that the changes in the intestinal wall were not associated with enterocyte death in the studied cohort. The lack of a significant increase in I-FABP could be explained by the clinical condition of the patients: most of the patients enrolled in Giron's study had mild to moderate symptoms (outpatients, hospitalized in regular wards), and only a few patients were critically ill. In our study, all patients were treated in the ICU for the most severe symptoms of COVID-19; all had respiratory failure and required mechanical ventilation (ARDS diagnosed in 85%), nearly half had septic shock, and 30% required renal replacement therapy to treat acute kidney failure. In this cohort, the higher levels of I-FABP in COVID-19 patients were associated with a diagnosis of septic shock and bad treatment outcome. Hypoxia of enterocytes resulting from poor intestinal perfusion in combination with the direct effects of the SARS-CoV-2 virus may lead to increased intestinal permeability and impairment of the intestinal immune barrier function. In patients with septic shock, hypoperfusion of the intestinal mucosa is a potential mechanism of multi-organ failure [63]. In an animal model of shock, Chang et al. showed

severe damage to the intestinal barrier, which included the injury and atrophy of the intestinal mucosa and increased intestinal permeability [64]. Shock-associated intestinal ischemia may induce enterocyte damage. Piton et al. showed that almost half of critically ill patients had enterocyte damage on admission to the ICU, as evidenced by elevated levels of I-FABP in the plasma [38]. In our study, 40% of patients were diagnosed with septic shock on ICU admission, and the concentration of I-FABP was much higher in this group of patients compared with patients without shock throughout the study period, while the concentration of citrulline was similar in both groups. This indicates that impaired blood flow in septic shock can lead to the damage of intestinal cells and the release of cytosolic proteins into the bloodstream.

The overall mortality from COVID-19-induced sepsis proved to be higher than from previous viral-induced sepsis [65–70]. In our study, the mortality rate was high, and considering the mid-pandemic crisis management, prompted the need for evaluating survival chances of individual patients in the ICU. With 57% mortality in this study vs. 30–60% in patients admitted to the ICU as reported by previous studies, our result is relatively high. It should be noted, however, that during the pandemic there were large differences in the availability of ICU beds, which significantly influenced the admission time and the clinical condition of patients admitted to the ICU, important factors affecting the outcome of treatment. Rapid deterioration of a patient's health requires the implementation of advanced therapies, often limited to large clinical centers and not accessible to every patient. Therefore, it is important to be able to make decisions early in the first days after ICU admission, and a model that could identify patients with viral sepsis who are at high risk of death would be important in planning effective treatment for these patients. In the search for such a model, intestinal biomarkers are being analyzed based on the fact that gastrointestinal symptoms are one of the major problems in critically ill COVID-19 patients, often complicating therapy by limiting therapeutic options [71].

I-FABP and citrulline levels have been shown to correlate with outcome in critically ill patients [38,40,72]. We found that the I-FABP level measured on admission to the ICU predicted mortality with an AUC of 0.710 and with an optimal cut-off point of 668.57 pg/mL. Moreover, according to the results of multivariate logistic regression analysis, the best model predicting mortality included initial I-FABP, creatinine, and PCT, a diagnosis of septic shock on ICU admission, and the APACHE II score. An initial I-FABP above 668.57 pg/mL, a high APACHE II score, and the presence of septic shock indicated a significantly higher risk of 28-day mortality in the analyzed cohort; the initial PCT and creatinine levels had no statistical significance in the model. In recent studies multiple models have been used to predict mortality in COVID-19 patients.

In a study by Leoni et al. focusing on critically-ill ICU patients, a multivariable mortality prediction model identified age, obesity, procalcitonin, the SOFA score and PaO_2/FiO_2 as significant elements associated with 28-day mortality [73]. Our study did not confirm these findings. The observed differences between prediction models were often related to the clinical condition of the studied cohort and the parameters included in the multivariate analysis. All patients in our study required immediate respiratory support in the form of mechanical ventilation or ECMO, while in the Leoni study 27% of patients were treated with less invasive methods of respiratory support (such as CPAP—ventilation using continuous positive airway pressure) on admission to the ICU. It should be emphasized that the new parameter, i.e., the level of I-FABP, included in our model confirms the importance of examining the degree of intestinal wall damage in predicting mortality in COVID-19 patients.

Limitation

This study had several limitations. The analysis was carried out on the basis of data from one facility and we realize that the criteria for admission to the ICU in our hospital may differ from the criteria of other hospitals. During the pandemic, our ICU acted as the regional ECMO center and only admitted COVID-19 patients with the most severe

respiratory symptoms. The results obtained are, therefore, particularly relevant to critical COVID-19 patients who required mechanical ventilation or ECMO.

5. Conclusions

In critically ill COVID-19 patients, intestinal cell damage is associated with shock and 28-day mortality. Our findings underline the importance of assessing the degree of intestinal wall damage in predicting patient mortality on ICU admission. Assessing mortality risk based on intestinal markers might be useful in clinical decisions concerning intestinal injury, imaging diagnostics and potential surgical interventions in isolated patients. It should be noted, however, that mortality may depend on the severity of the patients' condition in the hospital, staff experience, and available resources, which can vary significantly between ICUs.

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Data Availability Statement: The data presented in the study are available on request from the corresponding author. The data have not been made publicly available because they contain information that could compromise the privacy of the study participants.

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References

1. Acosta, M.A.T.; Singer, B.D. Pathogenesis of COVID-19-induced ARDS: Implications for an ageing population. *Eur. Respir. J.* **2020**, *56*, 2002049. [CrossRef]
2. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.-D.; Coopersmith, C.M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA-J. Am. Med. Assoc.* **2016**, *315*, 801–810. [CrossRef] [PubMed]
3. World Health Organization. Improving the Prevention, Diagnosis and Clinical Management of Sepsis. Available online: https://apps.who.int/gb/ebwha/pdf_files/WHA70/A70_13-en.pdf (accessed on 24 November 2022).
4. Sakr, Y.; Jaschinski, U.; Wittebole, X.; Szakmany, T.; Lipman, J.; Ñamendys-Silva, S.A.; Martin-Loeches, I.; Leone, M.; Lupu, M.-N.; Vincent, J.-L. Sepsis in Intensive Care Unit Patients: Worldwide Data from the ICON Audit. *Open Forum Infect. Dis.* **2018**, *5*, ofy313. [CrossRef] [PubMed]
5. Lin, G.-L.; McGinley, J.P.; Drysdale, S.B.; Pollard, A.J. Epidemiology and Immune Pathogenesis of Viral Sepsis. *Front. Immunol.* **2018**, *9*, 2147. [CrossRef] [PubMed]
6. Smiechowicz, J.; Barteczko-Grajek, B.; Adamik, B.; Bojko, J.; Gozdzik, W.; Lipinska-Gediga, M. Influenza in patients with respiratory failure admitted to intensive care units in Poland and the use of extracorporeal respiratory support: A survey-based multicenter study. *BMC Infect. Dis.* **2021**, *21*, 954. [CrossRef] [PubMed]
7. Karakike, E.; Giamarellos-Bourboulis, E.J.; Kyprianou, M.; Fleischmann-Struzek, C.; Pletz, M.W.; Netea, M.G.; Reinhart, K.; Kyriazopoulou, E. Coronavirus Disease 2019 as Cause of Viral Sepsis. *Crit. Care Med.* **2021**, *49*, 2042–2057. [CrossRef]
8. Tarragon, B.; Valdenebro, M.; Serrano, M.L.; Maroto, A.; Llopez-Carratala, M.R.; Ramos, A.; Rubio, E.; Huerta, A.; Marques, M.; Portoles, J. Acute kidney failure in patients admitted due to COVID-19. *Nefrologia* **2021**, *41*, 34–40. [CrossRef]
9. Italia, L.; Tomasoni, D.; Bisegna, S.; Pancaldi, E.; Stretti, L.; Adamo, M.; Metra, M. COVID-19 and Heart Failure: From Epidemiology during the Pandemic to Myocardial Injury, Myocarditis, and Heart Failure Sequelae. *Front. Cardiovasc. Med.* **2021**, *8*, 713560. [CrossRef]

10. de Roquetaillade, C.; Bredin, S.; Lascarrou, J.-B.; Soumagne, T.; Cojocaru, M.; Chousterman, B.G.; Leclerc, M.; Gouhier, A.; Piton, G.; Pène, F.; et al. Timing and causes of death in severe COVID-19 patients. *Crit. Care* **2021**, *25*, 224. [CrossRef]
11. Spearman, C.W.; Aghemo, A.; Valenti, L.; Sonderup, M.W. COVID-19 and the liver: A 2021 update. *Liver Int.* **2021**, *41*, 1988–1998. [CrossRef]
12. Dumas, A.; Bernard, L.; Poquet, Y.; Lugo-Villarino, G.; Neyrolles, O. The role of the lung microbiota and the gut-lung axis in respiratory infectious diseases. *Cell. Microbiol.* **2018**, *20*, e12966. [CrossRef] [PubMed]
13. Dickson, R.P.; Singer, B.; Newstead, M.W.; Falkowski, N.R.; Erb-Downward, J.R.; Standiford, T.J.; Huffnagle, G.B. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat. Microbiol.* **2016**, *1*, 16113. [CrossRef] [PubMed]
14. Kaliszewski, K.; Diakowska, D.; Nowak, Ł.; Tokarczyk, U.; Sroczyński, M.; Sępek, M.; Dudek, A.; Sutkowska-Stępień, K.; Kiliś-Pstrusińska, K.; Matera-Witkiewicz, A.; et al. Assessment of Gastrointestinal Symptoms and Dyspnea in Patients Hospitalized due to COVID-19: Contribution to Clinical Course and Mortality. *J. Clin. Med.* **2022**, *11*, 1821. [CrossRef]
15. Kang, M.K.; Kim, K.O.; Kim, M.C.; Cho, J.H.; Kim, S.B.; Gil Park, J.; Kim, K.H.; Lee, S.H.; Jang, B.I.; Kim, T.N. Clinical characteristics of coronavirus disease 2019 patients with diarrhea in Daegu. *Korean J. Intern. Med.* **2020**, *35*, 1261–1269. [CrossRef] [PubMed]
16. Liu, R.; Paz, M.; Siraj, L.; Boyd, T.; Salamone, S.; Lite, T.-L.V.; Leung, K.M.; Chirinos, J.D.; Shang, H.H.; Townsend, M.J.; et al. Feeding Intolerance in Critically Ill Patients with COVID-19. *Clin. Nutr.* **2022**, *41*, 3069–3076. [CrossRef]
17. Lamers, M.M.; Beumer, J.; van der Vaart, J.; Knoops, K.; Puschhof, J.; Breugem, T.I.; Ravelli, R.B.G.; van Schayck, J.P.; Mykytyn, A.Z.; Duimel, H.Q.; et al. SARS-CoV-2 productively infects human gut enterocytes. *Science* **2020**, *369*, 50–54. [CrossRef]
18. Li, W.; Moore, M.J.; Vasilieva, N.; Sui, J.; Wong, S.K.; Berne, M.A.; Somasundaran, M.; Sullivan, J.L.; Luzuriaga, K.; Greenough, T.C.; et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **2003**, *426*, 450–454. [CrossRef]
19. Zhou, P.; Yang, X.-L.; Wang, X.-G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.-R.; Zhu, Y.; Li, B.; Huang, C.-L.; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020**, *579*, 270–273. [CrossRef]
20. Harmer, D.; Gilbert, M.; Borman, R.; Clark, K.L. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett.* **2002**, *532*, 107–110. [CrossRef]
21. Xiao, F.; Tang, M.; Zheng, X.; Liu, Y.; Li, X.; Shan, H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* **2020**, *158*, 1831–1833.e3. [CrossRef]
22. Rhodes, A.; Evans, L.E.; Alhazzani, W.; Levy, M.M.; Antonelli, M.; Ferrer, R.; Kumar, A.; Sevransky, J.E.; Sprung, C.L.; Nunnally, M.E.; et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med.* **2017**, *43*, 304–377. [CrossRef] [PubMed]
23. Blaser, A.R.; Preiser, J.-C.; Fruhwald, S.; Wilmer, A.; Wernerman, J.; Benstoem, C.; Casaer, M.P.; Starkopf, J.; van Zanten, A.; Rooyackers, O.; et al. Gastrointestinal dysfunction in the critically ill: A systematic scoping review and research agenda proposed by the Section of Metabolism, Endocrinology and Nutrition of the European Society of Intensive Care Medicine. *Crit. Care* **2020**, *24*, 224. [CrossRef] [PubMed]
24. Blaser, A.R.; Padar, M.; Mändul, M.; Elke, G.; Engel, C.; Fischer, K.; Giabicani, M.; Gold, T.; Hess, B.; Hiesmayr, M.; et al. Development of the Gastrointestinal Dysfunction Score (GIDS) for critically ill patients—A prospective multicenter observational study (iSOFA study). *Clin. Nutr.* **2021**, *40*, 4932–4940. [CrossRef] [PubMed]
25. Blaser, A.R.; Malbrain, M.L.N.G.; Starkopf, J.; Fruhwald, S.; Jakob, S.M.; De Waele, J.; Braun, J.-P.; Poeze, M.; Spies, C. Gastrointestinal function in intensive care patients: Terminology, definitions and management. Recommendations of the ESICM Working Group on Abdominal Problems. *Intensiv. Care Med.* **2012**, *38*, 384–394. [CrossRef] [PubMed]
26. Pelsers, M.M.; Hermens, W.T.; Glatz, J.F. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin. Chim. Acta* **2005**, *352*, 15–35. [CrossRef] [PubMed]
27. Voth, M.; Duchene, M.; Auner, B.; Lustenberger, T.; Relja, B.; Marzi, I. I-FABP is a Novel Marker for the Detection of Intestinal Injury in Severely Injured Trauma Patients. *World J. Surg.* **2017**, *41*, 3120–3127. [CrossRef]
28. Sun, D.-L.; Cen, Y.-Y.; Li, S.-M.; Li, W.-M.; Lu, Q.-P.; Xu, P.-Y. Accuracy of the serum intestinal fatty-acid-binding protein for diagnosis of acute intestinal ischemia: A meta-analysis. *Sci. Rep.* **2016**, *6*, srep34371. [CrossRef]
29. Coufal, S.; Kokesova, A.; Tlaskalova-Hogenova, H.; Frybova, B.; Snajdauf, J.; Rygl, M.; Kverka, M. Urinary I-FABP, L-FABP, TFF-3, and SAA Can Diagnose and Predict the Disease Course in Necrotizing Enterocolitis at the Early Stage of Disease. *J. Immunol. Res.* **2020**, *2020*, 3074313. [CrossRef]
30. Tang, W.H.W.; Li, D.Y.; Hazen, S.L. Dietary metabolism, the gut microbiome, and heart failure. *Nat. Rev. Cardiol.* **2019**, *16*, 137–154. [CrossRef]
31. Kitai, T.; Kim, Y.-H.; Kiefer, K.; Morales, R.; Borowski, A.G.; Grodin, J.L.; Tang, W.W. Circulating intestinal fatty acid-binding protein (I-FABP) levels in acute decompensated heart failure. *Clin. Biochem.* **2017**, *50*, 491–495. [CrossRef]
32. Adamik, B.; Kübler, A.; Gozdzik, A.; Gozdzik, W. Prolonged Cardiopulmonary Bypass is a Risk Factor for Intestinal Ischaemic Damage and Endotoxaemia. *Heart Lung Circ.* **2017**, *26*, 717–723. [CrossRef] [PubMed]
33. Windmueller, H.G.; Spaeth, A.E. Source and fate of circulating citrulline. *Am. J. Physiol. Metab.* **1981**, *241*, E473–E480. [CrossRef] [PubMed]

