ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 26(1): 157-164, 2021
DOI: 10.5505/ejm.2021.25986

Effects of Parenteral Glutamine Supplementation on Endocan Levels in Septic Patients

Basak Kilic[1], Hulya Cicek[2], Ayşe Özlem Mete[3], Suleyman Ganidagli[4], Berna Kaya Ugur[4*]

[1]Gaziantep Ersin Arslan Training Hospital, Department of Anesthesiology and Reanimation, Gaziantep, Turkey
[2]Gaziantep University, Faculty of Medicine, Department of Biochemistry, Gaziantep, Turkey
[3]Gaziantep University, Faculty of Medicine, Department of Infectious Diseases, Gaziantep, Turkey
[4]Gaziantep University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Gaziantep, Turkey

ABSTRACT

The purpose of this study was to investigate the effect of parenteral Glutamine (Gln) supplementation on Endocan levels in patients with sepsis receiving parenteral nutrition.

A total of 60 patients with a diagnosis of sepsis were enrolled to the study. Patients were randomly divided into two groups to receive either standard parenteral nutrition or standard parenteral nutrition plus the parenteral dipeptide Gln-Ala. The Gln-Ala dipeptide dosage was calculated to be 0.35 mg/kg. Demographic data, CRP, ESR, lactate, WBC, Endocan serum levels, APACHE II scores at admission to ICU, and SOFA scores at day 0, 3rd and 7th days were all recorded. The outcomes of the patients were also recorded after 28-day follow up.

Results: There was no statistically significant difference between the Endocan values of patients receiving parenteral dipeptide Gln-Ala plus the standard parenteral nutrition vs standard parenteral nutrition. Also, there was no significant difference in mortality and septic shock development rates between the two groups.

According to our results, there was not a significant difference regarding outcomes of the patients in both study groups, including mortality rates and there was no beneficial effect of indiscriminate GLN supplementation. Further prospective studies with larger sample size are needed in order to make conclusive comments that there is no beneficial effect of administering GLN.

Key Words: Endocan, Glutamine, Parenteral Nutrition, Sepsis-3

Introduction

Sepsis is a known lethal clinical situation, in which mortality and abnormal organ functions are mainly due to dysfunction of the endothelium. Contractions induced by endothelial barrier during the infectious state, deterioration of protective regulatory mechanisms, and changes in the permeability of endothelium are among factors that take part in the dysfunction of the endothelium (1). Leukocytes help access areas of inflammation and various factors are secreted from endothelial tissue (2). Endocan becomes significant in endothelium following sepsis. Endocan, in other words, endothelial cell-specific molecule-1 (ESM-1), is a molecule with a proteoglycan structure that is found fundamentally in human endothelium. In septic situations, the endocan release is a reaction to cytokines and vascular endothelial growth factor (3). Glutamine (GLN) is an essential amino acid in the course of various diseases and in reactions to the state of stress (4,5). Therefore, GLN may have theoretical advantageous effects on intermediary metabolism when chronic dysfunction of endothelium is present and used as an alimentary supplement in critical patients (6,7). GLN supplementation can also stabilize vascular function and improve mitochondrial abnormal function. GLN administration may help to replenish the end reactions of the Krebs cycle, which possibly restore vascular functions. But there are controversial data regarding glutamine supplementation. While some studies state that GLN supplementation improves endothelial functions in sepsis, others mention the potentially harmful effects of GLN supplementation in sepsis (8,9).

In this study, we compared the effect of intravenous GLN supplementation to endocan levels of patients with sepsis.

*Corresponding Author: Dr. Berna Kaya Ugur, Gaziantep Universitesi, Tıp Fakültesi, Sahinbey Uygulama ve Arastirma Hastanesi, Anesteziyoloji ve Reanimasyon AD, 27090, Sahinbey, Gaziantep, Turkey
E-mail: bernakayaugur@hotmail.com, Tel: +90 (532) 572 76 51
ORCID ID: Basak Kilic: 0000-0002-8252-9850, **Hulya Cicek:** 0000-0003-1045-9619, **Ayşe Özlem Mete:** 0000-0003-0994-4465, **Suleyman Ganidagli:** 0000-0002-9644-7688, **Berna Kaya Ugur:** 0000-0003-0044-363X
Received: 25.10.2020, **Accepted:** 25.11.2020

Materials and Methods

This study is performed after the approval of the Gaziantep University Clinical Researches Ethical Committee (Approval no: 2016/154), according to the Helsinki Declaration in a prospective, single-centered, randomized, and controlled manner. Verbal and written informed consent was obtained from patients or their next-of-kin before enrollment in the study. The study is performed in Gaziantep University Faculty of Medicine Reanimation and Intensive Care Unit between June 1st, 2016, and March 1st, 2017. A total of 60 patients with a diagnosis of sepsis were enrolled in the study.

