Chinese Journal of Physiology 60(6): 320-326, 2017
DOI: 10.4077/CJP.2017.BAG495

Serum Glutamine Levels as a Potential Diagnostic Biomarker in Sepsis following Surgery for Peritonitis

Chun-Ju Yang[1], Ting-Shuo Huang[2, 3, 4], Tung-Liang Lee[5], Kang-Chung Yang[3], Shin-Sheng Yuan[6], Ruey-Hwa Lu[7], Chung-Ho Hsieh[7], and Yu-Chiau Shyu[3, 8, 9, 10]

[1]Institute of Biopharmaceutical Sciences, National Yang-Ming University, Taipei 11221
[2]Department of General Surgery, Keelung Chang Gung Memorial Hospital, Keelung 20445
[3]Community Medicine Research Center, Keelung Chang Gung Memorial Hospital, Keelung 20445
[4]College of Chinese Medicine, Chang Gung University, Taoyuan 33302
[5]Department of Microbiology, Soochow University, Taipei 11112
[6]Institute of Statistical Science, Academia Sinica, Taipei 11529
[7]Department of Surgery, Taipei City Hospital, Taipei 10341
[8]Department of Nursing, Chang Gung University of Science and Technology, Taoyuan 33302
[9]Department of Nutrition and Health Sciences, Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 33302
and
[10]Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan, Republic of China

Abstract

Few diagnostic biomarkers for sepsis after emergency peritonitis surgery are available to clinicians, and, thus, it is important to develop new biomarkers for patients undergoing this procedure. We investigated whether serum glutamine and selenium levels could be diagnostic biomarkers of sepsis in individuals recovering from emergency peritonitis surgery. From February 2012 to March 2013, patients who had peritonitis diagnosed at the emergency department and underwent emergency surgery were screened for eligibility. Serum glutamine and selenium levels were obtained at pre-operative, post-operative and recovery time points. The average level of pre-operation serum glutamine was significantly different from that on the recovery day (0.317 ± 0.168 *vs.* 0.532 ± 0.155 mM, $P < 0.001$); moreover, serum glutamine levels were unaffected by surgery. Selenium levels were significantly lower on the day of surgery than they were at recovery (106.6 ± 36.39 *vs.* 130.68 ± 56.98 ng/mL, $P = 0.013$); no significant difference was found between pre-operation and recovery selenium levels. Unlike selenium, glutamine could be a sepsis biomarker for individuals with peritonitis. We recommend including glutamine as a biomarker for sepsis severity assessment in addition to the commonly used clinical indicators.

Key Words: glutamine, peritonitis, selenium, sepsis, surgery

Corresponding author: Yu-Chiau Shyu, Community Medicine Research Center, Keelung Chang Gung Memorial Hospital, Keelung 20445, Taiwan. Tel: +011-886-2-2432-9292 #3391. E-mail: yuchiaushyu@gmail.com
Received: January 10, 2017; Revised: March 26, 2017; Accepted: June 16, 2017.
 ISSN : 0304-4920. http://www.cps.org.tw

Introduction

Septicemia is a leading cause of death in hospitalized patients. The rate of mortality from sepsis is 28.3% in the United States and 41.1% in Europe (1). In 2013, sepsis was the twelfth leading cause of death in Taiwan (2). Despite physicians' adherence to evidence-based clinical practice protocols and guidelines from the Surviving Sepsis Campaign (SSC), mortality from severe sepsis in Taiwan is still high at 45.9% (3). Sepsis mortality remains high in Taiwan because of delays in treatment and a lack of sepsis biomarkers for timely diagnosis. Anti-infectious therapies such as the use of antibiotics or antivirals are major current treatments for sepsis, and SSC guidelines recommend administration as soon as possible to improve outcomes (4). However, physicians often cannot decide which kinds of anti-infectious drugs to administer until after they have seen a culture report, which may take several days to produce and may delay drug treatment. Therefore, rapid and convenient biomarkers are necessary for prompt evaluation of sepsis severity.