34. Dossin, O.; Rupassara, S.; Weng, H.-Y.; Williams, D.; Garlick, P.; Schoeman, J. Effect of Parvoviral Enteritis on Plasma Citrulline Concentration in Dogs. *J. Vet. Intern. Med.* **2011**, *25*, 215–221. [CrossRef]
35. Herbers, A.H.; Feuth, T.; Donnelly, J.P.; Blijlevens, N.M. Citrulline-based assessment score: First choice for measuring and monitoring intestinal failure after high-dose chemotherapy. *Ann. Oncol.* **2010**, *21*, 1706–1711. [CrossRef]
36. Shen, L.-J.; Guan, Y.-Y.; Wu, X.-P.; Wang, Q.; Wang, L.; Xiao, T.; Wu, H.-R.; Wang, J.-G. Serum citrulline as a diagnostic marker of sepsis-induced intestinal dysfunction. *Clin. Res. Hepatol. Gastroenterol.* **2015**, *39*, 230–236. [CrossRef]
37. Giron, L.B.; Dweep, H.; Yin, X.; Wang, H.; Damra, M.; Goldman, A.R.; Gorman, N.; Palmer, C.S.; Tang, H.-Y.; Shaikh, M.W.; et al. Plasma Markers of Disrupted Gut Permeability in Severe COVID-19 Patients. *Front. Immunol.* **2021**, *12*, 686240. [CrossRef] [PubMed]
38. Piton, G.; Belon, F.; Cypriani, B.; Regnard, J.; Puyraveau, M.; Manzon, C.; Navellou, J.-C.; Capellier, G. Enterocyte Damage in Critically Ill Patients Is Associated With Shock Condition and 28-Day Mortality. *Crit. Care Med.* **2013**, *41*, 2169–2176. [CrossRef] [PubMed]
39. Guedj, K.; Uzzan, M.; Soudan, D.; Trichet, C.; Nicoletti, A.; Weiss, E.; Manceau, H.; Nuzzo, A.; Corcos, O.; Treton, X.; et al. I-FABP is decreased in COVID-19 patients, independently of the prognosis. *PLoS ONE* **2021**, *16*, e0249799. [CrossRef]
40. Piton, G.; Manzon, C.; Monnet, E.; Cypriani, B.; Barbot, O.; Navellou, J.-C.; Carbonnel, F.; Capellier, G. Plasma citrulline kinetics and prognostic value in critically ill patients. *Intensiv. Care Med.* **2010**, *36*, 702–706. [CrossRef]
41. World Health Organization. *Clinical Management of Severe Acute Respiratory Infection (SARI) When COVID-19 Disease is Suspected: Interim Guidance*; World Health Organization: Geneva, Switzerland, 2020.
42. Logan, M.; MacKinder, M.; Clark, C.M.; Kountouri, A.; Jere, M.; Ijaz, U.Z.; Hansen, R.; McGrogan, P.; Russell, R.K.; Gerasimidis, K. Intestinal fatty acid binding protein is a disease biomarker in paediatric coeliac disease and Crohn's disease. *BMC Gastroenterol.* **2022**, *22*, 260. [CrossRef]
43. Elkhatib, I.; Buchman, A.L. Plasma Citrulline Concentration as a Marker for Disease Activity in Patients With Crohn's Disease. *J. Clin. Gastroenterol.* **2012**, *46*, 308–310. [CrossRef] [PubMed]
44. World Medical Association. Declaration of Helsinki World Medical Association Declaration of Helsinki. *Bull. World Health Organ.* **2013**, *79*, 373–374.
45. Alhazzani, W.; Møller, M.H.; Arabi, Y.M.; Loeb, M.; Gong, M.N.; Fan, E.; Oczkowski, S.; Levy, M.M.; Derde, L.; Dzierba, A.; et al. Surviving Sepsis Campaign: Guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). *Intensive Care Med.* **2020**, *46*, 854–887. [CrossRef] [PubMed]
46. Parasa, S.; Desai, M.; Chandrasekar, V.T.; Patel, H.K.; Kennedy, K.F.; Roesch, T.; Spadaccini, M.; Colombo, M.; Gabbiadini, R.; Artifon, E.L.A.; et al. Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients with Coronavirus Disease 2019: A Systematic Review and Meta-Analysis. *JAMA Netw. Open* **2020**, *3*, e2011335. [CrossRef]
47. Gu, J.; Han, B.; Wang, J. COVID-19: Gastrointestinal Manifestations and Potential Fecal–Oral Transmission. *Gastroenterology* **2020**, *158*, 1518–1519. [CrossRef] [PubMed]
48. Boraschi, P.; Giugliano, L.; Mercogliano, G.; Donati, F.; Romano, S.; Neri, E. Abdominal and gastrointestinal manifestations in COVID-19 patients: Is imaging useful? *World J. Gastroenterol.* **2021**, *27*, 4143–4159. [CrossRef]
49. Camilleri, M. Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut* **2019**, *68*, 1516–1526. [CrossRef]
50. Sarikaya, M.; Ergul, B.; Dogan, Z.; Filik, L.; Can, M.; Arslan, L. Intestinal Fatty Acid Binding Protein (I-FABP) as a Promising Test for Crohn's Disease: A Preliminary Study. *Clin. Lab.* **2015**, *61*, 87–91. [CrossRef]
51. Adriaanse, M.P.M.; Tack, G.J.; Passos, V.L.; Damoiseaux, J.G.M.C.; Schreurs, M.W.J.; Van Wijck, K.; Riedl, R.G.; Masclee, A.A.M.; Buurman, W.A.; Mulder, C.J.J.; et al. Serum I-FABP as marker for enterocyte damage in coeliac disease and its relation to villous atrophy and circulating autoantibodies. *Aliment. Pharmacol. Ther.* **2013**, *37*, 482–490. [CrossRef]
52. Crenn, P.; Coudray–Lucas, C.; Thuillier, F.; Cynober, L.; Messing, B. Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. *Gastroenterology* **2000**, *119*, 1496–1505. [CrossRef]
53. Fragkos, K.C.; Forbes, A. Citrulline as a marker of intestinal function and absorption in clinical settings: A systematic review and meta-analysis. *United Eur. Gastroenterol. J.* **2018**, *6*, 181–191. [CrossRef]
54. van Vliet, M.J.; Tissing, W.J.; Rings, E.H.; Koetse, H.A.; Stellaard, F.; Kamps, W.A.; de Bont, E.S. Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. *Pediatr. Blood Cancer* **2009**, *53*, 1188–1194. [CrossRef]
55. Ware, L.B.; Magarik, J.A.; Wickersham, N.; Cunningham, G.; Rice, T.W.; Christman, B.W.; Wheeler, A.P.; Bernard, G.R.; Summar, M.L. Low plasma citrulline levels are associated with acute respiratory distress syndrome in patients with severe sepsis. *Crit. Care* **2013**, *17*, R10. [CrossRef] [PubMed]
56. Ansone, L.; Briviba, M.; Silamikelis, I.; Terentjeva, A.; Perkons, I.; Birzniece, L.; Rovite, V.; Rozentale, B.; Viksna, L.; Kolesova, O.; et al. Amino Acid Metabolism is Significantly Altered at the Time of Admission in Hospital for Severe COVID-19 Patients: Findings from Longitudinal Targeted Metabolomics Analysis. *Microbiol. Spectr.* **2021**, *9*, e00338-21. [CrossRef]
57. Obayan, A.O.E. Overview of the Rationale for L-Glutamine Treatment in Moderate-Severe COVID-19 Infection. *J. Infect. Dis. Epidemiol.* **2021**, *7*, 187. [CrossRef]
58. Mehandru, S.; Poles, M.; Tenner-Racz, K.; Horowitz, A.; Hurley, A.; Hogan, C.; Boden, D.; Racz, P.; Markowitz, M. Primary HIV-1 Infection Is Associated with Preferential Depletion of CD4+ T Lymphocytes from Effector Sites in the Gastrointestinal Tract. *J. Exp. Med.* **2004**, *200*, 761–770. [CrossRef]

59. Brenchley, J.M.; Price, D.A.; Douek, D.C. HIV disease: Fallout from a mucosal catastrophe? *Nat. Immunol.* **2006**, *7*, 235–239. [CrossRef]
60. Skowyra, A.; Mikula, T.; Suchacz, M.; Skowyra, A.; Wiercińska-Drapało, A. The role of serum I-FABP concentration in assessment of small intestine mucosa among HIV-infected patients. *Eur. J. Inflamm.* **2015**, *13*, 75–81. [CrossRef]
61. El Kamari, V.; Sattar, A.; Mccomsey, G.A. Brief Report: Gut Structural Damage: An Ongoing Process in Chronically Untreated HIV Infection. *JAIDS J. Acquir. Immune Defic. Syndr.* **2019**, *80*, 242–245. [CrossRef]
62. Saia, R.S.; Giusti, H.; Luis-Silva, F.; Pedroso, K.J.B.; Auxiliadora-Martins, M.; Morejón, K.M.L.; Degiovani, A.M.; Cadelca, M.R.; Basile-Filho, A. Clinical investigation of intestinal fatty acid-binding protein (I-FABP) as a biomarker of SARS-CoV-2 infection. *Int. J. Infect. Dis.* **2021**, *113*, 82–86. [CrossRef]
63. van Haren, F.M.P. Prognostic Value of Enterocyte Injury in Critically Ill Patients: No Gut, No Glory? *Crit. Care Med.* **2013**, *41*, 2238–2239. [CrossRef] [PubMed]
64. Chang, J.-X.; Chen, S.; Ma, L.-P.; Jiang, L.-Y.; Chen, J.-W.; Chang, R.-M.; Wen, L.-Q.; Wu, W.; Jiang, Z.-P.; Huang, Z.-T.; et al. Functional and Morphological Changes of the Gut Barrier during the Restitution Process after Hemorrhagic Shock. *World J. Gastroenterol.* **2005**, *11*, 35. [CrossRef] [PubMed]
65. Arentz, M.; Yim, E.; Klaff, L.; Lokhandwala, S.; Riedo, F.X.; Chong, M.; Lee, M. Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA-J. Am. Med. Assoc.* **2020**, *323*, 1612–1614. [CrossRef] [PubMed]
66. Myers, L.C.; Parodi, S.M.; Escobar, G.J.; Liu, V.X. Characteristics of Hospitalized Adults With COVID-19 in an Integrated Health Care System in California. *JAMA-J. Am. Med. Assoc.* **2020**, *323*, 2195–2198. [CrossRef]
67. Sharma, S.; Badami, V.; Rojas, E.; Mittal, A.; Stansbury, R.; Rana, B.; Wilson, A.; Wen, S. ICU Mortality in Patients With Coronavirus Disease 2019 Infection: Highlighting Healthcare Disparities in Rural Appalachia. *Crit. Care Explor.* **2021**, *3*, e547. [CrossRef]
68. Bhatraju, P.K.; Ghassemieh, B.J.; Nichols, M.; Kim, R.; Jerome, K.R.; Nalla, A.K.; Greninger, A.L.; Pipavath, S.; Wurfel, M.M.; Evans, L.; et al. COVID-19 in Critically Ill Patients in the Seattle Region—Case Series. *N. Engl. J. Med.* **2020**, *382*, 2012–2022. [CrossRef]
69. Auld, S.C.; Caridi-Scheible, M.; Blum, J.M.; Robichaux, C.; Kraft, C.; Jacob, J.T.; Jabaley, C.S.; Carpenter, D.; Kaplow, R.; Hernandez-Romieu, A.C.; et al. ICU and Ventilator Mortality Among Critically Ill Adults with Coronavirus Disease 2019. *Crit. Care Med.* **2020**, *48*, e799–e804. [CrossRef]
70. Carbonell, R.; Urgelés, S.; Rodríguez, A.; Bodí, M.; Martín-Loeches, I.; Solé-Violán, J.; Díaz, E.; Gómez, J.; Trefler, S.; Vallverdú, M.; et al. Mortality comparison between the first and second/third waves among 3,795 critical COVID-19 patients with pneumonia admitted to the ICU: A multicentre retrospective cohort study. *Lancet Reg. Health-Eur.* **2021**, *11*, 100243. [CrossRef]
71. Chen, T.-H.; Hsu, M.-T.; Lee, M.-Y.; Chou, C.-K. Gastrointestinal Involvement in SARS-CoV-2 Infection. *Viruses* **2022**, *14*, 1188. [CrossRef]
72. Sekino, M.; Funaoka, H.; Sato, S.; Okada, K.; Inoue, H.; Yano, R.; Matsumoto, S.; Ichinomiya, T.; Higashijima, U.; Matsumoto, S.; et al. Intestinal fatty acid-binding protein level as a predictor of 28-day mortality and bowel ischemia in patients with septic shock: A preliminary study. *J. Crit. Care* **2017**, *42*, 92–100. [CrossRef]
73. Leoni, M.L.G.; Lombardelli, L.; Colombi, D.; Bignami, E.G.; Pergolotti, B.; Repetti, F.; Villani, M.; Bellini, V.; Rossi, T.; Halasz, G.; et al. Prediction of 28-day mortality in critically ill patients with COVID-19: Development and internal validation of a clinical prediction model. *PLoS ONE* **2021**, *16*, e0254550. [CrossRef] [PubMed]

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Citrulline, Intestinal Fatty Acid-Binding Protein and the Acute Gastrointestinal Injury Score as Predictors of Gastrointestinal Failure in Patients with Sepsis and Septic Shock

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Abstract: Gastrointestinal (GI) failure can be both a cause of sepsis and a consequence of the systemic pro-inflammatory response in sepsis. Changes in biomarkers of enterocyte damage, citrulline and I-FABP (intestinal fatty acid binding protein), may indicate altered intestinal permeability and damage. The study group consisted of patients with sepsis (N = 28) and septic shock (N = 30); the control group included patients without infection (N = 10). Blood samples were collected for citrulline and I-FABP and a 4-point AGI score (acute GI injury score) was calculated to monitor GI function on days 1, 3, 5, 7, and 10. Citrulline concentrations in the study group were lower than in the control. Lower values were also noted in septic patients with shock when compared to the non-shock group throughout the study period. I-FABP was higher in the septic shock group than in the sepsis group only on days 1 and 3. Citrulline was lower in patients with GI failure (AGI III) when compared to AGI I/II, reaching significance on days 7 (p = 0.034) and 10 (p = 0.015); moreover, a higher AGI score was associated with an increased 28 day mortality (p = 0.038). The results indicate that citrulline measurements, along with the AGI assessment, have clinical potential in monitoring GI function and integrity in sepsis.

Keywords: citrulline; I-FABP; biomarker; sepsis; septic shock; intestinal injury; intensive care

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1. Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a "dysregulated host response to infection" [1]. Gastrointestinal (GI) disorders are common in patients with sepsis and are associated with higher mortality [2]. GI complications can be both the cause of sepsis, as in patients with peritonitis, where the primary source of infection is the abdominal cavity, and the consequence of the systemic pro-inflammatory response observed in sepsis and septic shock. Changes to the intestinal-blood barrier over the course of sepsis are complex and include impaired regulation of splanchnic blood flow and intestinal mucosal hypoperfusion, increased permeability of the intestinal wall, coagulation-related local tissue perfusion disorders, and changes in the microbiome [3–11]. Another explanation for GI disorders may be disturbed intestinal motility in sepsis/shock as a result of the intensive use of sedatives and prolonged mechanical ventilation [12]. GI disorders in sepsis patients can have a multifaceted clinical presentation, including malnutrition, feeding intolerance, ulceration and bleeding, and bacterial translocation, all of which may contribute to worsening organ failure in sepsis and poor treatment outcome [13,14].

Diagnostics and monitoring of GI function in the intensive care unit (ICU) is a challenge for clinicians. Several scoring systems are routinely used to monitor the clinical condition of patients with sepsis, the most common being the Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), or the Simplified Acute Physiology Score (SAPS) score; unfortunately, the function of the GI system is not part of any of these scoring systems. Both an acute gastrointestinal injury (AGI) grading scheme and the recently developed Gastrointestinal Dysfunction Score (GIDS) score have been proposed for assessing the severity of GI disorders in ICU patients [2,15,16].

Therefore, the question arises as to whether biomarkers can be useful in monitoring the function of the GI tract in patients with sepsis as a method to support clinical assessment and scoring systems. Citrulline, a potential biomarker for assessing GI function in ICU patients, is mainly produced in humans by enterocytes in the small intestine and then released into the bloodstream; therefore, blood levels of citrulline may reflect the function and mass of intestinal epithelial cells [17]. Previous studies have shown that in critically ill patients, citrulline levels were often below the normal range of 30 to 50 mmol/mL; furthermore, low citrulline levels in this patient population correlated with clinical signs of GI dysfunction and failure, and with higher mortality [18–21]. Most studies evaluating changes in citrulline levels have been conducted in a general population of critically ill ICU patients; studies in the sepsis/septic shock population are rare and more research is needed in this category [20,22].

The second protein we studied as a potential biomarker of intestinal damage was I-FABP (intestinal fatty acid binding protein). It is a cytosolic protein specifically localized in the enterocytes of the small and large intestine. It is released into the bloodstream upon enterocyte destruction; therefore, I-FABP can be considered to be a marker of enterocyte function and intestinal mucosal injury [23]. Several studies have examined the association of I-FABP and citrulline with poor prognosis in sepsis, but the results have been inconclusive [24–26]. The results from our previous study showed that plasma levels of I-FABP, but not citrulline, were associated with significantly higher mortality in a group of patients with viral sepsis (COVID-19) [27].

The main objective of this study was to evaluate changes in citrulline and I-FABP plasma concentrations in a group of patients with bacterial sepsis. We hypothesized that shock-associated intestinal ischemia may induce damage of the intestinal barrier and functional enterocyte mass reduction. The effect of septic shock on intestinal cell integrity was studied using biomarker measurements and the current AGI classification system. The AGI score is a measure of GI disorders that can range from transient, partial impairment of GI function to permanent, long-term, and life-threatening damage. The relationship between AGI score and the biomarkers' levels was assessed. A secondary objective was to study the predictive ability of ICU baseline citrulline and I-FABP levels for 28 day mortality.