Diagnosis of sepsis was made parallel to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) (10).

Inclusion criteria were patients, older than 18 years with sepsis and receiving parenteral nutrition. Patients who were in septic shock at admission to ICU, malignant diseases, rheumatologic diseases, tuberculosis, HIV positive, hepatic and renal failure, congestive heart failure, and pregnant were excluded from the study. Patients who meet the inclusion criteria were randomized into two groups via computerized randomization method to Group G (n=30) (Gln=standard parenteral nutrition+iv Gln) and Group C (n=30) (control= standard parenteral nutrition).

Demographic data of the patients in both groups including age, gender, primary diagnosis at admission, WBC, ESR, CRP, lactate levels at 0, 3rd, 7th day, SOFA scores at 0, 3rd, 7th day, and APACHE-II scores at admission were recorded. Day 0 was accepted as the day of the diagnosis of sepsis. Standard sepsis therapy according to the guidelines (10) had been initiated immediately after the diagnosis. Patients in Group G (n=30) had standard olive oil-based parenteral nutrition solution (OliClinomel® N7-1000E2), which is a 1500 ml triple chambered parenteral nutrition bag that includes olive oil-based lipid solution, amino acid solution, and glucose solution. It contains Lipid emulsion, 400 ml Amino acid solution, 800 ml Glucose solution of 800 ml in a total of 2000ml. Composition of a 1000 ml bag (g) N4-550E N7-1000E

Refined olive oil + refined soya oil* 20.00 40.00, Alanine 4.56 8.28, Arginine 2.53 4.60, Glycine 2.27 4.12, Histidine 1.92, Isoleucine 2.40, Leucine 2.92, Lysine 2.32, Methionine 1.60,Phenylalanine 2.24, Proline 2.72, Serine 2.00, Threonine 1.68, Tryptophan 0.72, Tyrosine 0.16, Valine 2.32, Sodium Acetate, 3H2O 2.45,Sodium glycerophosphate, 5H2O 2.14, Potassium chloride 1.79 Magnesium chloride, 6H2O 0.45, Anhydrous glucose 160.00, (As glucose monohydrate) (88.00) (176.00), Calcium chloride, 2H2O 0.30. Caloric needs of the patients were calculated according to the Harris-Benedict formula (11) as follows:

Basal energy needs (BEN) for women=65,5+(9,5x body weight/kg+1,8x height/cm)-(4,7x age/year)

Basal energy needs (BEN) for men=66+(13,7x body weight/kg+5,0x height/cm)-(6,8x age/year)

Glutamine-alanine dipeptide solution (Dipeptiven® 20%, Fresenius Kabi İlaç san. ve Tic. Ltd. Şti., Türkiye) had been supplemented to patients in Group G with a dose of 0.35 mg/kg. 100 ml of Dipeptiven® contains 20 g N (2)-L-alanyl-L-glutamine (=8.20 g L-alanine, 13.46 g L-glutamine).

Blood samples (4 ml) were obtained from patients on the first day of sepsis diagnosis, (Day 0), and at the following 3rd and 7th days at ICU.

Blood samples were centrifuged at 3000 rpm for 20 minutes and sera were kept at -80°C until the day of endocan analysis. ELISA method was utilized for the analyses and measurements were done twice for each sample. Quantitative assessment of levels of Human endocan, according to the instructions of the manufacturer (Shanghai Sunred Biological Technology Co., Ltd, Shanghai/China) with catalog number 201-12-1978. The double-antibody sandwich enzyme immunoassay technique was utilized for the analysis. All concentration/absorption graphic curves and calculations were performed on the program of the Biotek ELx808 (Winooski, Vermont, USA) device. The sensitivity of the test for human endocan was determined as 7.506 ng/L and detection range of 8-2000 ng/L. Normal range of endocan is 0.15 and 2.5 ng/L. Intra-assay and inter-precision assay variation coefficients were found as 8.2% and 5.4%, respectively.

