

Immunological Effects of Glutamine Supplementation in Polytrauma Patients in Intensive Care Unit.

Antonella Cotoia (✉ antonella.cotoia@unifg.it)

University of Foggia

Leonarda Pia Cantatore

University of Foggia

Renata Beck

University of Foggia

Alessandra De Gregorio

University of Foggia

Flavia Marchese

University of Foggia

Giuseppe Ferrara

University of Foggia

Gilda Cinnella

University of Foggia

Research Article

Keywords: Glutamine, polytrauma, Immunoglobulin A, CD3+/CD4+ T helper lymphocytes, CD3+/CD8+ T suppressor lymphocytes, CD3+/CD19+ B lymphocytes

Posted Date: October 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-959494/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Immunological effects of glutamine supplementation in polytrauma patients in Intensive Care

Unit

Antonella COTOIA^{1*}, Leonarda Pia CANTATORE¹, Renata BECK¹, Alessandra DE GREGORIO¹,
Flavia MARCHESE¹, Giuseppe FERRARA¹, Gilda CINNELLA¹

*¹Anesthesia and Intensive Care Unit, Department of Medical and Surgical Sciences, Policlinico Riuniti
Foggia, University of Foggia, Foggia;*

**Corresponding author: Antonella Cotoia, ¹Anesthesia and Intensive Care Unit, Department of
Medical and Surgical Sciences, Policlinico Riuniti Foggia, University of Foggia, Foggia. E-mail:
antonella.cotoia@unifg.it. ORCID ID: 0000-0002-0154-0418*

Abstract

Background

In polytrauma intensive care unit (ICU) patients, glutamine (GLN) become a "conditionally essential" amino acid; its role has been extensively studied in numerous clinical trials but their results are inconclusive.

We evaluated the IgA-mediated humoral immunity after GLN supplementation in polytrauma ICU patients.

Results

All consecutive patients with polytrauma who required mechanical ventilation and enteral nutrition (EN) provided within 24 hours since the admission in ICU at the University Hospital of Foggia from September 2016 to February 2017 were included.

Thereafter, two groups were identified: patients treated by conventional EN (25 kcal/kg/die) and patients who have received conventional EN enriched with 50 mg/kg/ideal body weight of alanyl-GLN 20% intravenously.

We analyzed plasmatic concentration of IgA, CD3+/CD4+ T Helper Lymphocytes, CD3+/CD8+ T suppressor Lymphocytes, CD3+/CD19+ B Lymphocytes, IL-4 and IL-2 at admission, at 4, 8 days.

We identified 30 patients, with 15 subjects per group. IgA levels increased significantly in GLN vs control group at T0, T4 and T8. CD3+/CD4+ T helper lymphocytes and CD3+/CD8+ T suppressor lymphocytes levels significantly increased in GLN vs control group at T4 and T8. CD3+/CD19+ B

lymphocytes levels increased significantly in GLN vs control group only at T8.IL-2 and IL-4 levels showed no significant differences when comparing GLN with control group.

Conclusions

Our study showed that there was an improvement in humoral and cell-mediated immunity with GLN supplementation in polytrauma ICU patients using recommended doses.

Keywords

Glutamine; polytrauma; Immunoglobulin A; CD3+/CD4+ T helper lymphocytes; CD3+/CD8+ T suppressor lymphocytes; CD3+/CD19+ B lymphocytes.

Background

Glutamine (GLN) is classified as a non-essential amino acid and it is released from skeletal muscle to be a constituent of proteins (1). Furthermore, GLN acts as an immune stimulator as an essential component for lymphocyte proliferation and cytokine production, macrophage phagocytic and secretory activities, and for neutrophil bacterial killing (2).

Under stable conditions GLN can be produced in sufficient amounts and stored in the muscle tissue.

These stores actually represent greater than 50% of the total free amino acid pool in the body.