2. Materials and Methods

From January to December 2019, a single-centre, prospective observational study of patients with sepsis or septic shock admitted to the ICU of the Wrocław University Hospital was conducted. The study protocol was accepted by the local Bioethical Committee (No. KB—822/2018). Written informed consent was obtained from the patient or a legally authorized representative. The study protocol complies with the 1975 Declaration of Helsinki, as revised in 1983.

2.1. Patients and Data Collection

The inclusion criteria for patients in the study group were the diagnosis of sepsis or septic shock upon admission to the ICU in accordance with the SEPSIS-3 definition and suspected or confirmed bacterial infection [1]. The exclusion criteria were as follows: pregnancy, terminal illness with no chance for meaningful recovery, expected ICU length of stay of 24 h or less, age <18 years old, and a history of previous severe gGI disabilities

(chronic inflammatory diseases, such as ulcerative colitis, Lesniowski–Crohn disease, and viral hepatitis), chronic kidney failure, or pre-existing RRT dependency.

The primary sepsis management was conducted In accordance with the Surviving Sepsis Campaign guidelines [28]. Continuous renal replacement therapy (CRRT) was used as needed. On days 1, 3, 5, 7, and 10, clinical and laboratory data were recorded, and blood samples were taken from each patient. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was used to assess patients' baseline status. The Sequential Organ Failure Assessment (SOFA) score was used to monitor organ function each day. In patients with sepsis, these scores are commonly used as predictive tools in the ICU. APACHE II is a scale that includes both laboratory and clinical parameters (oxygenation status, cardiovascular assessment, consciousness, fever, essential electrolytes, complete blood count, and history of organ failure). The SOFA score focuses on the function of the vital systems: cardiovascular, respiratory, blood coagulation, liver, kidney, and the level of consciousness.

The Acute Gastrointestinal Injury (AGI) score was used to monitor GI function. The AGI score is a measure of GI failure in severe diseases, primarily evaluated in an ICU setting. As described by the ESICM Working Group on Abdominal Problems, the AGI score consists of 4 points [15]. Briefly,

1. AGI score I: (the patient is at risk of developing GI dysfunction) the function of the digestive tract is partially impaired; GI symptoms are associated with a known cause and transient;
2. AGI score II: (the patient developed GI dysfunction) the GI tract is unable to function properly to meet patient's needs for nutrients and fluids;
3. AGI score III: (the patient developed GI failure) severe GI damage which does not respond to normal treatment and the general condition of the patient is not improving;
4. AGI score IV: (the patient developed GI failure with severe impact on distant organ function) persistent, long-term damage, resulting in worsening of multi-organ dysfunction syndrome or shock; life-threatening and requiring surgical intervention.

In our ICU practice, the AGI score is assessed daily to monitor GI function in patients with sepsis. Feeding intolerance (FI), described as high gastric residual volumes (GRV >500 mL), diarrhea (3 or more bowel movements with stool $\geq$200–300 g per day or volume >250 mL), or constipation (absence of bowel movements for 3 or more days), was recorded. The route of feeding administration (enteral/parenteral), type of nutrition product, and volume of feeding were recorded. In addition, 28-day mortality and length of stay in ICU and hospital were recorded.

2.2. Control Group

In order to compare the level of citrulline and I-FABP in ICU patients with and without sepsis, a control group was included in the study. The inclusion criteria in the control group were as follows: admission for first-time planned coronary artery bypass grafting under cardiopulmonary bypass, age >18 years old. The exclusion criteria in the control group were as follows: infectious complications after surgery, pre-existing RRT dependency, the need for RRT after surgery, ejection fraction <40%, and other co-morbidities involving diabetes and renal or liver failure. After the surgery, patients were routinely transferred to the ICU. In the control group, citrulline and I-FABP plasma concentrations were measured in blood samples collected on the first day of the ICU stay.

2.3. Sample Collection and Measurement of the Biomarkers

For plasma concentration measurements of I-FABP and citrulline, blood samples were collected (2.7 mL, tubes containing 0.109 M sodium citrate as an anticoagulant, BD Vacutainer, BD, NJ) through an arterial cannula routinely inserted at ICU admission. Samples were collected on days 1, 3, 5, 7, and 10, always in the morning. All patients (both fed enterally or receiving total parenteral nutrition through a central venous catheter) were infused with nutrients at a constant rate and there was no fasting period; as such, samples

were taken while the patients were receiving food. Plasma separation was performed immediately after centrifugation for 10 min at 2000× *g*. The plasma was then stored at −70 °C. Commercial ELISA kits were used to analyze both I-FABP and citrulline levels (Quantikine ELISA Human FABP2/I-FABP Immunoassay, R&D Systems, Minneapolis, MN, USA; Human citrulline ELISA Kit, Cusabio Biotech Co., Ltd., Houston, TX, USA).

2.4. Statistical Analysis

The data were analyzed with Statistica 13 software (StatSoft, Inc., Tulsa, OK, USA) and R 3.6.01: R Core Team (2013). Continuous variables were summarized with three statistics: the median and the interquartile range between the 25th and 75th percentiles, and categorical as frequencies with percentages. The distribution of data was not normal based on the Shapiro–Wilk test and the analysis was performed with nonparametric tests. The Mann–Whitney U test was used to compare continuous variables between study groups at each time point. Categorical variables were analyzed using the Chi-square test, and contingency tables were used to analyze the frequency distribution of categorical variables. The predictive accuracy of the biomarkers measurements on admission to the ICU was tested using receiver operating characteristic curve (ROC) analysis, by calculating the area under the curve (AUC). The Youden's statistic was used to select the optimum cut-off point for prognosis of GI failure (AGI III or AGI IV). Survival analysis of time to death was performed using the Kaplan–Meier curve and a log-rank test. The Kruskal–Wallis ANOVA by ranks was used for comparison of biomarker levels between subgroups with different AGI scores. The Friedman ANOVA was used to analyze within-group changes in performance overtime. *p*-values less than 0.05 were regarded as significant. The multivariable logistic regression analysis was performed to evaluate the association between values of the studied biomarkers and covariates and for the development of GI failure during ICU stay. The statistical metrics of the fitted model, including accuracy, sensitivity, specificity, and ROC AUC, were reported. The significance of predictors was analyzed considering the 95% confidence intervals and the resulting *p*-values.

3. Results

In the 12 month period, 58 septic patients met the inclusion criteria and were included in the analysis. Of these, 52% of patients had septic shock diagnosed on admission to the ICU, and 48% had sepsis. The median APACHE II score for the entire group was 25 points, the median SOFA score was 10 points, 71% of patients required mechanical ventilation on ICU admission, and 31% required continuous renal-replacement therapy (CRRT). The 28 day mortality was 38%. In order to compare the level of biomarkers in ICU patients with and without sepsis, a control group was included in the study. The control group consisted of ten adult patients after coronary artery bypass grafting, treated in the ICU after the surgery. Blood samples in the control group were collected on the day of admission to the ICU. The median age in the control group was 67 years (IQR 61–70 years), and males accounted for 70%. The septic group and the control group did not differ in terms of age ($p = 0.828$) and gender ($p = 0.377$). Immediately after surgery, all patients in the control group were mechanically ventilated, extubated, and breathing normally within 24 h of admission to the ICU. After extubation, all control group patients received a standard postoperative diet; there was no enteral tube feeding or parenteral feeding in this group. None of the patients in the control group developed infectious complications or kidney failure, and all patients survived. Patient characteristics are summarized in Table 1.

3.1. Levels of Citrulline and I-FABP in Septic Patients with and without Shock

First, the level of biomarkers measured in patients with sepsis and in the control group, i.e., patients without infection treated in the ICU, was compared. Blood samples in the control group were collected only once (day 1) and the concentrations of citrulline and I-FABP were compared with the values measured in the study group collected on day 1. The median citrulline level was significantly lower in the septic group when compared to

the control group: 26.98 nmol/mL (IQR 21.45–32.28) vs. 39.82 nmol/mL (IQR 36.58–42.80), respectively, $p < 0.001$. The median I-FABP level was higher in the septic group than in the control group (719.66 pg/mL, (IQR 238.00–1346.78) vs. 545.67 pg/mL, (IQR 477.33–645.66), respectively), but the observed difference was not significant ($p = 0.549$). None of the patients in the control group developed infectious complications or organ failure during ICU treatment and all were transferred from the ICU within an average of 2 days (IQR 2.0–3.0).

Table 1. Baseline characteristics of patients.

Parameter	Control	Septic Patients		
	N = 10	Shock (+), *N* = 30	Shock (−), *N* = 28	*p* *
Age, years	67.5 (61.0–70.0)	66.0 (60.0–73.0)	61.5 (55.5–72.5)	0.198
Male, n (%)	7.0 (70.0)	18.0 (60.0)	16.0 (57.0)	0.825
BMI, kg/m^2	27.0 (24.0– 29.1)	27.8 (25.2–30.9)	26.1 (24.3–29.7)	0.171
APACHE II score	10.5 (10–11)	28.0 (24.0–32.0)	24 (18–28)	0.046
SOFA score	2 (1–3)	10 (8.0–13.0)	9 (8–12)	0.388
ICU admission *n* (%):				0.360
Medical	0.0	18.0 (60.0)	20.0 (71.0)	
Surgical	10.0 (100.0)	12.0 (40.0)	8.0 (29)	
Lactate [mmol/L]	0.9 (0.7–1.1)	4.6 (2.6–8.2)	1.7 (1.2–1.8)	<0.001
PLT [10^3/uL]	159.0 (137.0–181.0)	210.0 (124.0–309.0)	174.5 (120.0–364.5)	0.803
Fibrinogen [g/L]	2.9 (3.6–6.5)	4.6 (3.6–6.0)	5.6 (3.7–6.6)	0.481
D-dimer [mg/L]	0.7 (0.4–2.1)	6.2 (3.9–15.7)	6.2 (2.7–10.1)	0.395
WBC [10^3/uL]	12.6 (11.2–16.1)	17.1 (11.3–27.6)	13.3 (9.1–21.1)	0.543
CRP [mg/L]	61.6 (35.5–106.7)	194.6 (104.1–328.4)	255.6 (164.5–344.5)	0.358
PCT [ng/mL]	0.1 (0.0–0.1)	10.6 (3.6–34.2)	8.7 (3.5–23.4)	0.528
Treatment *n* (%):				
CRRT	0.0	12.0 (40.0)	6.0 (21.0)	0.126
Mechanical ventilation	10.0 (100)	19.0 (63.0)	22.0 (79.0)	0.202
ICU LOS [day]	2.0 (2.0–3.0)	7.5 (2.0–17.5)	11 (5.0–21.0)	0.093
Mortality, 28 days (%)	0.0	50.0	25.0	0.049

Values are presented as the median and the interquartile range or as frequencies with percentages; * the *p*-value represents the difference between patients with and without septic shock. Abbreviations: BMI, Body Mass Index; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; ICU, Intensive Care Unit; PLT, Platelets; WBC, White Blood Cells; CRP, C-Reactive Protein; PCT, Procalcitonin; CRRT, Continuous Renal-Replacement Therapy; LOS, Length of Stay.

Next, to assess the effect of septic shock on intestinal cell damage, we compared the levels of biomarkers between septic patients with and without septic shock. Citrulline was significantly lower in patients with septic shock than in patients without shock on days 1, 3, 5, 7, and 10 (Figure 1, left panel). The median I-FABP concentrations were higher in the septic shock group than in the non-shock group, with statistically significant differences between the groups on day 1 and day 3 of the study (Figure 1, right panel).

As with other amino acids, some citrulline can be removed during renal replacement therapy; therefore, in patients with sepsis/septic shock, we compared citrulline levels between subgroups of patients who required/did not require continuous renal-replacement therapy (CRRT(+)/CRRT(−)). CRRT was used in 31% of patients to support renal function on admission to the ICU. Median citrulline levels were similar in the CRRT(+) and CRRT(−) subgroups (27.81 nmol/mL vs. 26.62 nmol/mL, respectively, $p = 0.506$) at ICU admission; there were also no significant differences in citrulline concentrations between the CRRT((+)) and CRRT(−) subgroups on days 3, 5, 7, and 10 ($p > 0.05$). Similar results were obtained for I-FABP, with no differences in I-FABP concentrations between the CRRT((+)) and CRRT(−) subgroups at ICU admission (821.78 pg/mL vs. 651.33 pg/mL, $p = 0.464$). There were also no significant differences in I-FABP concentrations between the CRRT((+)) and CRRT(−) subgroups on days 3, 5, 7, and 10 ($p > 0.05$).

We also assessed whether liver dysfunction affects citrulline and I-FABP levels. On admission to the ICU, 36 patients had no symptoms of liver dysfunction with normal bilirubin levels (<1.2 mg/dL) and 22 patients had bilirubin levels above normal range.

Citrulline concentrations were similar in both subgroups (26.46 nmol/mL IQR 19.16–29.93 vs. 28.84 nmol/mL, IQR 24.52–33.30, respectively, $p = 0.273$). I-FABP concentrations were also similar in both subgroups (729.70 pg/mL, IQR 238.00–1216.33 vs. 719.66 pg/mL, IQR 293.00–2740.66, respectively, $p = 0.696$). There were also no significant differences in citrulline and I-FABP concentrations between these subgroups on days 3, 5, 7, and 10 ($p > 0.05$).

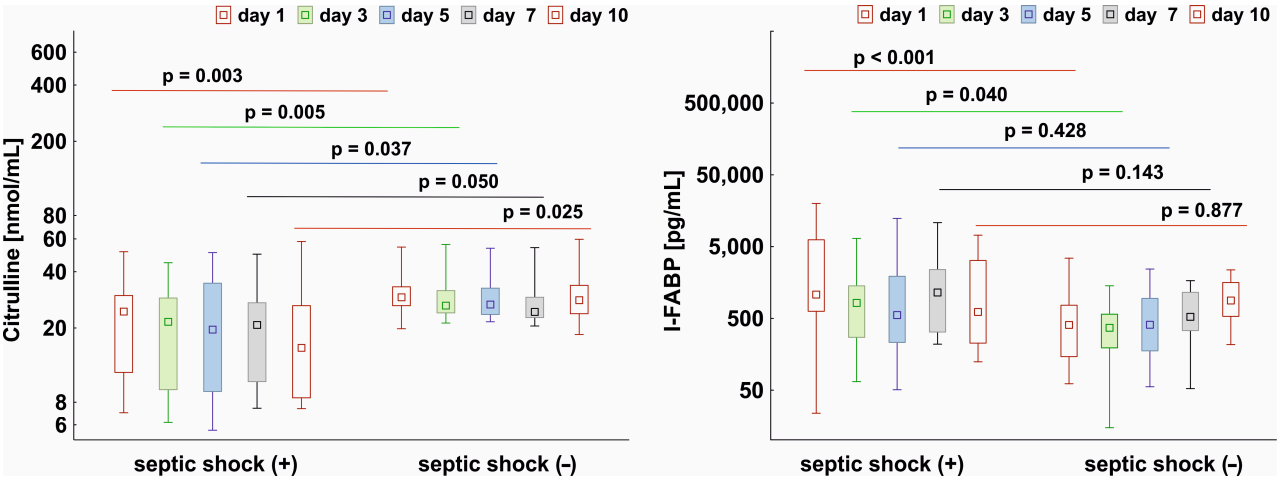

Figure 1. Graphs comparing the levels of Citrulline (**left**) and Intestinal Fatty Acid Binding Protein (I-FABP) (**right**) in the blood of patients with and without septic shock.

The *p*-value shows the differences between groups on corresponding days; a logarithmic scale was used to plot the data. The box plots represent the median values (midpoint) with upper and lower quartiles (box); the whiskers represent the minimum and maximum values.

3.2. The Relationship between AGI Score and Biomarker Levels

All septic patients were assessed using the 4-point AGI score on ICU admission (day 1) and on days 3, 5, 7, and 10. A detailed description of the AGI score is provided below in the Section 2. On ICU admission, 69% of patients were graded with AGI I (indicating that the function of the digestive tract is partially impaired, and that patients are at a risk of developing GI dysfunction); 31% were graded with AGI II (indicating the development of GI dysfunction). GI failure was not found in any of the patients on admission to the ICU; therefore, none were classified as AGI III or IV. In the following days, the number of patients with AGI score III (indicating GI failure) increased up to 2%, 29%, 47%, and 50% on day 5, 7, and 10, respectively; none of the patients were classified as AGI IV. Feeding intolerance was 18%, 31%, 29%, 29%, and 23% on days 1, 3, 5, 7, and 10, respectively. Enteral nutrition was administered to 26%, 52%, 57%, 66%, and 61% of patients on days 1, 3, 5, 7, and 10, respectively. In the control group, all patients on admission to the ICU were qualified as AGI I because the operation itself is associated with the risk of developing GI dysfunction. All patients in the control group received a standard postoperative diet after extubation and no enteral tube feeding or parenteral feeding was used in this group; no patient from the control group developed GI dysfunction (AGI II) or GI failure (AGI III).