Statistical Analysis: A comparison of the variables between the two groups was assessed with the Student t-test when distribution was normal, and the Mann Whitney U test was used to compare non-normally distributed variables in 2 groups. Freidman and Dunn multiple comparison tests were performed for comparison of non-normal data between 3 different time points.

Descriptive parameters were presented as frequency, percentage (%), and mean ± standard derivation (mean ± SD), median (min, max and interquartile range). Statistical analysis was

completed with SPSS for Windows version 22.0 and a p-value < 0.05 was recognized as statistically significant.

Results

Sixty patients were enrolled in the content of study according to the Sepsis-3 criteria (9). There was no significant difference between the two groups when age and gender are compared (p=0.019). Regardless of the group, 51.7% of the patients included in the study were male (n=31) and 48.3% (n=29) were female. The primary clinical diagnoses of the patients at admission to ICU are presented in Table 1.

Infection sources of sepsis regardless of groups are given in Table 2.

Day 0 APACHE-II scores of the patients in group G and C were 18,96±5,67 and 18,53±5,21; respectively. There was no statistically significant difference in terms of APACHE-II scores between the groups (p=0.75). No difference was revealed between the groups regarding WBC, ESR, CRP, and lactate levels at day 0, 3rd, and 7th days (Table 3). SOFA day 0 values of Group C were significantly higher than the Group G (p=0.02). SOFA scores of the patients at day 0, 3rd, and 7th day are also shown in Table 3. Distribution and comparison of SOFA scores between the groups by days are given in Table 4.

According to intra-group analysis, a significant difference between SOFA scores on day 0 and 7th day in Group G was revealed (p=0.004). In the control group, a significant increase was observed in both the 3rd and 7th days compared to day 0 (p=0.012 and 0.02). The intra-group comparison of SOFA scores at day 0, 3rd, and 7th days is demonstrated in Table 4.

No significant difference was observed between Groups G and C in terms of Endocan levels recorded at 0, 3rd, and 7th days. A comparison of the endocan levels of the two groups is demonstrated in Table 5.

There was septic shock in 15 patients (50%) in group G, and 19 patients in group C (63.3%). No statistically significant difference was revealed between the groups in terms of the development of septic shock (p=0.29).

Total mortality rate of the patients were 60% (n=36). Among these patients 31.6% (n=19) was in Group G, 28.3% (n=17) was in Group C (p=0.79).

Although there was no statistically significant difference, the average of levels of Endocan at 3rd day was found higher in Group G than Group C, and also Endocan 7th-day values were found to be higher for Group C in patients compared to Group G.

Also, there was no statistically significant difference between the patients that died in the groups G and C regarding the mean Endocan values (ng/L) of on day 0 (220,29±168,04 vs 175,11±195,08), 3rd day (250,99±183,47 vs 171,71±156,73) and 7th day (216,83±143,18 vs 193,55±146,37) according to study days (days 0, 3, and 7, p = 0.129, 0.0129, 0.707; respectively). According to the three study days, a significant difference was observed among the groups in terms of the mean Endocan values of the patients who were died (days 0, 3rd, 7th respectively p = 0.129, 0.0129, 0.707).

Discussion

Sepsis is a fatal disease process with endothelial dysfunction taking part as the major contributor. There are different proposed mechanisms in the pathogenetic pathways related to the dysfunction of endothelial tissue in severe sepsis. Some of these are contractions induced by endothelial barrier during the infectious state, deterioration of protective regulatory mechanisms and changes in the permeability of endothelium are among factors that take part in the dysfunction of the endothelium (1,12). Endocan is one of the landmarks that are observed in human endothelium during sepsis and is synthesized from endothelial cells. It has a proteoglycan structure with soluble properties. Cytokines boost Endocan expression during the pathogenesis of sepsis.

GLN is an alimentary supplement used in patients with serious health conditions such as cancer and hematologic disorders. GLN is a precursor of a-ketoglutarate, may have advantageous interactions on intermediary metabolism when there is a chronic dysfunction of the endothelium.