However, GLN levels normally decrease in intensive care unit (ICU) patients both due to an

hypercatabolic status, especially in trauma and sepsis, and an increased requirement by the gut, the

immune system, liver and kidneys which exceeds the individual's ability to produce it in sufficient

amounts. In critically ill patients, GLN may even become a "conditionally essential" amino acid (3):

it is considered a "fuel for the immune system", where a low blood concentration may impair immune

cell function, resulting in poor clinical outcomes and increased risk of mortality and so its

supplementation is recommended in ICU patients(4). Interestingly, some authors showed that GLN

supplements of 10–20 g/d (or with high-dose >0.2g/kg/d), plus standard enteral nutrition (EN) formulas

reduced the rates of pneumonia, sepsis, bacteremia in ICU patients with shortened hospital stays, better

immune function and lower hospital costs (5-12). It is important to note that humoral immunity is a

process of adaptive immunity mediated by immunoglobulins such as IgA secreted by B lymphocytes

(5). Cell-mediated immunity is responsible for destroying the intracellular pathogens with T

Lymphocytes, which consequently produce inflammatory mediators such as Interleukin. More clinical trial and new studies on these topics are necessary to focused on cell-mediated and humoral immune responses in severely injured trauma patients and rule on GLN depletion on immune functioning (9-14).

We hypothesize that GLN could enhance both humoral and cell-mediated immunity. The aim of our study is to investigate the effect of GLN enriched EN on cell-mediated and humoral immune system in ICU polytrauma patients.

Results

46 patients were screened for eligibility. Of all patients 5 were not eligible due to an ICU stay < 96 h and 11 were excluded from the study because died within 8 days in ICU.

30 patients were enrolled and divided in two groups based on different treatments.

No patients reported any intolerance, hypersensitivity or side effects related to treatment.

No difference was found in age, gender and weight of patients' characteristics between two groups.

No significant differences were found either in ISS, SAPS or in APACHE II score.

The ICU length of stay and 28-day mortality were similar in both groups.

Furthermore, there was no significant difference in mechanical ventilation (MV) time between GLN and control group (Table 1).

Intragroup analysis showed increased IgA levels at T8 (vs T4 $p=0.04$) in GLN group. On the contrary, IgA levels trend was always constant in control group ($p=1$). Intergroup analysis showed that IgA levels increased significantly in GLN vs control at T0 ($253,6\pm 126,9$ mg/mL vs $161\pm 44,4$ mg/mL; $p=0.03$), at T4 ($256,4\pm 128,7$ mg/mL vs $163,2\pm 43,5$ mg/mL; $p=0.03$) and T8 ($280\pm 148,3$ mg/mL vs $153,6\pm 32$ mg/mL; $p<0,01$) (Fig. 1).

Intragroup analysis showed that in GLN group CD3+/CD4+ T helper lymphocytes significantly increased in each time during the study period ($p<0,001$), while in control group CD3+/CD4+ T helper lymphocytes were stable over the time. Instead, intergroup analysis showed that CD3+/CD4+ T helper lymphocytes were $518,3\pm 115,7$ cells/ μ L in GLN group and $519,6\pm 162,8$ cells/ μ L in control at

baseline ($p = 0,98$) and their levels significantly increased in GLN vs control group at T4 ($767,6 \pm 232$ cells/ μL vs $539,7 \pm 165,5$ cells/ μL ; $p < 0,01$) and T8 ($903,2 \pm 257,2$ cells/ μL vs $555,6 \pm 157,7$ cells/ μL ; $p < 0,001$) (Fig. 2).

Intragroup analysis showed that in GLN group CD3+/CD8+ T suppressor lymphocytes highly increased in each time during the study period ($p < 0,001$), while in control group they increased only at T4 (vs T0: $p < 0,001$) and T8 (vs T4: $p = 0,002$). Intergroup analysis showed that at T0, CD3+/CD8+ T suppressor lymphocytes were $498,5 \pm 153,2$ cells/ μL in GLN group and $482,5 \pm 242$ cells/ μL in control ($p = 0,8$) and their levels significantly increased in GLN vs control group at T4 ($680,3 \pm 186,1$ cells/ μL vs $530,6 \pm 226,9$ cells/ μL ; $p < 0,05$) and T8 ($839,5 \pm 162,6$ cells/ μL vs $571,3 \pm 225,7$ cells/ μL ; $p < 0,001$) (Fig. 3).

Intragroup analysis showed that in GLN group CD3+/CD19+ B lymphocytes significantly increased in each time during the study period ($p < 0,001$), while in control group they were stable. Intergroup analysis, at T0, CD3+/CD19+ B lymphocytes levels were $169,3 \pm 69$ cells/ μL in GLN group and $157,6 \pm 65$ cells/ μL in control ($p = 0,6$) and increased significantly in GLN vs control group only at T8 ($266,3 \pm 131,7$ cells/ μL vs $163,8 \pm 67,3$ cells/ μL ; $p = 0,01$) (Fig. 4).