To assess the relationship between AGI scores and biomarkers level in sepsis, the changes in citrulline and I-FABP levels over time were analyzed in three subgroups, according to highest AGI score calculated during ICU stay: AGI I as the highest calculated score occurred in 20 patients, AGI II in 19, and 19 patients developed severe GI damage and were diagnosed with GI failure (AGI score III). The level of citrulline significantly decreased over time in patients with AGI III ($p = 0.027$), while it did not change significantly in patients with AGI I ($p = 0.561$) and AGI II ($p = 0.406$). The level of I-FABP did not change

significantly over time in patients with AGI I ($p = 0.063$), AGI II ($p = 0.062$), and AGI III ($p = 0.157$). We than compared biomarker levels between AGI I vs. AGI III and AGI II vs. AGI III at each time point; results are shown in Table 2. Citrulline levels were significantly reduced in the AGI III subgroup when compared to AGI II throughout the study, while the difference between the AGI III and AGI I subgroups was significant only at day 10. Similar associations were not observed with I-FABP (Table 2).

Table 2. The relationship between the AGI score and the level of biomarkers. During ICU stay, AGI I was the highest calculated result in 20 patients, AGI II in 19 patients, and in 19 patients severe damage to the GI tract occurred and GI failure was diagnosed (AGI III).

	Day 1	Day 3	Day 5	Day 7	Day 10
			Citrulline [nmol/mL]		
AGI I	27.36 (13.36–30.58)	25.03 (23.30–30.92)	26.72 (23.63–46.54)	24.11 (23.42–24.40)	33.81 (27.55–35.22)
AGI II	31.00 (27.26–33.30)	27.56 (25.62–36.87)	32.66 (25.62–36.87)	29.20 (21.33–31.45)	29.40 (23.84–47.95)
AGI III	24.59 (12.30–26.85)	23.42 (12.64–27.47)	22.14 (9.92–26.81)	21.50 (10.32–25.88)	18.46 (8.88–26.33)
* p	0.244	0.197	0.053	0.205	0.037
# p	<0.001	0.018	0.005	0.027	0.022
			I-FABP [pg/mL]		
AGI I	476.61 (146.33–1095.36)	400.71 (303.84–812.14)	546.00 (155.27–1633.57)	493.21 (484.00–610.67)	2125.36 (694.00–3204.29)
AGI II	956.33 (429.67–2191.00)	414.67 (254.67–1002.54)	421.33 (286.33–2432.86)	1146.33 (431.00–1443.00)	937.87 (373.00–1139.67)
AGI III	832.57 (125.80–1148.27)	283.76 (198.17–831.07)	416.78 (173.33–690.00)	550.35 (322.33–1673.00)	698.10 (225.71–1479.29)
* p	0.693	0.424	0.793	0.753	0.231
# p	0.293	0.447	0.451	0.887	0.650

Values are presented as the median and the interquartile range; * p-value represents the difference in biomarker levels in AGI I vs. AGI III; # p-value represents the difference in biomarker levels in AGI II vs. AGI III. Abbreviations: AGI, Acute Gastrointestinal Injury; I-FABP, Intestinal Fatty Acid Binding Protein.

Next, to assess the effect of septic shock on intestinal cell damage, we compared the distribution of AGI scores between patients with and without septic shock: the distributions of the scores were comparable between septic patients with and without shock on day 1, 3, 5, and 7 of ICU stay. At the end of observation, distributions of the AGI scores differed significantly and most of the patients (75%) with shock were graded with AGI III on day 10. In contrast, in the non-shock group, only 29% of patients on day 10 had AGI III (Figure 2).

3.3. Biomarker Levels as a Tool for Predicting the Development of GI Failure

Citrulline had the ability to predict the development of GI failure with an AUC of 0.738 (95% CI 0.607–0.869, $p < 0.001$). The optimal cut-off value for the baseline citrulline level was 27.03 ng/mL, with sensitivity of 78.9% and specificity of 68.8%. The I-FABP did not have the ability to predict the development of GI failure ($p > 0.05$).

In addition, a multivariable logistic regression analysis was performed to create a model predicting the development of GI failure (i.e., AGI score III) during ICU stay in the analyzed cohort. The aim was to check whether the biomarkers measured on admission to the ICU (citrulline, I-FABP) and covariates calculated at ICU admission (APACHE II and SOFA scores, the presence of septic shock, and the presence of GI dysfunction (i.e., AGI score II) at the time of admission to the ICU) predict the development of GI failure during ICU stay. The fitted model reached an accuracy of 0.72 (95% CI 0.60–0.84), a sensitivity of 0.87 (95% CI 0.78–0.96), and a specificity of 0.42 (95% CI 0.29–0.55), when the threshold was set to 0.5. The ROC AUC for the model was 0.77 (95% CI 0.64–0.89) indicating decent discriminative power. The analysis showed that only two out of six predictors were statistically significant: the presence of GI dysfunction at ICU admission

(*p*-value = 0.037) and the level of citrulline (*p*-value = 0.036). The results can be interpreted as follows: (1) lower levels of citrulline slightly increased the risk of developing GI failure, and (2) patients with GI dysfunction at the time of admission to the ICU had a 5-fold higher risk of developing GI failure during their stay in the ICU. Figure 3 shows a forest plot with details of the odds ratios for each predictor.

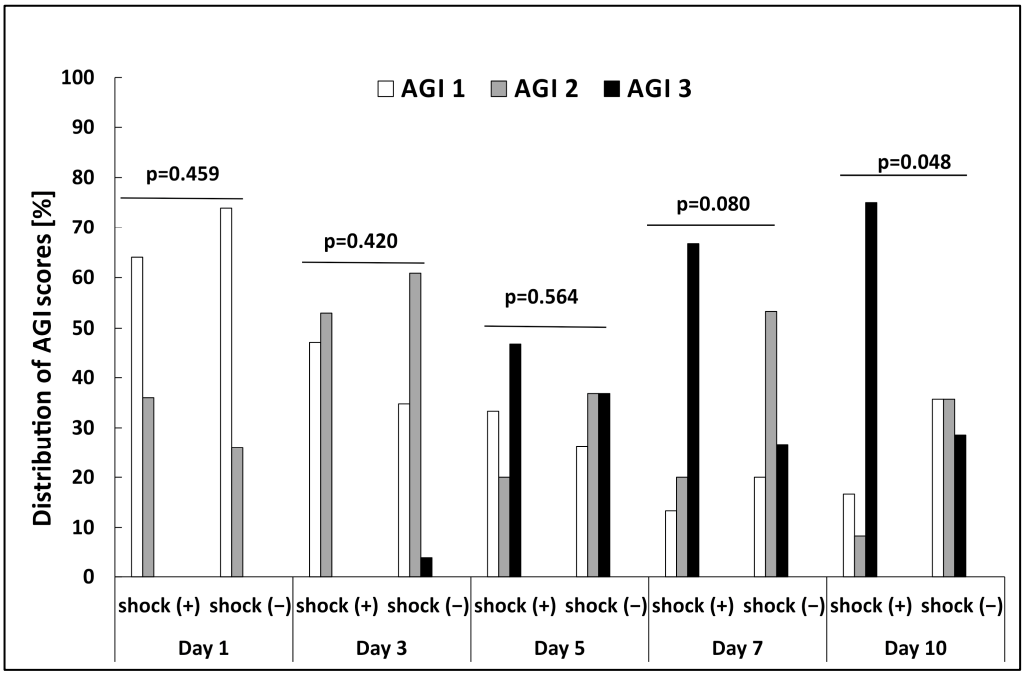

Figure 2. Distribution of the Acute Gastrointestinal Injury (AGI) scores among sepsis patients with and without shock calculated on days 1, 3, 5, 7, and 10.

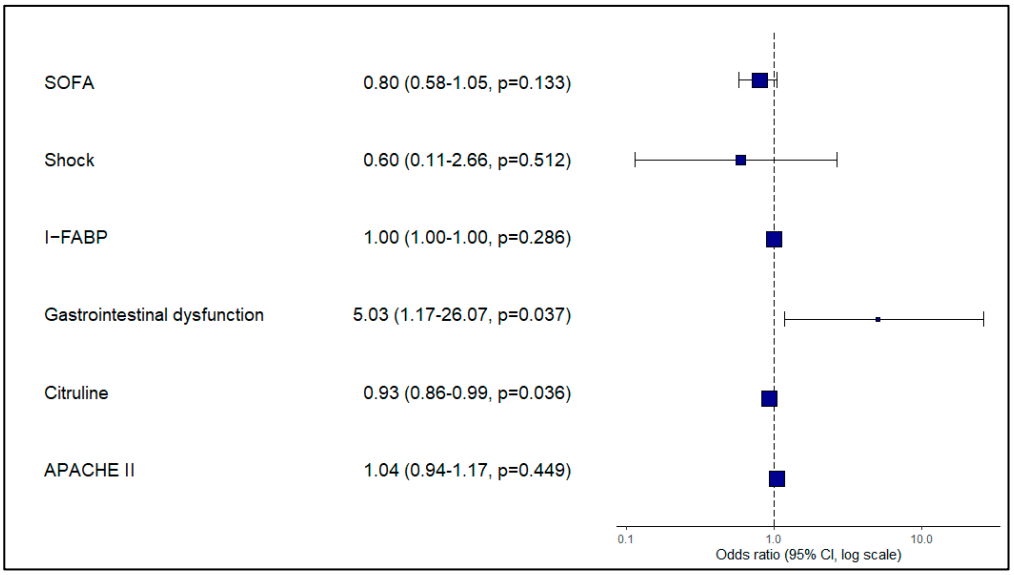

Figure 3. Forest plot of the multivariate logistic regression analysis for predicting the development of gastrointestinal failure during ICU stay (odds ratio, 95% CI, *p*-value are provided). Parameters were calculated on admission to the ICU. (abbreviations) SOFA, Sequential Organ Failure Assessment; I-FABP, intestinal fatty acid binding protein; APACHE II, Acute Physiology and Chronic Health Evaluation II; CI, confidence interval.

3.4. AGI Score, Biomarkers, and 28-Day Mortality

On ICU admission, 69% of patients were graded with AGI I and 31% with AGI II. GI failure was not found in any of the patients on admission to the ICU; therefore, none were

classified as AGI III. There was a significant difference in 28 day survival between groups with different AGI scores calculated at ICU admission: an AGI II score was associated with a worse prognosis when compared to AGI I (log-rank test $p = 0.038$). Figure 4 shows the Kaplan–Meier curves stratified on the AGI score for 28 day mortality in the studied population. However, there were no differences in citrulline or I-FABP levels between patients who died and those who survived throughout the study period ($p > 0.05$).

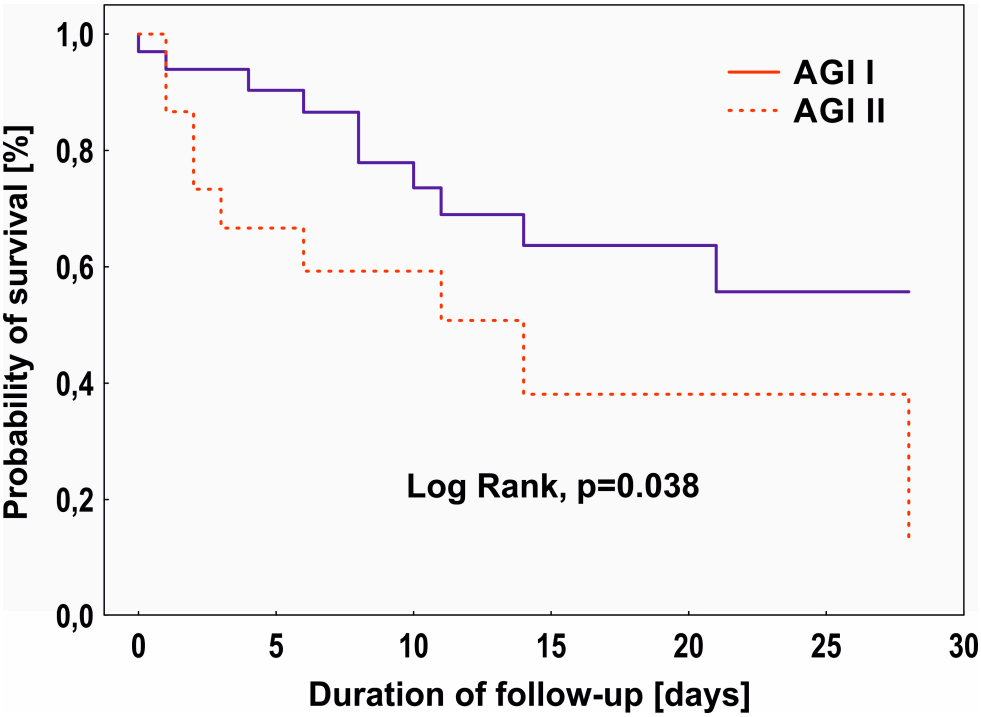

Figure 4. Kaplan–Meier curves stratified by the Acute Gastrointestinal Injury (AGI) scores (AGI I vs. AGI II) calculated in patients with sepsis at Intensive Care Unit (ICU) admission for 28 day mortality. *p*-value was for differences across the AGI scores by log-rank test.

4. Discussion

In our study, we assessed changes in citrulline and I-FABP concentrations in a well-defined group of ICU patients diagnosed with bacterial sepsis/septic shock. We found significant alterations in citrulline and I-FABP levels during sepsis, with decreased citrulline levels possibly indicating a reduction in functional enterocyte mass and elevated I-FABP levels suggesting enterocyte damage. In addition, citrulline concentrations were significantly lower in patients with septic shock, reflecting shock-related intestinal ischemia and damage to enterocytes and the intestinal barrier. Our results showed that plasma level of citrulline and the diagnosis of GI dysfunction at the time of admission to the ICU were associated with significantly higher risk of developing GI failure during ICU stay in the analyzed cohort. The predictive ability of baseline levels of citrulline and I-FABP for 28 day mortality was low; therefore, these biomarkers do not seem to be good candidates as independent predictors of mortality in our sepsis patient population. On the other hand, the AGI score calculated in septic patients on the first day of ICU admission was a strong predictor for 28 day mortality.

Various biomarkers have been studied in recent years to assess intestinal damage in critically ill patients, and I-FABP and citrulline appear to be the most promising [29–31]. I-FABP, a protein responsible for binding fatty acids in the intestine, is produced by the intestinal epithelium and released upon intestinal villi ischemia and damage [32,33]. Its importance as a clinical tool has been demonstrated in type 2 diabetes, necrotizing enterocolitis, mesenteric ischemia, and abdominal trauma [34–36]. Citrulline is an amino acid synthesized mainly in the small-bowel epithelium [17]. Intestinal damage leads to a

decrease in the enterocyte mass, and thus to a reduction in synthesis of the amino acid; this results in a low concentration of citrulline in plasma [37]. Citrulline has also turned out to be a useful predictive tool in chronic inflammatory intestinal diseases [38].

The application of citrulline and I-FABP as biomarkers of intestinal damage was also evaluated in animal models of sepsis and in patients with sepsis and septic shock. In an animal model of sepsis, damage to the intestinal mucosa was evident with changes in intestinal villi length and mucosal thickness. Consequently, the level of citrulline was lower and I-FABP higher than in the control group (animals without sepsis), reflecting changes in both the number and function of intestinal epithelial cells; citrulline in animals with sepsis decreased as a result of reduced production by damaged enterocytes and I-FABP increased in the bloodstream in the presence of intestinal ischemia. Thus, the measurement of both citrulline and I-FABP may be useful as a non-invasive tool for the diagnosis of sepsis-induced intestinal damage [39]. To date, only a few studies have been conducted evaluating the usefulness of citrulline as a marker of intestinal damage and prognosis in a population of patients with sepsis. In a pediatric population of critically ill patients, a decrease in citrulline concentration was associated with worse prognosis (increased acute phase parameters, longer mechanical ventilation, and a longer ICU stay) [40]. Another study on a general population of ICU patients by Piton et al. showed that low plasma citrulline levels on ICU admission were associated with high CRP, higher rates of nosocomial infections, and 28 day mortality [21]. Patients with septic shock accounted for 7% of the population studied by Piton, but the mean levels of citrulline concentrations in this group were <20 nmol/mL throughout the observation period. In our study, all patients were diagnosed with sepsis and patients with septic shock accounted for 52%. We hypothesized that septic shock-related intestinal ischemia and hypoperfusion may have resulted in damage to the intestinal barrier and a reduction in enterocyte mass. In fact, plasma citrulline levels were significantly reduced in these patients when compared to the values measured in the non-shock septic group, confirming our hypothesis. It is worth noting that in the entire study group of patients with sepsis (both with and without septic shock), the levels of citrulline were already lower on admission to the ICU when compared to previously reported values in healthy subjects; the optimal cut-off value for the baseline citrulline level to predict the development of GI failure was 27.03 ng/mL [41].