GLN administration may help to replenish the end reactions of the Krebs cycle, which possibly restore vascular functions and also enhance mitochondrial dysfunction.

L-Glutamine acts as a major substrate of amino groups and carbon skeletons and is also a precursor of different biological mechanisms (9).

In general, 70-80% of GLN is absorbed in the intestines and levels can be monitored in blood samples. A number of effects related to GLN administration are recognized. Glucose utilization is enhanced when there is insulin resistance, HSP

Table 1. The clinical diagnoses of the patients at admission

Clinical diagnosis	Percentage (%)	Group	N
COPD exacerbation	16.7%	Group G	4
		Group C	6
Cerebrovascular events	15%	Group G	5
		Group C	4
HELLP/ eclampsia	13.3%	Group G	5
		Group C	3
Urosepsis	11.7%	Group G	3
		Group C	4
Trauma	10%	Group G	3
		Group C	3
Pneumonia	15%	Group G	4
		Group C	5
Meningitis	5%	Group G	2
		Group C	1
Cardiac arrest/ Post-CPR	13.3%	Group G	4
		Group C	4

COPD: Chronic Obstructive Pulmonary Disease, HELLP: Hemolysis, Elevated Liver Enzymes, Low Platelet Count, CPR: Cardio Pulmonary Resuscitation

Table 2. Infection Sources of Sepsis

Infection sources	Percentage (%)	n
Respiratory system	71.6	43
Urinary tract	18.3	11
Wound	5	3
Central nervous system	5	3

70 is stimulated, some anti-inflammatory and immune regulator effects, improvement of glutathione synthesis together with enhanced anabolic routes (13). GLN serves as an essential substrate in mitochondrion for the protection of the cellular environment from oxidative stress, maintaining a-ketoglutarate dehydrogenase activity, and improving the ATP content of the cell (14). GLN can refill intermediate molecules of the Krebs cycle and therefore is a strong anaplerotic molecule (15,16). GLN may be used for the treatment of disease processes where Krebs cycle activation is anticipated.

Nonpressor doses of L-NMMA administered in long-term to mice can produce a model of early asymptomatic dysfunction of endothelium that can be treated with administration of L-glutamine through replenishing of the Krebs cycle (9,17).

Results of a study stated that endocan levels might be a better predictor of the severity and outcome of sepsis (18). Mortality rates for sepsis and septic shock have commonly been referred between 20% and 50%, and we detected a mortality rate of 45% in this study (10).

Infections of the respiratory, gastrointestinal, and urinary tract were the most common causes of sepsis in the study of Mihajlovic et al as stated as similar to most epidemiologic studies (18), and similarly respiratory infections were the major source in our study. Despite advances in respiratory care and antibiotherapy, unfortunately, respiratory infections were still the leading source of sepsis in our clinic as the most epidemiologic studies (19). The diagnoses regarding infections at admission to ICU consisted of urosepsis, pneumonia, and meningitis and the total percentage of these patients was 31.7%. However, the rest of the patients in our study (68.3%) did not suffer from any kind of infection at admission to ICU. This may reflect us hospital-acquired infections are the main cause of sepsis in our ICU so early detection of sepsis with a more assertive approach such as biochemical markers is essential. Endocan and thrombomodulin shows better

Table 3. WBC, ESR, CRP, Lactate Levels and SOFA Scores of Patients day 0, 3rd, and 7th days

	Group G (n=30) (mean±standard deviation)	Group C (n=30) (mean±standard deviation)	p
WBC 0	13,23±6,67	11,90±5,52	0,38
WBC 3	13,78±7,80	11,43±6,16	0,14
WBC 7	11,45±6,47	12,31±6,60	0,91
ESR 0	35,20±23,48	41,56±27,70	0,34
ESR 3	49,40±29,02	46,23±25,02	0,17
ESR 7	55,33±28,90	49,76±19,37	0,67
CRP 0	100,29±96,30	110,98±98,50	0,67
CRP 3	136,35±117,12	129,31±108,82	0,72
CRP 7	159,80±140,89	127,06±127,87	0,29
Lactate 0	2,48±3,07	2,19±1,42	0,26
Lactate 3	2,33±2,82	2,19±1,19	0,11
Lactate 7	3,29±3,47	1,95±1,09	0,16
SOFA 0	3,86±2,01	5,65±3,14	0,02
SOFA 3	4,73±2,21	5,96±2,94	0,08
SOFA 7	5,53±3,73	6,00±3,79	0,56