Intragroup analysis showed that in GLN group IL-2 levels significantly increased in each time during the study period ($p < 0,05$); while in control group they significantly increased only at T4 (vs T0: $p = 0,002$) and T8 (vs T4: $p = 0,002$) (Fig. 5). Instead, intergroup analysis showed that no differences were

observed between GLN and control group at $T0(5,2\pm0,9 \text{ pg/mL vs } 5,6\pm1,3\text{pg/mL}; p = 0,4)$,
 $T4(5,6\pm0,9\text{pg/mL vs } 5,9\pm1,4 \text{ pg/mL}; p = 0,5)$ and $T8(6,2\pm0,9 \text{ pg/mL vs } 6,1\pm1,5 \text{ pg/mL}; p = 0,8)$.

Intragroup analysis showed higher IL-4 levels in each time point in GLN group ($p < 0.004$), and constant IL-4 levels in control group. In intergroup analysis *IL-4* levels showed no significant differences when comparing GLN with control group (Fig.6).

Table 1 Demographic, laboratory and clinical characteristics

	Control EN Group 1 n.15	GLN-enriched EN Group 2 n.15	P value
Age (y)	53.5±11.4	54.0±11.7	0.57
Weight (kg)	76±16.4	74.53±16.9	0.48
Height (cm)	165±9.2	165±7.5	0.22
BMI (kg/cm ²)	28.7±8.1	29.1±8.9	0.19
Sex (M/F)	8/7	10/5	0,17
Albumin (g/dl)	2.5±0.9	2.8±0.6	0.82
Proteins (g/dl)	5.3±0,9	6.2±0.8	0.91
Plasmatic Urea Level(mmoL/liter)	41±9.2	45±8.1	0.46

GCS Score	11±2	12±3	0.40
ISS Score	27.5±10.1	40.9±19.8	0.09
SAPS Score	45.4±12.1	45.8±9.8	0.9
APACHES II Score	23.6±3.6	21.4±6.3	0.76
ICU Length of Stay (days)	25±5	20±7	0.39
ICU Mortality n (%)	6 (40)	5 (33,3)	
MV time (d)	9.26±1.3	9±1.4	0.31

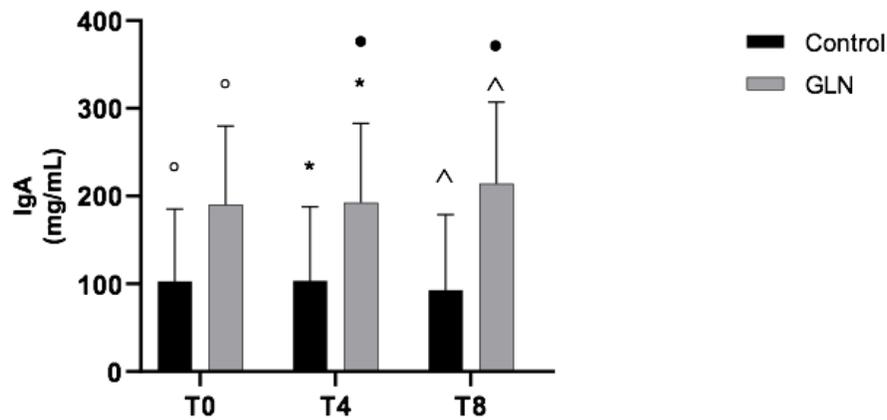


Figure 1: IgA levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. °, *, ^, v = Significant difference in GLN vs control at T0 ($p < 0,05$), at T4 ($p < 0,05$) and at T8 ($p < 0,01$); • = Significant difference ($p < 0,05$) in GLN group between T4 and T8.