I-FABP is a protein that is expressed almost exclusively in enterocytes and is released from damaged cells into the bloodstream [33,42]. Therefore, elevation in plasma I-FABP may indicate damage to the intestinal wall. In healthy people, enterocytes undergo apoptosis without extracellular release of intracellular proteins including proteolytic enzymes and other DAMPs (damage-associated molecular patterns) and I-FABP plasma levels are very low or even undetectable [43]. In a study by Haan et al., a median level of 87 pg/mL was established based on measurements of plasma collected from 57 healthy volunteers [44]. In our study, both the septic group and the ICU control group had I-FABP levels well above this reference value. We could also confirm that plasma levels of citrulline and I-FABP were associated with septic shock. On each of the study days, our patients with septic shock had significantly lower citrulline levels than those without shock, but I-FABP was significantly higher only on days 1 and 3 in the septic shock group. This may be the result of treatment leading to attenuation of the systemic inflammatory response to infection and thus less damage to the enterocytes. Intestinal damage, as indicated by low citrulline levels and high I-FABP levels, has been shown to be associated with shock [20,45]. Derikx et al. showed a direct relationship between splanchnic hypoperfusion, measured with gastric mucosal tonometry, and intestinal mucosal damage, confirmed by high plasma I-FABP levels in the early phase of abdominal sepsis [24]. The release of cytosolic proteins into the bloodstream as a result of intestinal cell damage has also been reported in COVID-19 patients complicated by septic shock, who had higher I-FABP levels when compared to non-shock patients [27]. This may suggest that the release of I-FABP from damaged enterocytes during septic shock is independent of the type of causative pathogen and occurs in both bacterial and viral sepsis; further research is needed to confirm this observation.

The Acute Gastrointestinal Injury (AGI) scale is one of the few useful tools to assess the degree of intestinal injury in ICU settings. It was developed by ESICM in 2012 [15] and validated in 2017 [2]. Many studies have used the AGI score as a major indication of intestinal dysfunction and failure in variety of clinical settings, e.g., in critically ill COVID-19 patients, in patients with foodborne sepsis, and to evaluate enteral feeding protocol in critically ill patients and gut rest strategy [46–50]. In the present study, biomarker levels measured on days 1, 3, 5, 7, and 10 were assessed by comparison with the severity of intestinal dysfunction determined by the AGI score on respective days. Our results confirm that declining citrulline levels are associated with an increased risk of GI dysfunction and failure in patients with sepsis, similar to what was shown in the IN-PANCIA study and another study by Teng et al. in a general ICU population [19,51]. Unlike citrulline, changes in I-FABP levels were not associated with the AGI score in sepsis patients on any study day. A similar result was shown by Li et al., where a high level of I-FABP did not increase the odds of any AGI score in a general population of critically ill ICU patients [31]. In addition, we attempted to create a model predicting the development of GI failure during ICU stay. According to the results of multivariate logistic regression analysis, an elevated AGI score already at admission to the ICU (indicating GI dysfunction) and a decreased citrulline concentration were associated with a significantly higher risk of developing GI failure during ICU stay.

The distribution of AGI scores in our study between patients with and without septic shock was comparable on the first days of the study and significantly different at the end of observation. Most patients with shock had AGI score III (75%); among those who were not in shock, only 29% had AGI III on day 10. This indicates severe intestinal injury, manifested by persistent feeding intolerance despite treatment. Our results are consistent with previously published results by Sun et al., which showed that higher scores of AGI were associated with worse clinical variables, higher rates of septic shock, and higher 28 day mortality in a cohort of critically ill COVID-19 patients [47]. The relationship between AGI score and ICU outcome was studied by Klanovicz et al. [52], who found that AGI III present within the first 48 h after ICU admission was a significant risk factor for ICU mortality in patients with septic shock undergoing mechanical ventilation. In septic and septic shock patients, AGI score on admission turns out to be a good 28 day survival predictive tool. We found that AGI II score calculated at ICU admission was associated with a significantly worse prognosis of survival when compared to AGI I. As confirmed by a recent meta-analysis, the incidence of acute GI injury in critically ill patients is high (40%) and is associated with higher mortality; therefore, ESICM-initiated stratification may facilitate the identification and treatment of patients at risk of acute GI injury, and consequently better treatment outcomes [53].

5. Limitations

We are aware of our study limitations. First, the study was conducted in a single center with a relatively small patient population and needs to be validated in a much larger cohort of patients with sepsis and septic shock and critically ill ICU patients without infection. In a multivariate logistic regression analysis performed to model GI failure, the 95% confidence interval for the AGI score calculated at ICU admission is wide, which can be attributed to the small size of the dataset. Future research on a larger data set is needed. However, it should be emphasized that our study group is well defined and limited to sepsis and septic shock of bacterial etiology. Also, the control group is small but is well defined as a group with no prior infection and no infectious complications during the ICU stay. Second, it is still not clear how to interpret low citrulline results in patients with sepsis/septic shock: does low citrulline value require a specific therapeutic approach, and what value of citrulline should be considered as the cut-off point for supplementation? These issues need to be addressed in future studies.

6. Conclusions

GI dysfunction in septic patients on the first day of ICU admission, confirmed by a worse AGI score, is a predictor for 28 day mortality. In addition, an elevated AGI score on ICU admission, together with a decrease in citrulline levels, were associated with a higher risk of developing GI failure during ICU stay. Intestinal wall ischemia associated with hypoperfusion in septic shock may result in damage to the intestinal barrier and a reduction in enterocyte mass and function, as indicated by a significant decrease in plasma citrulline and I-FABP in the first days upon ICU admission. However, it should be noted that the levels of citrulline and I-FABP may be elevated in many conditions associated with disorders of the GI tract, kidneys, or liver, which may occur simultaneously in patients with sepsis.

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References

1. Singer, M.; Deutschman, C.S.; Seymour, C.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.D.; Coopersmith, C.M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **2016**, *315*, 801–810. [CrossRef]
2. Hu, B.; Sun, R.; Wu, A.; Ni, Y.; Liu, J.; Guo, F.; Ying, L.; Ge, G.; Ding, A.; Shi, Y.; et al. Severity of Acute Gastrointestinal Injury Grade Is a Predictor of All-Cause Mortality in Critically Ill Patients: A Multicenter, Prospective, Observational Study. *Crit. Care* **2017**, *21*, 188. [CrossRef] [PubMed]
3. Adelman, M.W.; Woodworth, M.H.; Langelier, C.; Busch, L.M.; Kempker, J.A.; Kraft, C.S.; Kraft, C.S.; Martin, G.S.; Martin, G.S. The Gut Microbiome's Role in the Development, Maintenance, and Outcomes of Sepsis. *Crit. Care* **2020**, *24*, 278. [CrossRef] [PubMed]
4. Fay, K.T.; Klingensmith, N.J.; Chen, C.W.; Zhang, W.; Sun, Y.; Morrow, K.N.; Liang, Z.; Burd, E.M.; Ford, M.L.; Coopersmith, C.M. The Gut Microbiome Alters Immunophenotype and Survival from Sepsis. *FASEB J.* **2019**, *33*, 11258–11269. [CrossRef] [PubMed]
5. Adamik, B.; Gozdzik, W.; Jakubczyk, D.; Welna, M.; Kübler, A. Coagulation Abnormalities Identified by Thromboelastometry in Patients with Severe Sepsis: The Relationship to Endotoxemia and Mortality. *Blood Coagul. Fibrinolysis* **2017**, *28*, 163–170. [CrossRef]
6. Bray, M.A.; Sartain, S.E.; Gollamudi, J.; Rumbaut, R.E. Microvascular Thrombosis: Experimental and Clinical Implications. *Transl. Res.* **2020**, *225*, 105–130. [CrossRef]
7. Dempfle, C.E. Coagulopathy of Sepsis. *Thromb. Haemost.* **2004**, *91*, 213–224. [CrossRef]
8. de Backer, D.; Creteur, J.; Preiser, J.C.; Dubois, M.J.; Vincent, J.L. Microvascular Blood Flow Is Altered in Patients with Sepsis. *Am. J. Respir. Crit. Care Med.* **2002**, *166*, 98–104. [CrossRef]
9. Ellis, C.G.; Jagger, J.; Sharpe, M. The Microcirculation as a Functional System. *Crit. Care* **2005**, *9*, S3–S8. [CrossRef]
10. Altshuler, A.E.; Lamadrid, I.; Li, D.; Ma, S.R.; Kurre, L.; Schmid-Schönbein, G.W.; Penn, A.H. Transmural Intestinal Wall Permeability in Severe Ischemia after Enteral Protease Inhibition. *PLoS ONE* **2014**, *9*, e96655. [CrossRef]

11. Jiang, L.-Y.; Zhang, M.; Zhou, T.-E.; Yang, Z.-F.; Wen, L.-Q.; Chang, J.-X. Changes of the Immunological Barrier of Intestinal Mucosa in Rats with Sepsis. *World J. Emerg. Med.* **2010**, *1*, 138–143. [PubMed]
12. Reintam Blaser, A.; Preiser, J.C.; Fruhwald, S.; Wilmer, A.; Wernerman, J.; Benstoem, C.; Casaer, M.P.; Starkopf, J.; van Zanten, A.; Rooyackers, O.; et al. Gastrointestinal Dysfunction in the Critically Ill: A Systematic Scoping Review and Research Agenda Proposed by the Section of Metabolism, Endocrinology and Nutrition of the European Society of Intensive Care Medicine. *Crit. Care* **2020**, *24*, 224. [CrossRef]
13. Doudakmanis, C.; Bouliaris, K.; Kolla, C.; Efthimiou, M.; Koukoulis, G.D. Bacterial Translocation in Patients Undergoing Major Gastrointestinal Surgery and Its Role in Postoperative Sepsis. *World J. Gastrointest. Pathophysiol.* **2021**, *12*, 106–114. [CrossRef]
14. Crapser, J.; Ritzel, R.; Verma, R.; Venna, V.R.; Liu, F.; Chauhan, A.; Koellhoffer, E.; Patel, A.; Ricker, A.; Maas, K.; et al. Ischemic Stroke Induces Gut Permeability and Enhances Bacterial Translocation Leading to Sepsis in Aged Mice. *Aging* **2016**, *8*, 1049–1063. [CrossRef]
15. Blaser, A.R.; Malbrain, M.L.N.G.; Starkopf, J.; Fruhwald, S.; Jakob, S.M.; de Waele, J.; Braun, J.P.; Poeze, M.; Spies, C. Gastrointestinal Function in Intensive Care Patients: Terminology, Definitions and Management. Recommendations of the ESICM Working Group on Abdominal Problems. *Intensive Care Med.* **2012**, *38*, 384–394. [CrossRef]
16. Reintam Blaser, A.; Padar, M.; Mändul, M.; Elke, G.; Engel, C.; Fischer, K.; Giabicani, M.; Gold, T.; Hess, B.; Hiesmayr, M.; et al. Development of the Gastrointestinal Dysfunction Score (GIDS) for Critically Ill Patients—A Prospective Multicenter Observational Study (ISOFA Study). *Clin. Nutr.* **2021**, *40*, 4932–4940. [CrossRef]
17. Curis, E.; Nicolis, I.; Moinard, C.; Osowska, S.; Zerrouk, N.; Bénazeth, S.; Cynober, L. Almost All about Citrulline in Mammals. *Amino Acids* **2005**, *29*, 177–205. [CrossRef]
18. Noordally, S.O.; Sohawon, S.; Semlali, H.; Michely, D.; Devriendt, J.; Gottignies, P. Is There a Correlation between Circulating Levels of Citrulline and Intestinal Dysfunction in the Critically Ill? *Nutr. Clin. Pract.* **2012**, *27*, 527–532. [CrossRef] [PubMed]
19. Fagoni, N.; Piva, S.; Marino, R.; Chiarini, G.; Ferrari, D.; Grespi, E.; Bertuetti, R.; Barbieri, S.; Latronico, N.; Rasulo, F. The IN-PANCIA Study: Clinical Evaluation of Gastrointestinal Dysfunction and Failure, Multiple Organ Failure, and Levels of Citrulline in Critically Ill Patients. *J. Intensive Care Med.* **2020**, *35*, 279–283. [CrossRef]
20. Crenn, P.; Neveux, N.; Chevret, S.; Jaffray, P.; Cynober, L.; Melchior, J.C.; Annane, D. Plasma L-Citrulline Concentrations and Its Relationship with Inflammation at the Onset of Septic Shock: A Pilot Study. *J. Crit. Care* **2014**, *29*, 315.e1–315.e6. [CrossRef] [PubMed]
21. Piton, G.; Manzon, C.; Monnet, E.; Cypriani, B.; Barbot, O.; Navellou, J.C.; Carbonnel, F.; Capellier, G. Plasma Citrulline Kinetics and Prognostic Value in Critically Ill Patients. *Intensive Care Med.* **2010**, *36*, 702–706. [CrossRef] [PubMed]
22. Luiking, Y.C.; Poeze, M.; Ramsay, G.; Deutz, N.E.P. Reduced Citrulline Production in Sepsis Is Related to Diminished de Novo Arginine and Nitric Oxide Production. *Am. J. Clin. Nutr.* **2009**, *89*, 142–152. [CrossRef] [PubMed]
23. Kanda, T.; Fujii, H.; Tani, T.; Murakami, H.; Suda, T.; Sakai, Y.; Ono, T.; Hatakeyama, K. Intestinal Fatty Acid-Binding Protein Is a Useful Diagnostic Marker for Mesenteric Infarction in Humans. *Gastroenterology* **1996**, *110*, 339–343. [CrossRef]
24. Derikx, J.P.M.; Poeze, M.; van Bijnen, A.A.; Buurman, W.A.; Heineman, E. Evidence for Intestinal and Liver Epithelial Cell Injury in the Early Phase of Sepsis. *Shock* **2007**, *28*, 544–548. [CrossRef] [PubMed]
25. Padar, M.; Starkopf, J.; Starkopf, L.; Forbes, A.; Hiesmayr, M.; Jakob, S.M.; Rooijackers, O.; Wernerman, J.; Ojavee, S.E.; Reintam Blaser, A. Enteral Nutrition and Dynamics of Citrulline and Intestinal Fatty Acid-Binding Protein in Adult ICU Patients. *Clin. Nutr. ESPEN* **2021**, *45*, 322–332. [CrossRef] [PubMed]
26. Sekino, M.; Funaoka, H.; Sato, S.; Okada, K.; Inoue, H.; Yano, R.; Matsumoto, S.; Ichinomiya, T.; Higashijima, U.; Matsumoto, S.; et al. Intestinal Fatty Acid-Binding Protein Level as a Predictor of 28-Day Mortality and Bowel Ischemia in Patients with Septic Shock: A Preliminary Study. *J. Crit. Care* **2017**, *42*, 92–100. [CrossRef]
27. Tyszko, M.; Lipińska-Gediga, M.; Lemańska-Perek, A.; Kobylińska, K.; Gozdzik, W.; Adamik, B. Intestinal Fatty Acid Binding Protein (I-FABP) as a Prognostic Marker in Critically Ill COVID-19 Patients. *Pathogens* **2022**, *11*, 1526. [CrossRef]
28. Rhodes, A.; Evans, L.E.; Alhazzani, W.; Levy, M.M.; Antonelli, M.; Ferrer, R.; Kumar, A.; Sevransky, J.E.; Sprung, C.L.; Nunnally, M.E.; et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med.* **2017**, *43*, 304–377. [CrossRef]
29. Piton, G.; Capellier, G. Biomarkers of Gut Barrier Failure in the ICU. *Curr. Opin. Crit. Care* **2016**, *22*, 152–160. [CrossRef]
30. Jenkins, B.; Calder, P.C.; Marino, L.V. A Scoping Review Considering Potential Biomarkers or Functional Measures of Gastrointestinal Dysfunction and Enteral Feeding Intolerance in Critically Ill Adults. *Clin. Nutr. ESPEN* **2022**, *52*, 331–339. [CrossRef]
31. Li, H.; Chen, Y.; Huo, F.; Wang, Y.; Zhang, D. Association between Acute Gastrointestinal Injury and Biomarkers of Intestinal Barrier Function in Critically Ill Patients. *BMC Gastroenterol.* **2017**, *17*, 45. [CrossRef] [PubMed]
32. Pelsers, M.M.A.L.; Namiot, Z.; Kisielewski, W.; Namiot, A.; Januszkiewicz, M.; Hermens, W.T.; Glatz, J.F.C. Intestinal-Type and Liver-Type Fatty Acid-Binding Protein in the Intestine. Tissue Distribution and Clinical Utility. *Clin. Biochem.* **2003**, *36*, 529–535. [CrossRef] [PubMed]
33. Lieberman, J.M.; Sacchettini, J.; Marks, C.; Marks, W.H. Human Intestinal Fatty Acid Binding Protein: Report of an Assay with Studies in Normal Volunteers and Intestinal Ischemia. *Surgery* **1997**, *121*, 335–342. [CrossRef]
34. Heida, F.H.; Hulscher, J.B.F.; Schurink, M.; Timmer, A.; Kooi, E.M.W.; Bos, A.F.; Bruggink, J.L.M.; Kasper, D.C.; Pones, M.; Benkoe, T. Intestinal Fatty Acid-Binding Protein Levels in Necrotizing Enterocolitis Correlate with Extent of Necrotic Bowel: Results from a Multicenter Study. *J. Pediatr. Surg.* **2015**, *50*, 1115–1118. [CrossRef] [PubMed]