*Significant at 0.05 levels. WBC: White blood cell count (/mm^3), ESR: Erythrocyte sedimentation rate (cm), CRP: C-reactive protein (mg/L), SOFA score: Sequential Organ Failure Assessment score

Table 4. Intra-Group Comparison of SOFA Scores at day 0, 3rd and 7th days

Group	Time	p
G	SOFA 0-SOFA 3rd day	0.081
	SOFA 0-SOFA 7th day	0.004
	SOFA 3rd day-SOFA 7th day	0.245
C	SOFA 0-SOFA 3rd day	0.012
	SOFA 0-SOFA 7th day	0.020
	SOFA 3rd day-SOFA 7th day	0.846

*Significant at 0.05 levels. SOFA score: Sequential Organ Failure Assessment Score

discriminative power than procalcitonin (20). Considering the value of procalcitonin in the follow-up of infection, evaluating the value of endocan levels during for sepsis follow-up becomes more prominent. Additionally the addition of endocan to follow-up markers significantly contributes to the SOFA score in the logistic regression model (20). Our study revealed no significant difference between Groups G and C regarding the levels of endocan recorded at day 0, 3rd, and 7th days. Moreover, endocan measurement is recommended as an appropriate laboratory marker for the postmortem analysis of sepsis. Significantly higher endocan levels were detected in the sera of the postmortem sepsis cases (21). On the contrary, serum endocan levels were increased in the control group. It would be so pretentious to conclude the beneficial effect of intravenous GLN supplementation in sepsis according to our results, especially in this small study group. The small sample size and the difficulty of homogenization of comorbid circumstances were the major limitations of our study.

The studies concerning the effects of GLN supplementation in septic patients are still controversial. It is a well-known factor that endogenous GLN may become insufficient in critical patients. The deficiency of GLN can be assessed with diminished plasma levels. It can be used as a prognostic factor for assessing worse outcomes of septic patients (22). Significantly reduced hospital mortality, rates of complications regarding infections, and length of hospitalization have been reported in seriously ill patients that are administered intravenous GLN according to the recommendations of the clinical guidelines for balanced nutritional support (23).

Table 5. Comparison of the Endocan Levels of the two Groups

	Group G (n=30) (mean±standard deviation)	Group C(n=30) (mean±standard deviation)	p value
ENDOCAN- Day 0	160.47 ± 101.62	158.9 ± 168.91	0.209
ENDOCAN- 3rd day	203.88 ± 165.31	158.53 ± 144.54	0.188
ENDOCAN- 7th day	166.32 ± 102.62	200.75 ± 178.16	0.824
p value	P=1.000	P=0.273	

*Significant at 0.05 levels. Endocan Levels are ng/L

There is moderate evidence that GLN alimentation decreased rates of infections and the duration of mechanical ventilation. It is suggested that GLN alimentation decreases the duration of hospital stay among critically ill patients with low-quality evidence. But there is a negligible effect of GLN alimentation regarding the risk of mortality and duration of ICU stay. There are indefinite data regarding the effects on serious side effect risks. Today, there is no evidence regarding the supplementation of individual amino acids, and GLN during serious illness may even be detrimental. There is uncertainty regarding optimal timing, dosage, and content of the amino acid combination for seriously ill patients (25).

Another important limitation of this study is that we did not measure the levels of GLN at the time of admission due to insufficient financial resources. However, based on the common view in the literature, we accepted that glutamine levels were low in ICU patients in our study (26).

Hypothetically, enteral nutrition is a preferable choice compared with parenteral nutrition for a long time regarding presumed better morbidity and mortality scores. But almost all studies supporting this idea were biased due to patient selection. No significant difference was revealed in outcomes between both nutritional routes when compared in eligible patients in a prospective manner (27).

Parenteral administration results in higher plasma concentrations of GLN than the enteral route when similar GLN doses or a GLN-comprising dipeptide are applied, so we preferred the intravenous route of GLN supplementation in patients who are supplemented by parenteral nutrition (28,29).