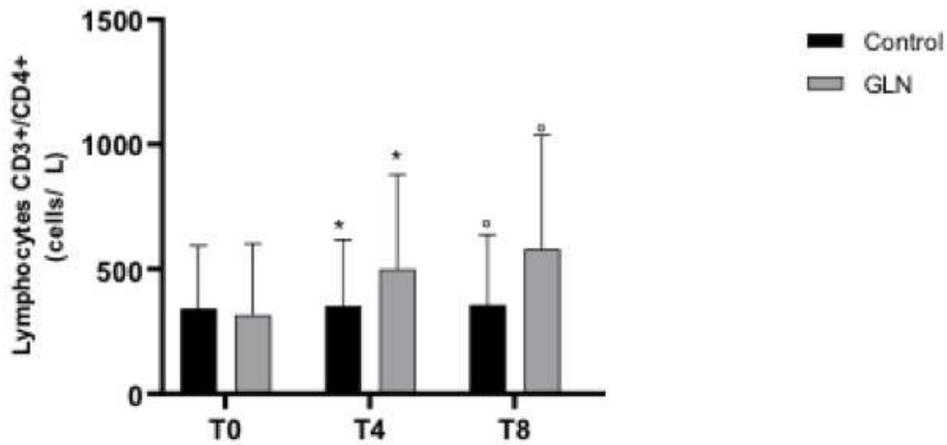


Figure 2: CD3+/CD4+ T Helper Lymphocytes levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. *,[°],[^] = Significant difference in GLN vs control at T4 ($p < 0,01$) and T8 ($p < 0,001$); v,[°],[•] = Statistically highly significant ($p < 0,001$) in GLN group.

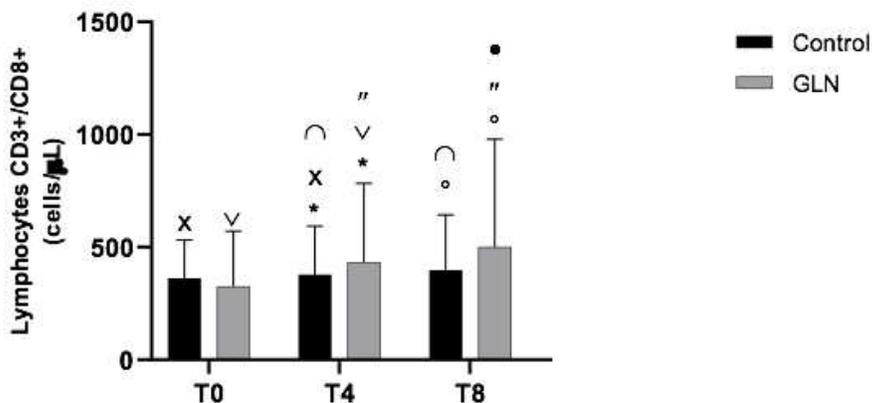


Figure 3: CD3+/CD8+ T suppressor Lymphocytes levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. *,[°],[^] = Significant difference in GLN vs control at T4 ($p < 0,05$), at T8 ($p < 0,001$); v,^{*},[^] = Statistically highly significant ($p < 0,001$) in GLN group; x,[^] = Significant difference in control group between T0 and T4 ($p < 0,001$) and T4 and T8 ($p = 0,002$).

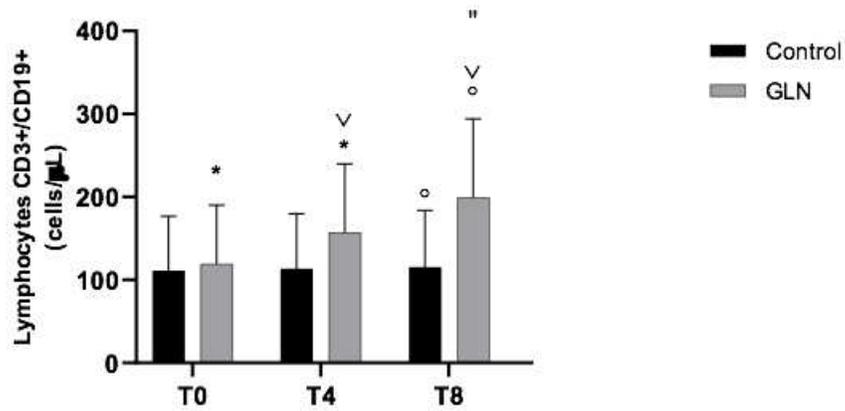


Figure 4: B Lymphocytes CD3+/CD19+ levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. °, ^ = Significant difference in GLN vs control at T8 ($p = 0,01$); *, v, ”: Statistically highly significant ($p < 0,001$) in GLN group.