35. Kanda, T.; Tsukahara, A.; Ueki, K.; Sakai, Y.; Tani, T.; Nishimura, A.; Yamazaki, T.; Tamiya, Y.; Tada, T.; Hirota, M.; et al. Diagnosis of Ischemic Small Bowel Disease by Measurement of Serum Intestinal Fatty Acid-Binding Protein in Patients with Acute Abdomen: A Multicenter, Observer-Blinded Validation Study. *J. Gastroenterol.* **2011**, *46*, 492–500. [CrossRef] [PubMed]
36. Voth, M.; Duchene, M.; Auner, B.; Lustenberger, T.; Relja, B.; Marzi, I. I-FABP Is a Novel Marker for the Detection of Intestinal Injury in Severely Injured Trauma Patients. *World J. Surg.* **2017**, *41*, 3120–3127. [CrossRef]
37. Crenn, P.; Coudray-Lucas, C.; Thuillier, F.; Cynober, L.; Messing, B. Postabsorptive Plasma Citrulline Concentration Is a Marker of Absorptive Enterocyte Mass and Intestinal Failure in Humans. *Gastroenterology* **2000**, *119*, 1496–1505. [CrossRef]
38. Elkhatib, I.; Buchman, A.L. Plasma Citrulline Concentration as a Marker for Disease Activity in Patients With Crohn's Disease. *J. Clin. Gastroenterol.* **2012**, *46*, 308–310. [CrossRef]
39. Shen, L.J.; Guan, Y.Y.; Wu, X.P.; Wang, Q.; Wang, L.; Xiao, T.; Wu, H.R.; Wang, J.G. Serum Citrulline as a Diagnostic Marker of Sepsis-Induced Intestinal Dysfunction. *Clin. Res. Hepatol. Gastroenterol.* **2015**, *39*, 230–236. [CrossRef]
40. Blasco-Alonso, J.; Sánchezyáñez, P.; Rosa Camacho, V.; Camacho Alonso, J.M.; Yahyaoui Macías, R.; Gil-Gómez, R.; Milano Manso, G. Citrulline and Arginine Kinetics and Its Value as a Prognostic Factor in Pediatric Critically Ill Patients. *An. Pediatría* **2015**, *83*, 257–263. [CrossRef]
41. Sarikaya, M.; Ergül, B.; Doğan, Z.; Filik, L.; Can, M.; Arslan, L. Intestinal Fatty Acid Binding Protein (I-FABP) as a Promising Test for Crohn's Disease: A Preliminary Study. *Clin. Lab.* **2015**, *61*, 87–91. [CrossRef] [PubMed]
42. Ockner, R.K.; Manning, J.A. Fatty Acid Binding Protein in Small Intestine. Identification, Isolation, and Evidence for Its Role in Cellular Fatty Acid Transport. *J. Clin. Investig.* **1974**, *54*, 326–338. [CrossRef]
43. Pelsers, M.M.A.L.; Hermens, W.T.; Glatz, J.F.C. Fatty Acid-Binding Proteins as Plasma Markers of Tissue Injury. *Clin. Chim. Acta* **2005**, *352*, 15–35. [CrossRef]
44. de Haan, J.J.; Lubbers, T.; Derikx, J.P.; Relja, B.; Henrich, D.; Greve, J.W.; Marzi, I.; Buurman, W.A. Rapid Development of Intestinal Cell Damage Following Severe Trauma: A Prospective Observational Cohort Study. *Crit. Care* **2009**, *13*, R86. [CrossRef] [PubMed]
45. Piton, G.; Belon, F.; Cypriani, B.; Regnard, J.; Puyraveau, M.; Manzon, C.; Navellou, J.C.; Capellier, G. Enterocyte Damage in Critically Ill Patients Is Associated with Shock Condition and 28-Day Mortality. *Crit. Care Med.* **2013**, *41*, 2169–2176. [CrossRef]
46. Zhang, D.; Li, H.; Li, Y.; Qu, L. Gut Rest Strategy and Trophic Feeding in the Acute Phase of Critical Illness with Acute Gastrointestinal Injury. *Nutr. Res. Rev.* **2019**, *32*, 176–182. [CrossRef] [PubMed]
47. Sun, J.K.; Liu, Y.; Zou, L.; Zhang, W.H.; Wang, Y.; Kan, X.H.; Chen, J.D.; Li, J.J.; Shi, Q.K.; Yuan, S.T. Acute Gastrointestinal Injury in Critically Ill Patients with COVID-19 in Wuhan, China. *World J. Gastroenterol.* **2020**, *26*, 6087–6097. [CrossRef]
48. Drakos, P.; Volteas, P.; Cleri, N.A.; Alkadaa, L.N.; Asencio, A.A.; Oganov, A.; Pryor, A.; Talamini, M.; Rubano, J.; Bannazadeh, M.; et al. Acute Gastrointestinal Injury and Feeding Intolerance as Prognostic Factors in Critically Ill COVID-19 Patients. *J. Gastrointest. Surg.* **2022**, *26*, 181–190. [CrossRef]
49. Ahn, J.W.; Scallan Walter, E.; White, A.E.; McQueen, R.B.; Hoffmann, S. Identifying Sepsis From Foodborne Hospitalization: Incidence and Hospitalization Cost by Pathogen. *Clin. Infect. Dis.* **2022**, *75*, 857–866. [CrossRef]
50. Zhang, Z.; Li, Q.; Jiang, L.; Xie, B.; Ji, X.; Lu, J.; Jiang, R.; Lei, S.; Mao, S.; Ying, L.; et al. Effectiveness of Enteral Feeding Protocol on Clinical Outcomes in Critically Ill Patients: A Study Protocol for before-and-after Design. *Ann. Transl. Med.* **2016**, *4*, 308. [CrossRef]
51. Teng, J.; Xiang, L.; Long, H.; Gao, C.; Lei, L.; Zhang, Y. The Serum Citrulline and D-Lactate Are Associated with Gastrointestinal Dysfunction and Failure in Critically Ill Patients. *Int. J. Gen. Med.* **2021**, *14*, 4125–4134. [CrossRef] [PubMed]
52. Klanovicz, T.M.; Franzosi, O.S.; Nunes, D.S.L.; Loss, S.H.; Batassini, É.; Turra, E.E.; Teixeira, C.; Vieira, S.R.R. Acute Gastrointestinal Failure Is Associated with Worse Hemodynamic and Perfusion Parameters over 48 h after Admission in Patients with Septic Shock: Retrospective Cohort Study. *Nutr. Clin. Pract.* **2022**. *Epub ahead of print*. [CrossRef] [PubMed]
53. Zhang, D.; Li, Y.; Ding, L.; Fu, Y.; Dong, X.; Li, H. Prevalence and Outcome of Acute Gastrointestinal Injury in Critically Ill Patients: A Systematic Review and Meta-Analysis. *Medicine* **2018**, *97*, e12970. [CrossRef] [PubMed]

4. OMÓWIENIE PUBLIKACJI STANOWIĄCYCH PRACĘ DOKTORSKĄ

W pierwszej z prezentowanych publikacji *Tyszko M, Lipińska-Gediga M, Lemańska-Perek A, Kobylińska K, Gozdzik W, Adamik B. Intestinal Fatty Acid Binding Protein (I-FABP) as a Prognostic Marker in Critically Ill COVID-19 Patients.* Pathogens*. 2022; 11(12):1526. https://doi.org/10.3390/pathogens11121526* przedstawiono wyniki badania, w którym analizowano zmiany stężenia biomarkerów jelitowych u pacjentów z sepsą lub wstrząsem septycznym o etiologii wirusowej (COVID-19) leczonych na OIT.

Pandemia SARS-CoV 2 spowodowała nieznane dotychczas obciążenie dla ochrony zdrowia, również w zakresie intensywnej terapii. Stanowiło to szczególne wyzwanie, by pomóc jak największej liczbie pacjentów przy ograniczonych środkach. Do czasu pandemii COVID-19 sepsa o etiologii wirusowej występowała stosunkowo rzadko, od 1 do 6%. [26–28] W czasie pandemii codziennie na OIT przyjmowano kolejnych pacjentów z COVID-19, u których w przebiegu ciężkiego wirusowego zapalenia płuc trzeba było wdrożyć wentylację mechaniczną. U tych chorych często dochodziło do rozwoju niewydolność wielonarządowej i groźnych powikłań, ze zgonem włącznie. Już sama aktywacja ogólnoustrojowej odpowiedzi immunologicznej obserwowana w sepsie jest znacznym ryzykiem wystąpienia powikłań wielonarządowych, w tym zaburzeń żołądkowo - jelitowych. Dodatkowo możemy mówić o zależności płuca-jelita, gdzie poprzez chłonkę mogą przedostawać się molekuły prozapalne. Zakażenie wirusem SARS-CoV-2 jest jeszcze groźniejsze z punktu widzenia objawów żołądkowo – jelitowych, ponieważ wirus może bezpośrednio infekować komórki nabłonkowe przewodu pokarmowego, powodując zmiany w strukturze tkanki i rozpad bariery nabłonkowej. [29–33] Dlatego skuteczne metody monitorowania funkcji jelit w przebiegu ciężkich zakażeń wirusowych są bardzo potrzebne. Obecnie stosowana ocena stopnia uszkodzenia jelit opiera się głównie na objawach klinicznych (nietolerancja pokarmu, objętość zalegająca, nudności, wymioty, konieczność interwencji farmakologicznych lub chirurgicznych). Objawy te są manifestowane często z pewnym opóźnieniem, a ocena objawów pacjenta jest często subiektywna. Dlatego też tak ważne jest poszukiwanie biomarkerów, które jednoznacznie, obiektywnie i na wczesnym etapie choroby pozwolą określić ryzyko rozwoju niewydolności żołądkowo – jelitowej.

Celem prezentowanego badania było analiza dynamiki zmian stężeń wybranych biomarkerów u pacjentów chorych na COVID-19 leczonych na oddziale Intensywnej Terapii oraz opracowanie modelu wykorzystującego pomiar stężenia biomarkerów razem z rutynowymi danymi klinicznymi i laboratoryjnymi do przewidywania zgonu pacjenta.

Do badania włączono chorych przyjętych na OIT Uniwersyteckiego Szpitala Klinicznego we Wrocławiu w okresie od stycznia 2020r. do stycznia 2021r. Kryteria włączenia obejmowały zakażenie SARS-CoV-2 potwierdzone badaniem molekularnym, ciężki przebieg COVID-19 według wytycznych WHO [34] oraz zdiagnozowaną sepsę lub wstrząs septyczny przy przyjęciu na OIT. [1] Kryteria wykluczenia to wiek poniżej 18 roku życia oraz ciężkie choroby żołądkowo-jelitowe lub stan po dużej operacji w obrębie jamy brzusznej. Od każdego pacjenta pobrano próbkę krwi pięciokrotnie: przy przyjęciu (1 doba) oraz w każdym z 4 kolejnych dni leczenia (2, 3, 4, 5 doba). W pobranym materiale oznaczano cytrulinę i I-FABP.

Stan kliniczny pacjentów oceniany był na podstawie uznanych skal takich jak APACHE II oraz SOFA. Oceniano także stopień dysfunkcji żołądkowo-jelitowej na podstawie skali AGI (Acute Gastrointestinal Injury Score): AGI 0 – bez ryzyka rozwinięcia dysfunkcji żołądkowo-jelitowej (Gastrointestinal, GI); AGI 1 – ryzyko rozwinięcia dysfunkcji GI, ryzyko spowodowane m.in. stanem po operacji, ciężkim zakażeniem; AGI 2 – pierwsze objawy dysfunkcji GI lub dysfunkcja GI prawidłowo odpowiadająca na leczenie; AGI 3 – niewydolność GI nie ustępują pomimo stosowanego leczenia, utrzymująca się lub pogarszająca się; AGI 4 – pacjent, u którego doszło do poważnych zaburzeń GI wymagających interwencji chirurgicznej.

Podczas 12-miesięcznego okresu do badania włączono 69 kolejnych pacjentów przyjętych na OIT z zakażeniem SARS-CoV-2. Spośród nich 27 pacjentów zostało wyłączonych ze względu na przeszłość chorobową, a 42 pacjentów spełniało kryteria włączenia do badania. Z grupy 42 pacjentów wyłączono 2, których próbki uległy hemolizie i oznaczenie biomarkerów było niemożliwe. Ostatecznie analizie poddano 40 pacjentów; wszyscy pacjenci w grupie badanej zostali przyjęci na OIT z rozpoznanym ciężkim wirusowym zapaleniem płuc, zaś u 85% z nich stwierdzono ARDS (Acute Respiratory Distress Syndrome). Przy przyjęciu na OIT wszyscy chorzy wymagali natychmiastowego wspomagania układu oddechowego, w tym u 29 chorych (73%) zastosowano wentylacją mechaniczną i u 11 (27%) wentylacją mechaniczną i V-V ECMO. Pacjentów przyporządkowano do grup na podstawie przeżywalności na OIT:

Grupa badana 1: pacjenci, którzy przeżyli (N = 17, 42,5%)

Grupa badana 2: pacjenci, którzy zmarli (N = 23, 57,5%)

Stężenia biomarkera I-FABP były wyższe w Grupie 2 w porównaniu z pacjentami z Grupy 1. Różnica ta była istotna statystycznie w dniach 1-3 i nieistotna statystycznie w dniach 4 i 5. Nie obserwowano podobnego trendu dla cytruliny, a stężenia były zbliżone w kolejnych dniach obserwacji.

Stan kliniczny pacjentów przy przyjęciu na podstawie punktacji w skali APACHE II był istotnie lepszy w Grupie 1 niż w Grupie 2 (14 vs 19 pkt., p=0,018), podobnie w skali SOFA, lecz różnica ta była nieistotna statystycznie (7 vs 9 pkt., p=0,126). Przy przyjęciu na OIT stopień dysfunkcji żołądkowo-jelitowej oceniono w skali AGI na 1/2 punkty i nie wystąpiły statystycznie istotne różnice pomiędzy badanymi grupami. Spośród standardowych badań laboratoryjnych wykonywanych przy przyjęciu na OIT znacząco różniło się stężenie kreatyniny (Grupa 1 vs 2: 0,86 vs 1,30 mg/dl, p=0,015) oraz mleczanów (Grupa 1 vs 2: 1,51 vs 2,21 mmol/L, p=0,024). Znacząco dłużej trwała hospitalizacja pacjentów z Grupy 1 niż z Grupy 2 (29 dni vs 16 dni, p=0,004) oraz pobyt na OIT (27 dni vs 13 dni, p=0,049). Wszyscy pacjenci przy przyjęciu na OIT mieli sepsę, a u 40% rozpoznano wstrząs septyczny. Stężenia I-FABP były wyższe w grupie pacjentów we wstrząsie w porównaniu z pacjentami bez wstrząsu przez cały okres badania, a poziomy cytruliny były podobne (p>0.05).

Do oceny wartości predykcyjnej I-FABP wykorzystano analizę ROC (receiver operating characteristic), wyliczono AUC (area under the ROC curve). Wykazano, że wyjściowe stężenie I-FABP jest predyktorem 28-dniowej śmiertelności (AUC 0.710 (95% CI 0.547–0.873, p=0.011). Optymalna wartość progowa dla I-FABP jako predyktora śmiertelności wyniosła 668.57 pg/mL (czułość 0.739, 95% CI 0.376–0.740; swoistość 0.765, 95% CI 0.274–0.863). Wyliczoną wartość progową zastosowano do wykonania analizy przeżycia Kaplan-Meiera, w której potwierdzono znacząco niższą przeżywalność pacjentów w grupie z I-FABP powyżej 668.57 pg/ml (log-rank test p=0.007). Krzywa ROC dla cytruliny nie miała istotnej wartości prognostycznej (p>0.05). Wykonano dodatkowo wieloczynnikową analizę regresji logistycznej w celu stworzenia modelu dla prognozowania 28-dniowej śmiertelności w badanej grupie. Wykazano, że wyjściowy wysoki poziom I-FABP (>668.57 pg/mL), wysoka punktacja w skali APACHE II i obecność wstrząsu septycznego przy przyjęciu były istotnymi wskaźnikami złego rokowania.