On contrary, GLN supplementation is recommended in various international guidelines. But there are controversial data regarding glutamine supplementation in sepsis and GLN supplementation may not be suitable for everybody. All treatment recommendations and study designs have included seriously ill patients. Data from recent papers and systematic reviews point out that unselective GLN administration in seriously ill patients may do more harm rather than benefits (30-32).

The proposal of administering high doses of combined enteral/parenteral supplementations to patients in ICU with severe septic patients with two or more organ failures was not suitable (33). There is no advantageous or harmful effect of unselective GLN administration (34-36).

The idea to normalize plasma GLN levels of the patients with low levels of GLN with parenteral supplementation at the admittance of the ICU has not been studied. There are different thoughts regarding testing pretreatment levels of GLN, and recent papers deem this effort as unnecessary (37).

According to another recent study, total mortality rates of sepsis in ICU and hospital were 25.8% and 35.3%, respectively. But it varied from 11.9% and 19.3% in Australia to 39.5% and 47.2% in Africa (38). The total mortality rate of the patients was 60% (n=36) in our study that is comparable between the groups. Significant higher total mortality rates may be due to the patient characteristics such as older age, inevitable comorbidities in parallel with senility, and the small sample size of our study.

The mortality rate in Group G was higher, so our results may bring to mind that GLN supplementation in septic patients is not beneficial. However, in this study, we hypothesized that if parenteral glutamine supplementation improves endothelial dysfunction, endocan levels would decrease in Group G. According to the intragroup analysis, endocan levels at 0, 3rd and 7th days in group G were not significantly changed. Also, there was no significant difference in terms of endocan levels between the GLN group and the controls. Our results did not support our hypothesis.

The higher mortality rates in group G than in group C seem to support the view that intravenous glutamine supplementation is not beneficial in patients with sepsis. However, due to

such a small sample size and the potential and inevitable bias in patient selection, reaching this conclusion may be pretentious.

According to our results, there was not a significant difference regarding the outcomes of the patients in both study groups, including mortality rates. Further prospective studies with larger sample size are needed in order to make conclusive comments that there is no beneficial effect of administering GLN.

References

1. De Backer D. Benefit-risk assessment of drotrecogin alfa (activated) in the treat- ment of sepsis, Drug Saf 2007; 30: 995-1010.
2. Qiao R., Wang H, Yan W, L.E. et al. Extracellular matrix hyaluronan is a determinant of the endothelial barrier, Am. J. Phys. Cell Phys 1995; 269: C103-C109.
3. Scherpereel A, Depontieu F, Grigoriu B, et al. Endocan, a new endothelial marker in human sepsis. Crit Care Med 2006; 34: 532-537.
4. Coeffier M, Dechelotte P. The role of glutamine in intensive care unit patients: mechanisms of action and clinical outcome. Nutrition reviews 2005; 63: 65-69.
5. Parry-Billings M, Evans J, Calder PC, Newsholme EA Does glutamine contribute to immunosuppression after major burns? Lancet 1990; 336: 523-525.
6. Wischmeyer PE. Glutamine: mode of action in critical illness. Critical care medicine 2007; 35; 541-544.
7. Wernerman J. Role of glutamine supplementation in critically ill patients. Current opinion in anaesthesiology 2008; 21: 155-159.
8. Addabbo F, Chen Q, Patel DP, et al. Glutamine supplementation alleviates vasculopathy and corrects metabolic profile in an in vivo model of endothelial cell dysfunction. *PloS one* 2013; *8*; e65458.
9. Vermeulen MA, Brinkmann SJ, Stehle P, van Leeuwen PA. Consequences of the REDOXS and METAPLUS Trials: the end of an era of glutamine and antioxidant supplementation for critically ill patients? JPEN J Parenter Enteral Nutr 2016; 40: 12.
10. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 23: 315: 801-810
11. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A*. 1918; 4: 370-373.
12. Recknagel P, Gonnert FA, Westermann M, et al. Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis, PLoS Med 2012; 9: e1001338.
13. Ziegler TR, Benfell K, Smith RJ, et al. Safety and metabolic effects of L-glutamine administration in humans. JPEN Journal of parenteral and enteral nutrition 1990; 14: 137-146.
14. Roth E. Nonnutritive effects of glutamine. The Journal of nutrition 2008; 138; 2025-2031.
15. Brunengraber H, Roe CR. Anaplerotic molecules: current and future. Journal of inherited metabolic disease 2006; 29: 327-331.
16. Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. The Journal of biological chemistry 2002; 277: 30409-30412.
17. O'Riordan E, Mendelev N, Patschan S, et al. Chronic NOS inhibition actuates endothelial-mesenchymal transformation. American journal of physiology Heart and circulatory physiology 2007; 292: 285-294.
18. Mihajlovic DM, Lendak DF, Brkic SV, et al. Endocan is useful biomarker of survival and severity in sepsis. Microvasc Res 2014; 93: 92-97.
19. Alberti C, Brun-Buisson C, Burchardi H, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med 2002; 28: 108-121.
20. Mihajlovic D, Brkic S, Lendak D, et al. Endothelial biomarkers in the light of new sepsis definition. Biomark Med 2019; 13: 341-351.
21. Palmiere C, Augsburger M. Endocan measurement for the postmortem diagnosis of sepsis. Leg Med (Tokyo) 2014; 16: 1-7.
22. Wernerman J. Glutamine supplementation. Ann Intensive Care 2011; 18; 1: 25.
23. Stehle P, Ellger B, Kojic D, et al. Glutamine dipeptide-supplemented parenteral nutrition improves the clinical outcomes of critically ill patients: A systematic evaluation of randomised controlled trials. Clin Nutr ESPEN. 2017; 17; 75-85.
24. Gunst J, Vanhorebeek I, Thiessen SE, Van den Berghe G. Amino acid supplements in critically ill patients. Pharmacol Res 2018; 130: 127-131.
25. Tao KM, Li XQ, Yang LQ, et al. Glutamine supplementation for critically ill adults. Cochrane Database Syst Rev 2014; 9: CD010050.