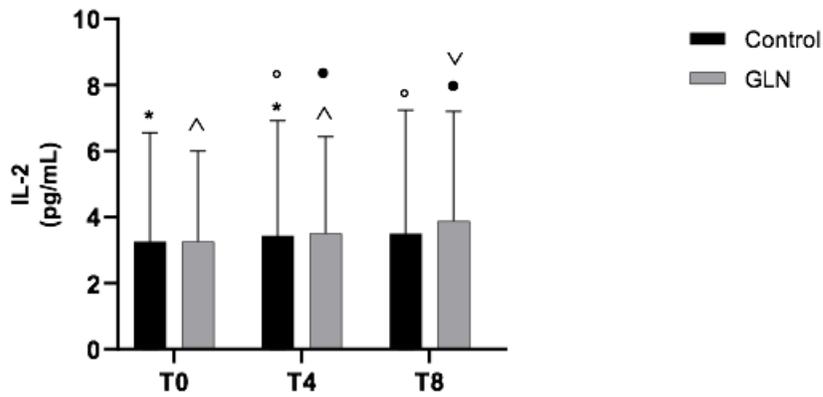


Figure 5: IL-2 levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. \wedge , \bullet , \vee = Significant difference in GLN group ($p < 0,05$); $*$, $^{\circ}$ = Significant difference in control group between T0 and T4 ($p = 0,002$) and T4 and T8 ($p = 0,002$).

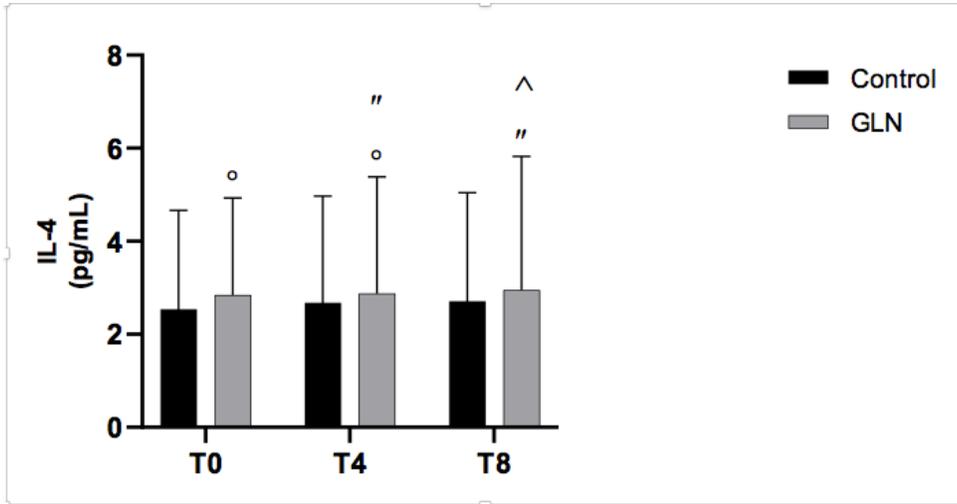


Figure 6: IL-4 levels in control and glutamine (GLN) groups. Data are presented as mean \pm SD. °, ", ^ = Significant difference ($p < 0.004$) in GLN group.

Discussion

Our study showed higher IgA levels (i), CD3+/CD4+ T helper lymphocytes and CD3+/CD8+ T suppressor lymphocytes (ii) in GLN group vs control group; IL-2 and IL-4 increased, but not significantly in GLN group vs control group (iii).

Our study is the first to investigate the IgA plasma levels –mediated humoral immunity in polytrauma patients treated with GLN supplementation, showing higher IgA levels. IgA is the most abundant immunoglobulin in the human body, and performs a very specialized role which involves mucosal immunity, development of tolerance and protection against infection. It is the key immunoglobulin in the respiratory and gastrointestinal tracts, which provide the first line of body defence(8).

Production of IgA is controlled by cytokine-producing T cells within the Gut-Associated Lymphoid Tissue(GALT) and possibly by cytokine released from the mucosa(9). The two most important compartments in which IgA are located are blood and mucous secretions: in the blood IgA is found as a monomer and is produced in bone marrow by plasma cells that derive from B cells activated in the lymphonodes; in mucous tissues IgA is secreted from IgA+ plasma cells after sensitization in the Peyer's patches as a dimer, bound by a J chain, and associated with the polymeric immunoglobulin receptor. Resulting immunoglobulin is known as secretory IgA (sIgA) (10-11). It is well known that GLN affects intestinal production of sIgA in humans and many other mammals, such as mice, pigs, and rats, and has critical roles in intestinal homeostasis by regulating immune responses *via* multiple mechanisms (12-14).