Wyniki niniejszego badania wskazują, że u pacjentów z COVID-19 leczonych na OIT uszkodzenie komórek jelitowych jest potencjalnym czynnikiem, który może przyczynić się do złego wyniku leczenia. Poziomy I-FABP, ale nie cytruliny, były znacznie wyższe u pacjentów, którzy ostatecznie zmarli z powodu COVID-19, niż u osób, które przeżyły. Przeprowadzona analiza sugeruje, że pomiar stężenia I-FABP we krwi u chorych na OIT mógłby być cennym narzędziem rokowniczym u pacjentów z sepsą wirusową (COVID-19). Właściwa ocena ryzyka zgonu na podstawie markerów jelitowych może być przydatna przy podejmowaniu decyzji klinicznych dotyczących uszkodzeń jelit, diagnostyce obrazowej i potencjalnych interwencjach chirurgicznych u izolowanych pacjentów.

W drugiej z prezentowanych publikacji *Tyszko M, Lemańska-Perek A, Śmiechowicz J, Tomaszewska P, Biecek P, Gozdzik W, Adamik B. Citrulline, Intestinal Fatty Acid-Binding Protein and the Acute Gastrointestinal Injury Score as Predictors of Gastrointestinal Failure in Patients with Sepsis and Septic Shock.* Nutrients. *2023; 15(9):2100. https://doi.org/10.3390/nu15092100* analizowano zmiany stężeń wybranych biomarkerów uszkodzenia jelita we krwi pacjentów przyjętych na OIT z sepsą lub wstrząsem septycznym o etiologii bakteryjnej. Uzyskane wyniki porównano z oceną w skali AGI (Acute Gastrointestinal Injury) i sprawdzono przydatność biomarkerów w określaniu ryzyka rozwoju niewydolności żołądkowo-jelitowej.

Diagnostyka i monitorowanie funkcji układu pokarmowego na Intensywnej Terapii jest wyzwaniem, szczególnie z powodu braku dokładnych i łatwo dostępnych metod oceny klinicznej i laboratoryjnej. Stosowane powszechnie skale kliniczne oceniające stan pacjenta takie jak APACHE II, SOFA czy SAPS nie obejmują w swojej punktacji funkcji układu pokarmowego. Jedną z niewielu skal o potwierdzonej skuteczności klinicznej jest AGI – Acute Gastrointestinal Injury oraz stosunkowo niedawno zaproponowane GIDS – Gastrointestinal Dysfunction Score, które mają na celu ocenić ciężkość zaburzeń żołądkowo-jelitowych u pacjentów OIT[35].

Badanie przeprowadzono w grupie chorych leczonych w Klinice Anestezjologii i Intensywnej Terapii USK we Wrocławiu przyjętych z rozpoznaniem sepsy lub wstrząsu septycznego o etiologii bakteryjnej pomiędzy styczniem 2019 a grudniem 2019.
Kryteria włączenia obejmowały pacjentów ze zdiagnozowaną sepsą lub wstrząsem septycznym zgodnie z definicją SEPSIS-3 przy przyjęciu na OIT i podejrzenie/potwierdzenie zakażenia bakteryjnego. Kryteriami wykluczenia był wiek poniżej 18r. życia, ciąża, nieuleczalna choroba w schyłkowej fazie, przewidywany krótki pobyt w OIT (24 godziny lub mniej), poważne choroby układu pokarmowego w historii: przewlekłe choroby zapalne (takie jak wrzodziejące zapalenie jelita grubego, choroba Leśniowskiego-Crohna, wirusowe zapalenie wątroby), przewlekła niewydolność nerek.

W trakcie leczenia stan kliniczny pacjentów oceniano stosując standardowe skale kliniczne, APACHE II i SOFA. Oceniano także stopień dysfunkcji żołądkowo-jelitowej na podstawie skali AGI (Acute Gastrointestinal Injury Score): AGI 0 – bez ryzyka rozwinięcia dysfunkcji żołądkowo-jelitowej (Gastrointestinal, GI); AGI 1 – ryzyko rozwinięcia dysfunkcji GI, ryzyko spowodowane m.in. stanem po operacji, ciężkim zakażeniem; AGI 2 – pierwsze

objawy dysfunkcji GI lub dysfunkcja GI prawidłowo odpowiadające na leczenie; AGI 3 – niewydolność GI nie ustępują pomimo stosowanego leczenia, utrzymująca się lub pogarszająca się; AGI 4 – pacjent, u którego doszło do poważnych zaburzeń GI wymagających interwencji chirurgicznej.

Od każdego pacjenta pobrano próbkę krwi przy przyjęciu oraz w 3, 5, 7 i 10 dobie leczenia. W pobranym materiale oznaczano, oprócz standardowych badań, stężenia biomarkerów: cytruliny i I-FABP.

Do badania włączono 58 pacjentów septycznych. Spośród badanych pacjentów, 48% miało sepsę, a 52% wstrząs septyczny przy przyjęciu. Mediana APACHE II dla całej grupy wyniosła 25 punktów, SOFA 10 punktów, 71% pacjentów wymagało wentylacji mechanicznej przy przyjęciu na OIT, a 31% wymagało ciągłej terapii nerkozastępczej. Śmiertelność 28-dniowa wyniosła 38%. W celu porównania wartości biomarkerów u pacjentów OIT z sepsą i bez sepsy, do badania włączono grupę kontrolną: pacjentów po planowym zabiegu kardiochirurgicznym pomostowania aortalno-wieńcowego leczonych na OIT bezpośrednio po zabiegu (N=10). U tych pacjentów biomarkery mierzone były tylko raz w próbkach krwi pobieranych w pierwszej dobie leczenia na OIT. Bezpośrednio po operacji wszyscy pacjenci z grupy kontrolnej byli wentylowani mechanicznie, następnie ekstubowani i oddychali normalnie w ciągu 24 godzin od przyjęcia na OIT. Po ekstubacji wszyscy pacjenci z grupy kontrolnej otrzymywali standardową dietę pooperacyjną; w tej grupie nie stosowano żywienia dojelitowego ani żywienia pozajelitowego. U żadnego z pacjentów z grupy kontrolnej nie wystąpiły powikłania infekcyjne ani niewydolność nerek, wszyscy pacjenci przeżyli.

W grupie pacjentów septycznych wykazano istotnie mniejsze stężenie cytruliny w porównaniu z grupą kontrolną (26.98 nmol/mL vs. 39.82nmol/mL, $p<0.001$). Stężenie I-FABP było wyższe w grupie pacjentów septycznych niż w grupie kontrolnej, jednak różnica była nieistotna statystycznie. Następnie w grupie pacjentów septycznych porównano stężenie biomarkerów w dwóch podgrupach: pacjentów ze wstrząsem i bez wstrząsu septycznego. Stężenie cytruliny było znacząco niższe u pacjentów ze wstrząsem septycznym niż bez wstrząsu w dniach 1, 3, 5, 7 i 10, a stężenia I-FABP były znacząco wyższe w podgrupie pacjentów ze wstrząsem septycznym niż bez wstrząsu w 1 i 3 dniu badania.

Przy przyjęciu na OIT wśród pacjentów septycznych 69% pacjentów było ocenionych w skali AGI na 1 pkt. (ryzyko wystąpienia dysfunkcji żołądkowo-jelitowej), a 31% na AGI 2 (obecne objawy dysfunkcji żołądkowo-jelitowej). U żadnego pacjenta septycznego nie stwierdzono objawów niewydolności żołądkowo-jelitowej przy przyjęciu na OIT. Natomiast w trakcie pobytu na OIT niewydolność żołądkowo-jelitowej oceniana na skali jako AGI 3 wystąpiła u

2% badanych 3 dniu, u 29% w 5 dniu, u 47% w 7 dniu, u 50% pacjentów w 10 dniu badania. Żaden pacjent w grupie badanej nie został oceniony na skali AGI na 4 pkt. W grupie kontrolnej, ze względu na stan po poważnej operacji, wszyscy byli oceniani na AGI 1 przy przyjęciu na OIT, u żadnego pacjenta z tej grupy nie doszło do rozwoju dysfunkcji ani niewydolności żołądkowo-jelitowej podczas leczenia na OIT.

Następnie, aby ocenić związek między punktacją AGI a poziomem biomarkerów w sepsie, przeanalizowano zmiany poziomów cytruliny i I-FABP w czasie (od 1 do 10 dnia obserwacji) w trzech podgrupach, zgodnie z najwyższą punktacją AGI obliczoną podczas pobytu na OIT:

podgrupa 1 - pacjenci septyczni bez dysfunkcji żołądkowo-jelitowej, ale z ryzykiem jej wystąpienia (AGI 1)

podgrupa 2 - pacjenci septyczni z niewielką dysfunkcją żołądkowo-jelitową w trakcie pobytu, reagującą na leczenie (AGI 2)

podgrupa 3 – pacjenci septyczni z niewydolnością żołądkowo-jelitową, niereagujący na standardowe leczenie (AGI 3)

AGI 1 jako najwyższy wyliczony wynik wystąpił u 20 pacjentów, AGI 2 u 19, a u 19 pacjentów doszło do ciężkiego uszkodzenia układu pokarmowego i zdiagnozowano u nich niewydolność żołądkowo-jelitową (AGI 3). Poziom cytruliny znacząco zmniejszał się w czasie od 1 do 10 dnia obserwacji w podgrupie AGI 3 ($p = 0.027$), podczas gdy nie obserwowano podobnego spadku w podgrupie AGI 1 ($p=0,561$) ani AGI 2 ($p=0,406$). Stężenie I-FABP nie zmieniało się istotnie statystycznie w trakcie 10-dniowej obserwacji w żadnej z podgrup.

Porównania stężenia biomarkerów między podgrupami zróżną punktacją w skali AGI wykazały, że poziomy cytruliny były znacząco obniżone w podgrupie AGI 3 w porównaniu z AGI 2 w każdym punkcie czasowym, podczas gdy różnica między podgrupami AGI 3 i AGI 1 była znacząca tylko w dniu 10. Podobnych powiązań nie zaobserwowano w przypadku I-FABP.

Następnie analizowano wartość predykcyjną biomarkerów dla przewidywania rozwoju niewydolności żołądkowo-jelitowej (AGI 3) stosując analizę ROC i ocenę pola pod krzywą (AUC). Wykazano, że wyjściowe stężenie cytruliny jest istotnym wskaźnikiem rozwoju niewydolności żołądkowo-jelitowej w trakcie leczenia na OIT (AUC 0,738; 95% CI 0.607 – 0.869, $p<0.001$), a optymalna wartość progowa dla cytruliny we krwi wynosiła 27.03 nmol/mL, przy czułości 78.9% i swoistości 68.8%. I-FABP nie wykazywało skuteczności w przewidywaniu niewydolności żołądkowo-jelitowej u pacjentów septycznych ($p>0.05$).

W kolejnym etapie wykonano wieloczynnikową analizę regresji logistycznej w celu stworzenia modelu do przewidywania rozwoju niewydolności żołądkowo-jelitowej (AGI 3) w

trakcie pobytu na OIT. Analiza wykazała, że dwa czynniki: wystąpienie dysfunkcji żołądkowo-jelitowej (AGI 2) już przy przyjęciu (OR 5,03) oraz stężenie cytruliny we krwi (OR 0,93) są statystycznie znaczące dla modelu, którego dokładność wyniosła 0.72 (95% Cl 0.60 – 0.84), czułość 0.87 (95% Cl 0.78 – 0.96) i swoistość 0.42 (95% Cl 0.29 – 0.55). ROC AUC dla modelu wyniosło 0.77 (95% Cl 0.64 – 0.89).

Uzyskane wyniki zgodne są w hipotezą, według której pomiar stężenia biomarkerów uszkodzenia jelita pozwala na przewidywanie rozwoju niewydolności układu pokarmowego u pacjentów z sepsą i wstrząsem septycznym. Wyższa ocena w skali AGI, razem ze zmniejszonym stężeniem cytruliny we krwi były powiązane ze zwiększonym ryzykiem rozwinięcia niewydolności żołądkowo-jelitowej w trakcie pobytu na OIT. Niedokrwienie ściany jelita związana z hipoperfuzją we wstrząsie septycznym może skutkować uszkodzeniem bariery jelitowej i zmniejszeniem funkcjonalnej masy erytrocytów, czego potwierdzeniem jest obniżenie stężenia cytruliny i podwyższenie stężenia I-FABP w pierwszych dniach od przyjęcia na OIT. Należy jednak zaznaczyć, że poziom cytruliny i I-FABP może być podwyższony w wielu stanach związanych z zaburzeniami funkcji przewodu pokarmowego, nerek czy wątroby, które mogą występować jednocześnie u pacjentów z sepsą.

5. PODSUMOWANIE

Przedstawione publikacje skupiają się na ocenie przydatności pomiarów biomarkerów uszkodzenia jelita u pacjentów z sepsą lub wstrząsem septycznym, hospitalizowanych na OIT, powiązując je z punktacjami skal klinicznych (APACHE II, SOFA, parametrami klinicznymi i laboratoryjnymi ocenianymi w pierwszych dobach pobytu, rozwojem niewydolności układu pokarmowego w trakcie pobytu na OIT i śmiertelnością. Możliwość korzystania z dodatkowych narzędzi, szczególnie u pacjentów sedowanych, u których objawy ze strony układu pokarmowego są często słabiej wyrażone i trudne do rozpoznania, mogłyby ułatwiać ocenę funkcjonowania przewodu pokarmowego i umożliwiać wczesne interwencje oraz wdrożenie adekwatnego leczenie i monitorowanie przed wystąpieniem poważnych powikłań. Poniżej przedstawiono najważniejsze wnioski z prezentowanego cyklu prac:

1. U pacjentów przyjmowanych na OIT z powodu sepsy lub wstrząsu septycznego obserwowane są zmiany w stężeniach biomarkerów jelitowych, co świadczy o uszkodzeniu komórek nabłonka jelita już we wczesnych dobach od przyjęcia.
2. Występuje zależność między wysokim stężeniem I-FABP, a śmiertelnością pacjentów przyjętych na OIT z powodu sepsy wirusowej. Pomiar stężenia biomarkerów mógłby być użytecznym narzędziem do przewidywania ryzyka zgonu u pacjentów z sepsą wirusową (COVID-19).
3. Występuje zależność między obniżonym stężeniem cytruliny a rozwinięciem się niewydolności żołądkowo-jelitowej, wyrażonej jako podwyższony wynik w skali AGI (AGI 3), u pacjentów przyjmowanych na OIT z powodu sepsy lub wstrząsu septycznego o etiologii bakteryjnej. Oznaczanie powyższego biomarkera uszkodzenia jelita mogłoby być przydatne przy podejmowaniu decyzji terapeutycznych dotyczących czasu, rodzaju i ilości wprowadzanego żywienia enteralnego oraz rozszerzania diagnostyki.
4. Stosując wieloczynnikową analizę regresji logistycznej utworzony został model do przewidywania przeżycia w grupie pacjentów przyjętych na OIT z powodu sepsy wirusowej. Wyjściowy wysoki poziom I-FABP, wysoka punktacja w skali APACHE II i obecność wstrząsu septycznego przy przyjęciu na OIT były istotnymi wskaźnikami złego rokowania.
5. Przeprowadzone badania pozwoliły na określenie wartości progowych dla badanych biomarkerów: wyjściowe stężenie I-FABP >668.57 pg/mL ma istotne znaczenie rokownicze dla przewidywania śmiertelności w grupie pacjentów z sepsą wirusową (COVID-19) oraz wyjściowe stężenie cytruliny <27.03 nmol/mL ma istotne znaczenie rokownicze dla przewidywania rozwoju niewydolności układu pokarmowego w grupie

pacjentów z sepsą bakteryjną. Ze względu na stosunkowo małą liczebność badanych grup, w celu potwierdzenia tych wartości, badania należy kontynuować na większych grupach pacjentów.

6. Badanie stężenia biomarkerów powinno być traktowane jako potencjalne narzędzie wspierające, a nie zastępujące stosowane skale kliniczne APACHE II i SOFA oraz inne parametry kliniczne i laboratoryjne rutynowo stosowane w praktyce klinicznej na OIT.

6. STRESZCZENIE

Wstęp: Zaburzenia żołądkowo-jelitowe są częste u pacjentów septycznych i powiązane ze zwiększoną śmiertelnością. Diagnostyka i monitorowanie funkcji układu pokarmowego na oddziale intensywnej terapii pozostaje wyzwaniem dla klinicystów. W związku z tym powstaje pytanie, czy pomiar biomarkerów może być przydatny do oceny uszkodzenia bariery jelitowej u pacjentów z sepsą.

Cel pracy: Głównym celem pracy była analiza zmian stężeń biomarkerów uszkodzenia jelita i ocena przydatności pomiarów stężeń biomarkerów do przewidywania wyników leczenia u pacjentów z sepsą lub wstrząsem septycznym.

Materiał i metody: Próbki krwi pacjentów pobierano przy przyjęciu na OIT oraz w kolejnych dniach pobytu. Przeprowadzono analizę dla cytruliny oraz I-FABP - potencjalnych markerów uszkodzenia komórek jelita. Do oceny stanu klinicznego pacjentów stosowano skale APACHE II, SOFA oraz AGI. Zgodnie ze skalą AGI, definiowano AGI 2 jako dysfunkcję żołądkowo-jelitową, a AGI 3 jako niewydolność żołądkowo-jelitową.