26. Buter H, Bakker AJ, Kingma WP, Koopmans M, Boerma EC. Plasma glutamine levels in patients after non-elective or elective ICU admission: an observational study. BMC Anesthesiol. 2016; 10; 16: 15.
27. Harvey SE, Parrott F, Harrison DA, et al. Trial of the route of early nutritional support in critically ill adults. N Engl J Med 2014; 371: 1673-1684.
28. Berg A, Rooyackers O, Norberg A, Wernerman J. Elimination kinetics of L-alanyl-L-glutamine in ICU patients. Amino Acids 2005; 29: 221-228.
29. Melis GC, Boelens PG, van der Sijp JR, et al. The feeding route (enteral or parenteral) affects the plasma response of the dipeptide Ala-Gln and the amino acids glutamine, citrulline and arginine, with the administration of Ala-Gln in preoperative patients. Br J Nutr 2005; 94: 19-26.
30. van Zanten AR, Hofman Z, Heyland DK. Consequences of the REDOXS and METAPLUS Trials: the end of an era of glutamine and antioxidant supplementation for critically ill patients? JPEN J Parenter Enteral Nutr 2015; 39: 890-892.
31. Wernerman J. What Is Actually Attributable to Glutamine? JPEN J Parenter Enteral Nutr. 2017; 41: 9.
32. Wernerman J. How to understand the results of studies of glutamine supplementation. Crit Care 2015; 19: 385.
33. Buijs N, Vermeulen MA, van Leeuwen PA. Glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 369: 484.
34. Vermeulen MA, Brinkmann SJ, Stehle P, van Leeuwen PA. Consequences of the REDOXS and METAPLUS Trials: the end of an era of glutamine and antioxidant supplementation for critically ill patients? JPEN J Parenter Enteral Nutr. 2016; 40: 12.
35. Buijs N, Vermeulen MA, van Leeuwen PA. Glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 369: 484.
36. Heyland D, Muscedere J, Wischmeyer PE, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med 2013; 368: 1489-1497.
37. Smedberg M, Wernerman J. Is the glutamine story over? Crit Care 2016; 10: 361.
38. Sakr Y, Jaschinski U, Wittebole X, et al. ICON Investigators. Sepsis in Intensive Care Unit Patients: Worldwide Data From the Intensive Care over Nations Audit. Open Forum Infect Dis. 2018; 19; 5; ofy313.