Herein, we showed higher T and B lymphocytes levels in GLN group. GLN appears to be fundamental in activation and proliferation of both CD3+/CD19+ B and CD3+/CD4+ and CD3+/CD8+ T lymphocytes. Specifically, it has been noticed that GLN enhances T lymphocytes' functions, playing a crucial role in Krebs cycle(15). Additionally, recent data showed that T cell activation is associated with rapid GLN uptake, by an amino acid transporter, ASCT2, as well as in vitro and in vivo conditions(16).

Regarding the cytokine profile, we analyzed IL-2 and IL-4, pro- and anti-inflammatory cytokines, respectively.

Concordly to in vitro experiments, our results showed increased IL-2 and IL-4 levels in patients treated with GLN.

Wei-KuoChang et al. found that 0.6mM GLN significantly enhanced IL-2 and IL-4 levels in vitro.

Furthermore, cytokine responses required the presence of optimal concentrations of GLN (11-17).

Another important item is the route of GLN administration (PN or EN).Although there are several reports of beneficial effects of GLN supplementation by the EN route in ICU patients (18,19) there is always an uncertainty whether or not the patient has received the prescribed dose. In addition, the complete absorption of GLN in the upper part of the intestine leaves very little extra GLN to other tissues as almost nothing passes though the liver into the general circulation. So these are arguments that favour the GLN administration by the PN route in addition to EN (20). For these reasons we have chosen PN GLN supplementation in ICU polytrauma patients.

Regarding the timing and dose of GLN supplementation, our patients were treated by conventional EN enriched with 50mg/kg/IBW of intravenous GLN for 7 consecutive days, in according to previously literature results. Recently, ESPEN guidelines recommend in critically ill trauma, additional EN doses of GLN (0.2-0.3 g/ kg/d) for the first five days with EN and in case of complicated wound healing for a longer period of 10 to 15 days (21).

This study showed a similar mortality and length of ICU stay in both polytrauma groups. However, contradictory findings have been recently reported in the literature regarding the linking between low or high GLN levels and mortality, so the debate is still open (22).

Our study showed that MV time was comparable between GLN and control groups.

Contrary, *Hai-bin Ni et al* showed that in critical patients with acute liver injury MV time was shorter in the GLN group than in the control group but they could not claim that this result is strictly addicted to GLN supplement (23).

However, animal researches demonstrated that GLN preserved breath muscle strength and reduced tissue damage in the organs (24, 25). Because of retrospective nature of our study, we could not evaluate any ultrasonographic diaphragmatic measurement such as diaphragmatic inspiratory excursion, time to peak inspiratory amplitude of the diaphragm, diaphragmatic thickness (DT), DT difference, and diaphragm thickening fraction, during the study period(26).

Another limitation of our study were: we could not measure GLN plasma levels, because of lack of kits based on high–performance chromatography, and, for its property of enhancing gut barrier functions, we did not analyze infectious setting because of high variability of antibiotic therapy.

Conclusions

This study showed that there was an improvement in humoral and cell-mediated immunity with GLN supplementation in polytrauma ICU patients using recommended doses.

The results of this study will provide pilot data for a larger clinical trial to investigate the exact mechanisms of any beneficial effects which have not be clearly understood.

Future research should confirm or refute whether a higher administration could translate any benefit for these patients.

Such studies should ensure that plasma GLN levels in treated patients are normalized.

Methods

Ethical approval was obtained from the Hospital Ethical Committee at the University Hospital of Foggia, on September 12 2019 (83/C.E./2019) and written informed consent was obtained from the patient during a planned follow-up visit. All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study included all consecutive patients with polytrauma (Injury Severity Score > 15) who required mechanical ventilation and EN provided within 24 hours since the admission in ICU at the University Hospital of Foggia from September 2016 to February 2017.