Wyniki: Pomiary stężeń biomarkerów uszkodzenia jelit wykazywały nieprawidłowe wartości u pacjentów septycznych i we wstrząsie septycznym. W grupie pacjentów z sepsą o etiologii bakteryjnej stężenia cytruliny w grupie septycznej były niższe niż w grupie kontrolnej (pacjenci OIT bez infekcji). Niższe wartości cytruliny i wyższe I-FABP odnotowano u pacjentów septycznych ze wstrząsem w porównaniu z pacjentami bez wstrząsu. Cytrulina była niższa u pacjentów z niewydolnością przewodu pokarmowego (AGI 3) w porównaniu z AGI 2. Analiza regresji wykazała, że pacjenci z wysokim ryzykiem wystąpienia niewydolności żołądkowo-jelitowej oraz z ryzykiem zgonu wykazywali znacząco odbiegające od normy wyniki pomiarów stężeń cytruliny i I-FABP.

Wnioski: Zaprezentowane prace wskazują, że stężenia biomarkerów uszkodzenia jelit, takich jak cytrulina i I-FABP, znacznie odbiegają od normy u pacjentów leczonych na OIT z powodu sepsy i wstrząsu septycznego. Istnieje związek między rokowaniem pacjenta i rozwinięciem niewydolności układu pokarmowego, a poziomem powyższych biomarkerów. W przypadku pacjentów septycznych leczonych na OIT ocena układu pokarmowego sprawia wiele trudności nawet dla doświadczonych klinicystów. Pomiar stężenia biomarkerów może stać się bardzo użytecznym narzędziem przy podejmowaniu decyzji terapeutycznych, decydowaniu o konieczności interwencji chirurgicznej lub rozpoczęciu żywienia enteralnego.

7. SUMMARY

Introduction: Gastrointestinal disorders are common in septic patients and are associated with increased mortality. Diagnosis and monitoring of gastrointestinal disorders in the ICU ward is a challenge for physicians. Therefore, the question arises whether the measurement of biomarkers can be useful to assess intestinal barrier damage in patients with sepsis.

Aim of the study: The main goal of the study was to analyze changes in the concentration of biomarkers of intestinal injury and to assess their usefulness of biomarker concentration measurements to predict treatment outcomes in patients with sepsis or septic shock.

Material and Methods: Blood samples were collected for biomarker analysis on admission to the ICU and on the following days of stay. Citrulline and I-FABP - potential markers of intestinal cell damage - were analyzed. The APACHE II, SOFA and AGI scores were used to assess the clinical condition of the patients. According to the AGI score, AGI 2 was defined as gastrointestinal dysfunction and AGI 3 as gastrointestinal failure.

Results: Measurements of intestinal injury biomarkers showed abnormal values in patients with septic and septic shock. In the group of patients with bacterial sepsis, citrulline concentration in the septic group was lower than in control group (ICU patients without an infection). Lower values of citrulline and higher of I-FABP were noted in septic patients in shock compared to non- shock patients. Citrulline was lower in patients with gastrointestinal failure (AGI 3) compared to patients with AGI 2. Multivariate regression analysis showed that patients at high risk of developing gastrointestinal failure and patients at high risk of death had significantly abnormal levels of citrulline and I-FABP.

Conclusion: The findings of the studies indicate that the concentrations of biomarkers of intestinal injury, such as citrulline and I-FABP, are significantly altered in ICU patients treated for sepsis and septic shock. There is a relationship between the patient's outcome or developing gastrointestinal failure and the level of these biomarkers. In ICU patients with sepsis, the examination of gastrointestinal symptoms may be difficult even for experienced physicians. Measurement of biomarker concentrations can become a very useful tool in making therapeutic decisions, decisions about the need for surgical intervention or the start of enteral feeding.

8. PIŚMIENNICTWO

[1] Singer, M.; Deutschman, C. S.; Seymour, C.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G. R.; Chiche, J. D.; Coopersmith, C. M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA - Journal of the American Medical Association*. American Medical Association February 23, 2016, pp 801–810. https://doi.org/10.1001/jama.2016.0287.

[2] Hu, B.; Sun, R.; Wu, A.; Ni, Y.; Liu, J.; Guo, F.; Ying, L.; Ge, G.; Ding, A.; Shi, Y.; et al. Severity of Acute Gastrointestinal Injury Grade Is a Predictor of All-Cause Mortality in Critically Ill Patients: A Multicenter, Prospective, Observational Study. *Crit Care*, **2017**, *21* (1). https://doi.org/10.1186/s13054-017-1780-4.

[3] Adelman, M. W.; Woodworth, M. H.; Langelier, C.; Busch, L. M.; Kempker, J. A.; Kraft, C. S.; Kraft, C. S.; Martin, G. S.; Martin, G. S. The Gut Microbiome's Role in the Development, Maintenance, and Outcomes of Sepsis. *Critical Care*. 2020. https://doi.org/10.1186/s13054-020-02989-1.

[4] Fay, K. T.; Klingensmith, N. J.; Chen, C. W.; Zhang, W.; Sun, Y.; Morrow, K. N.; Liang, Z.; Burd, E. M.; Ford, M. L.; Coopersmith, C. M. The Gut Microbiome Alters Immunophenotype and Survival from Sepsis. *FASEB Journal*, **2019**, *33* (10). https://doi.org/10.1096/fj.201802188R.

[5] Adamik, B.; Gozdzik, W.; Jakubczyk, D.; Welna, M.; Kübler, A. Coagulation Abnormalities Identified by Thromboelastometry in Patients with Severe Sepsis: The Relationship to Endotoxemia and Mortality. *Blood Coagulation and Fibrinolysis*, **2017**, *28* (2). https://doi.org/10.1097/MBC.0000000000000572.

[6] Bray, M. A.; Sartain, S. E.; Gollamudi, J.; Rumbaut, R. E. Microvascular Thrombosis: Experimental and Clinical Implications. *Translational Research*. 2020. https://doi.org/10.1016/j.trsl.2020.05.006.

[7] Dempfle, C. E. Coagulopathy of Sepsis. *Thrombosis and Haemostasis*. 2004. https://doi.org/10.1160/TH03-03-0182.

[8] De Backer, D.; Creteur, J.; Preiser, J. C.; Dubois, M. J.; Vincent, J. L. Microvascular Blood Flow Is Altered in Patients with Sepsis. *Am J Respir Crit Care Med*, **2002**, *166* (1). https://doi.org/10.1164/rccm.200109-016OC.

[9] Ellis, C. G.; Jagger, J.; Sharpe, M. The Microcirculation as a Functional System. *Critical Care*. 2005. https://doi.org/10.1186/cc3751.

[10] Altshuler, A. E.; Lamadrid, I.; Li, D.; Ma, S. R.; Kurre, L.; Schmid-Schönbein, G. W.; Penn, A. H. Transmural Intestinal Wall Permeability in Severe Ischemia after Enteral Protease Inhibition. *PLoS One*, **2014**, *9* (5). https://doi.org/10.1371/journal.pone.0096655.

[11] Jiang, L.-Y.; Zhang, M.; Zhou, T.-E.; Yang, Z.-F.; Wen, L.-Q.; Chang, J.-X. Changes of the Immunological Barrier of Intestinal Mucosa in Rats with Sepsis. *World J Emerg Med*, **2010**, *1* (2).

[12] Reintam Blaser, A.; Preiser, J. C.; Fruhwald, S.; Wilmer, A.; Wernerman, J.; Benstoem, C.; Casaer, M. P.; Starkopf, J.; van Zanten, A.; Rooyackers, O.; et al. Gastrointestinal Dysfunction in the Critically Ill: A Systematic Scoping Review and Research Agenda Proposed by the Section of Metabolism, Endocrinology and Nutrition of the European Society of Intensive Care Medicine. *Critical care (London, England)*. NLM (Medline) May 15, 2020, p 224. https://doi.org/10.1186/s13054-020-02889-4.

[13] Doudakmanis, C.; Bouliaris, K.; Kolla, C.; Efthimiou, M.; Koukoulis, G. D. Bacterial Translocation in Patients Undergoing Major Gastrointestinal Surgery and Its Role in Postoperative Sepsis. *World J Gastrointest Pathophysiol*, **2021**, *12* (6). https://doi.org/10.4291/wjgp.v12.i6.106.

[14] Crapser, J.; Ritzel, R.; Verma, R.; Venna, V. R.; Liu, F.; Chauhan, A.; Koellhoffer, E.; Patel, A.; Ricker, A.; Maas, K.; et al. Ischemic Stroke Induces Gut Permeability and Enhances Bacterial Translocation Leading to Sepsis in Aged Mice. *Aging*, **2016**, *8* (5). https://doi.org/10.18632/aging.100952.

[15] Reintam Blaser, A.; Padar, M.; Mändul, M.; Elke, G.; Engel, C.; Fischer, K.; Giabicani, M.; Gold, T.; Hess, B.; Hiesmayr, M.; et al. Development of the Gastrointestinal Dysfunction Score (GIDS) for Critically Ill Patients – A Prospective Multicenter Observational Study (ISOFA Study). *Clinical Nutrition*, **2021**, *40* (8), 4932–4940. https://doi.org/10.1016/j.clnu.2021.07.015.

[16] Curis, E.; Nicolis, I.; Moinard, C.; Osowska, S.; Zerrouk, N.; Bénazeth, S.; Cynober, L. Almost All about Citrulline in Mammals. *Amino Acids*. 2005. https://doi.org/10.1007/s00726-005-0235-4.

[17] Noordally, S. O.; Sohawon, S.; Semlali, H.; Michely, D.; Devriendt, J.; Gottignies, P. Is There a Correlation between Circulating Levels of Citrulline and Intestinal Dysfunction in the Critically Ill? *Nutrition in Clinical Practice*, **2012**, *27* (4). https://doi.org/10.1177/0884533612449360.

[18] Fagoni, N.; Piva, S.; Marino, R.; Chiarini, G.; Ferrari, D.; Grespi, E.; Bertuetti, R.; Barbieri, S.; Latronico, N.; Rasulo, F. The IN-PANCIA Study: Clinical Evaluation of Gastrointestinal Dysfunction and Failure, Multiple Organ Failure, and Levels of Citrulline in Critically Ill Patients. *J Intensive Care Med*, **2020**, *35* (3). https://doi.org/10.1177/0885066617742594.

[19] Crenn, P.; Neveux, N.; Chevret, S.; Jaffray, P.; Cynober, L.; Melchior, J. C.; Annane, D. Plasma L-Citrulline Concentrations and Its Relationship with Inflammation at the Onset of Septic Shock: A Pilot Study. *J Crit Care*, **2014**, *29* (2). https://doi.org/10.1016/j.jcrc.2013.11.015.

[20] Piton, G.; Manzon, C.; Monnet, E.; Cypriani, B.; Barbot, O.; Navellou, J. C.; Carbonnel, F.; Capellier, G. Plasma Citrulline Kinetics and Prognostic Value in Critically Ill Patients. *Intensive Care Med*, **2010**, *36* (4), 702–706. https://doi.org/10.1007/s00134-010-1751-6.

[21] Luiking, Y. C.; Poeze, M.; Ramsay, G.; Deutz, N. E. P. Reduced Citrulline Production in Sepsis Is Related to Diminished de Novo Arginine and Nitric Oxide Production. *American Journal of Clinical Nutrition*, **2009**, *89* (1). https://doi.org/10.3945/ajcn.2007.25765.

[22] Kanda, T.; Fujii, H.; Tani, T.; Murakami, H.; Suda, T.; Sakai, Y.; Ono, T.; Hatakeyama, K. Intestinal Fatty Acid-Binding Protein Is a Useful Diagnostic Marker for Mesenteric Infarction in Humans. *Gastroenterology*, **1996**, *110* (2). https://doi.org/10.1053/gast.1996.v110.pm8566578.

[23] Derikx, J. P. M.; Poeze, M.; Van Bijnen, A. A.; Buurman, W. A.; Heineman, E. Evidence for Intestinal and Liver Epithelial Cell Injury in the Early Phase of Sepsis. *Shock*, **2007**, *28* (5). https://doi.org/10.1097/shk.0b013e3180644e32.

[24] Padar, M.; Starkopf, J.; Starkopf, L.; Forbes, A.; Hiesmayr, M.; Jakob, S. M.; Rooijackers, O.; Wernerman, J.; Ojavee, S. E.; Reintam Blaser, A. Enteral Nutrition and Dynamics of Citrulline and Intestinal Fatty Acid-Binding Protein in Adult ICU Patients. *Clin Nutr ESPEN*, **2021**, *45*. https://doi.org/10.1016/j.clnesp.2021.07.026.

[25] Sekino, M.; Funaoka, H.; Sato, S.; Okada, K.; Inoue, H.; Yano, R.; Matsumoto, S.; Ichinomiya, T.; Higashijima, U.; Matsumoto, S.; et al. Intestinal Fatty Acid-Binding Protein Level as a Predictor of 28-

Day Mortality and Bowel Ischemia in Patients with Septic Shock: A Preliminary Study. *J Crit Care*, **2017**, *42*, 92–100. https://doi.org/10.1016/j.jcrc.2017.07.012.

[26] Sakr, Y.; Jaschinski, U.; Wittebole, X.; Szakmany, T.; Lipman, J.; Ñamendys-Silva, S. A.; Martin-Loeches, I.; Leone, M.; Lupu, M.-N.; Vincent, J.-L. Sepsis in Intensive Care Unit Patients: Worldwide Data from the ICON Audit. https://doi.org/10.1093/ofid/ofy313/5193171.

[27] Lin, G. L.; McGinley, J. P.; Drysdale, S. B.; Pollard, A. J. Epidemiology and Immune Pathogenesis of Viral Sepsis. *Frontiers in Immunology*. Frontiers Media S.A. September 27, 2018. https://doi.org/10.3389/fimmu.2018.02147.

[28] Smiechowicz, J.; Barteczko-Grajek, B.; Adamik, B.; Bojko, J.; Gozdzik, W.; Lipinska-Gediga, M. Influenza in Patients with Respiratory Failure Admitted to Intensive Care Units in Poland and the Use of Extracorporeal Respiratory Support: A Survey-Based Multicenter Study. *BMC Infect Dis*, **2021**, *21* (1). https://doi.org/10.1186/s12879-021-06672-w.

[29] Lamers, M. M.; Beumer, J.; Vaart, J. Van Der; Knoops, K.; Puschhof, J.; Breugem, T. I.; Ravelli, R. B. G.; Schayck, J. P. Van; Mykytyn, A. Z.; Duimel, H. Q.; et al. SARS-CoV-2 Productively Infects Human Gut Enterocytes. *Science (1979)*, **2020**, *369* (6499), 50–54. https://doi.org/10.1126/science.abc1669.

[30] Li, W.; Moore, M. J.; Vasllieva, N.; Sui, J.; Wong, S. K.; Berne, M. A.; Somasundaran, M.; Sullivan, J. L.; Luzuriaga, K.; Greeneugh, T. C.; et al. Angiotensin-Converting Enzyme 2 Is a Functional Receptor for the SARS Coronavirus. *Nature*, **2003**, *426* (6965). https://doi.org/10.1038/nature02145.

[31] Zhou, P.; Yang, X. Lou; Wang, X. G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H. R.; Zhu, Y.; Li, B.; Huang, C. L.; et al. A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin. *Nature*, **2020**, *579* (7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7.

[32] Harmer, D.; Gilbert, M.; Borman, R.; Clark, K. L. *Quantitative MRNA Expression Pro¢ling of ACE 2, a Novel Homologue of Angiotensin Converting Enzyme*.

[33] Xiao, F.; Tang, M.; Zheng, X.; Liu, Y.; Li, X.; Shan, H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology*, **2020**, *158* (6), 1831-1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055.

[34] World Health Organization. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance, 13 March 2020.

[35] Reintam Blaser A, Padar M, Mändul M, et al. Development of the Gastrointestinal Dysfunction Score (GIDS) for critically ill patients - A prospective multicenter observational study (iSOFA study). *Clin Nutr*. **2021**;40(8):4932-4940. doi:10.1016/j.clnu.2021.07.015

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OŚWIADCZENIE

Oświadczam, że w pracy *Tyszko M, Lipińska-Gediga M, Lemańska-Perek A, Kobylińska K, Gozdzik W, Adamik B. Intestinal Fatty Acid Binding Protein (I-FABP) as a Prognostic Marker in Critically Ill COVID-19 Patients.* Pathogens. *2022; 11(12):1526.* https://doi.org/10.3390/pathogens11121526 mój udział polegał na opracowaniu metodologii badania i wykonaniu pomiarów stężeń biomarkerów.

Podpis

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Podpis

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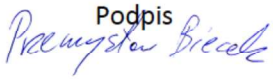
Podpis



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