Exclusion criteria were: age <18 years, renal failure (creatinine > 180 $\mu\text{mol/L}$) or liver failure (bilirubin > 40 $\mu\text{mol/L}$, alanine aminotransferase > 100 units/L and gamma glutamyl transferase > 100 units/L), patients underwent major abdominal surgery or receiving systemic steroids, known history of immunologic disorders and pregnancy.

Thereafter, two groups were identified: patients treated by conventional EN (25 kcal/kg/die) (control group) and patients who have received conventional EN enriched with 50 mg/kg/ideal body weight (IBW) of alanyl-GLN 20%(Dipeptiven, Fresenius-Kabi, Bad Homburg, Germany) intravenously in a central venous line (GLN group). GLN infusion was administrated on 7 consecutive days(6).

The IBW was calculated using the Broca formula(7): for men $\text{IBW} = [\text{height}(\text{cm}) - 100] - [\text{height}(\text{cm}) - 100] \times 10\%$; for women $\text{IBW} = [\text{height}(\text{cm}) - 100] + [\text{height}(\text{cm}) - 100] \times 15\%$.

Demographics, Simplified Acute Physiology Score (SAPS) II, Acute Physiologic Assessment and Chronic Health Evaluation Score (APACHES) II and Injury Severity Score (ISS), ICU length of stay and mortality were retrospectively recorded.

Additionally, albumin, total plasmatic proteins and plasmatic urea levels were recorded at admission in ICU. Plasmatic concentration of IgA, CD3+/CD4+ T Helper Lymphocytes, CD3+/CD8+ T suppressor Lymphocytes, CD3+/CD19+ B Lymphocytes, IL-4 and IL-2 were analyzed at T0 (T0), at 4 (T4), 8 (T8) days after ICU admission.

Statistical analysis

We identified a sample size of 30 patients, with 15 subjects per group.

The normality of distribution was assessed by *Shapiro Wilkison test*.

Since we found all of the data normally distributed, the data were expressed as means \pm SD. A paired sample t-test was used to detect changes within the groups.

Data were analyzed using one-ways *ANOVA* and repeated measurement analysis of variance.

Differences between the groups at each time point were examined post hoc using independent *sample t-test*.

A value of $p < 0.05$ was considered statistically significant.

Statistical analysis was performed by *Statistical Package for the Social Sciences* (SPSS Inc., Chicago, IL) Version 15.0 for Windows.

List of abbreviations

GLN: Glutamine;

ICU: Intensive care unit;

EN: enteral nutrition;

PN: parenteral nutrition;

IBW: Ideal body weight;

SAPS II: Simplified Acute Physiology Score II;

APACHES II: Acute Physiologic Assessment and Chronic Health Evaluation Score II;

ISS: Injury Severity Score;

SPSS: Statistical Package for the Social Sciences;

MV: mechanical ventilation;

sIgA: secretory Immunoglobulin A;

GALT: Gut-Associated Lymphoid Tissue;

DT: diaphragmatic thickness.

Declarations

Ethics approval and consent to participate

The local Ethical Committee (the Hospital Ethical Committee at the University Hospital of Foggia) approved the study (Protocol 83/C.E./2019) and written informed consent was obtained from the patient during a planned follow-up visit.

Consent for publication

Not applicable.

Availability of data and materials

The database used and analysed during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

None

Authors' contributions

AC: conceived, designed and coordinated the study, collected and analyzed the data, wrote the manuscript and statistical analysis. LC: collected and analyzed data, wrote the manuscript and statistical analysis. RB: monitored the study and revised the manuscript. ADeG and FM: collected and analyzed data. GF: collected data. GC: revised the manuscript.

All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Cruzat V, Rogero MM, Keane KN, Curi R, Newsholme P. Glutamine: Metabolism and immune function, supplementation and clinical translation. In: *Nutrients*. 2018.
2. Roth E. Nonnutritive Effects of Glutamine. *J Nutr*. 2008;138(10):2025S–2031S.
3. Cetinbas F, Yelken B, Gulbas Z. Role of glutamine administration on cellular immunity after total parenteral nutrition enriched with glutamine in patients with systemic inflammatory response syndrome. *J Crit Care*. 2010;
4. Lacey JM, Wilmore DW. Is Glutamine a Conditionally Essential Amino Acid? *Nutr Rev*. 1990;
5. Mitchell WA, Aspinall R. The immune system. In: *HIV and Aging*. 2008. p. 1–22.
6. Andrews FJ, Griffiths RD. Glutamine: essential for immune nutrition in the critically ill. *Br J Nutr*. 2002;87(S1):S3–8.
7. Laurent I, Astère M, Paul B, Liliane N, Li Y, Cheng Q, Li Q, Xiao X. The use of Broca index to assess cut- off points for overweight in adults: A short review. *Reviews in Endocrine and Metabolic Disorders*. 2020.
8. Singh K, Chang C, Gershwin ME. IgA deficiency and autoimmunity. Vol. 13, *Autoimmunity Reviews*. 2014. p. 163–77.
9. Wu Y, Kudsk KA, DeWitt RC, Tolley EA, Li J. Route and type of nutrition influence IgA-mediated intestinal cytokines. In: *Annals of Surgery*. 1999. p. 662–8.
10. Murphy K, Weaver C. *Janeway Immunologie*. Janeway Immunologie. 2018.
11. Kudsk KA, Wu Y, Fukatsu K, Zarzaur BL, Johnson CD, Wang R, Hanna KM, Sax H, Klimberg, Buchman A. Glutamine-enriched total parenteral nutrition maintains intestinal interleukin-4 and mucosal immunoglobulin A levels. *J Parenter Enter Nutr*. 2000;24(5):270–5.
12. Lai YN, Yeh SL, Lin MT, Shang HF, Yeh CL, Chen WJ. Glutamine supplementation enhances mucosal immunity in rats with Gut-Derived sepsis. *Nutrition*. 2004;
13. Glutamine in Parenteral Solutions Enhances Intestinal Mucosal Immune Function in Rats. *Nutrition Reviews*. 1993.

14. Ren W, Wang K, Yin J, Chen S, Liu G, Tan B, Wu G, Bazer F, Peng Y, Yin Y. Glutamine-induced secretion of intestinal secretory immunoglobulin A: A mechanistic perspective. Vol. 7, *Frontiers in Immunology*. 2016.
15. Shah AM, Wang Z, Ma J. Glutamine metabolism and its role in immunity, a comprehensive review. Vol. 10, *Animals*. 2020.
16. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, Blonska M, Lin X Sun S. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity*. 2014;40(5):692–705.
17. Chang WK, Yang KD, Shaio MF. Effect of glutamine on Th1 and Th2 cytokine responses of human peripheral blood mononuclear cells. *Clin Immunol*. 1999;93(3):294–301.
18. Jones C, Allan Palmer TE, Griffiths RD. Randomized clinical outcome study of critically ill patients given glutamine-supplemented enteral nutrition. *Nutrition*. 1999;15(2):108–15.
19. Houdijk APJ, Rijnsburger ER, Jansen J, Wesdorp R, Kweiss J, Mc Camish MA, Teerlink T Meuwissen S, Haarman H, Thijs L, Mvan L. Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. *Lancet*. 1998;352(9130):772–6.
20. Berg A, Forsberg E, Wernerman J. The local vascular tolerance to an intravenous infusion of a concentrated glutamine solution in ICU patients. *Clin Nutr*. 2002;21(2):135–9.
21. Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, et al. ESPEN guideline on clinical nutrition in the intensive care unit. *Clin Nutr*. 2019;38(1):48–79.
22. Blaauw R, Nel DG, Schleicher GK. Plasma glutamine levels in relation to intensive care unit patient outcome. *Nutrients*. 2020;12(2).
23. Ni H. Protective effect of glutamine in critical patients with acute liver injury. *World J Emerg Med*. 2011;2(3):210.
24. Hammarqvist F, Wernerman J, Von der Decken A, Vinnars E. Alanyl-glutamine counteracts the depletion of free glutamine and the postoperative decline in protein synthesis in skeletal muscle. *Ann Surg*. 1990;212(5):637–44.
25. Ribeiro SP, Villar J, Slutsky AS. Induction of the stress response to prevent organ injury. Vol. 3, *New Horizons: Science and Practice of Acute Medicine*. 1995. p. 301–11.
26. Theerawit P, Eksombatchai D, Sutherasan Y, Suwatanapongched T, Kiatboonsri C, Kiatboonsri S. Diaphragmatic parameters by ultrasonography for predicting weaning outcomes. *BMC Pulm Med*. 2018;18(1).