The biomarkers currently being used to assess the condition of a septic patient and to diagnose sepsis include procalcitonin (PCT), C-reactive protein (CRP), interleukin-6 (IL-6), triggering receptor expressed on myeloid cells-1 (TREM-1), lipopolysaccharide-binding protein (LBP) and urokinase plasminogen activator receptor (uPAR) (5). The newest SSC guidelines only suggest PCT and CRP for assessing sepsis (4). PCT is a precursor to calcitonin, which is involved in calcium homeostasis, and is the most investigated biomarker of sepsis (6, 7). A meta-analysis calculated that PCT has a sensitivity of 0.77 and a specificity of 0.79 for discriminating infectious sepsis from non-infectious conditions (8). CRP is an acute-phase protein that is stimulated by cytokines and released from the liver (9). CRP has traditionally been used as a marker of inflammation. CRP has only a sensitivity of 0.75 and a specificity of 0.67 for distinguishing bacterial infection from non-infectious disease (10). Although the sensitivity of PCT and CRP could be above 0.7 (8, 11), they are easily affected by other stressors such as non-infectious disease, oxidative stress and surgery (12). Sensitive, specific and precise biomarkers for assessing the condition of a septic patient are lacking.

Previous studies suggest that glutamine and selenium supplements can improve the outcomes of sepsis patients. Accordingly, the serum levels of glutamine and selenium are known to be decreased in sepsis patients. Glutamine is a non-essential amino acid that has numerous important roles including nitrogen balance, immune function, inflammatory response and antioxidant defense. In humans, glutamine participates in nitrogen transport. It is also a substrate for renal ammoniagenesis, and is also a maintainer of nitrogen balance (13). Glutamine is a fuel for lymphocytes and enterocytes, which facilitates cell-mediated immunity and maintains the integrity of the intestinal mucosa (15, 16). In addition, glutamine is a precursor to glutathione, an important endogenous antioxidant in humans (16). Individuals enduring severe metabolic stress, such as trauma, sepsis or major surgery, may be depleted of stored glutamine. Some observational studies in intensive care units have demonstrated that plasma glutamine depletion is correlated with unfavorable clinical outcomes (18, 19). A meta-analysis of randomized controlled trials showed that parenteral glutamine supplementation could shorten hospital stay and reduce complications (20, 21). We hypothesized that serum glutamine levels decrease during the acute stage of sepsis; thus, decreased levels of glutamine may reflect an individual's disease condition. Hence, glutamine may be used as a biomarker for sepsis.

Selenium is another sepsis biomarker candidate, as it is an essential micronutrient in humans. Selenium exerts antioxidant, pro-immunity, anti-viral and other pleiotropic effects (22). Serum selenium mainly occurs as selenoproteins, which improve immune functions by activating T-cells, especially $CD4^+$ cells, and facilitating the cytotoxicity of natural killer cells (23). The major selenoproteins are glutathione peroxidase (GPx), selenoenzyme GPx-1, selenoenzyme GPx-3 and selenoprotein P; these proteins can protect cells from free radical-induced oxidative stress (24). Selenium levels are reduced in sepsis patients, and are correlated inversely with infection and mortality rates (14, 25, 26). A meta-analysis suggested that high-dose selenium treatments might reduce mortality in sepsis patients (27). This implies that serum selenium is depleted in the disease state, and that selenium supplementation may improve clinical outcomes.

We hypothesized that altered patterns in glutamine or selenium levels are related to a patient's condition. If this changed pattern is indeed a response to the patient's septic condition, the pattern may then be useful as a biomarker for sepsis diagnosis, assessing disease severity, or for monitoring of the sepsis condition. The present study investigated serum glutamine and selenium levels to characterize a potential correlation between their behavior and the sepsis condition of the patients. We aimed, therefore, to develop a sensitive, precise and specific biomarker system for assessment of sepsis severity.

Materials and Methods

Patient Selection

This study was a collaboration between Taipei City Hospital, Taipei, and Chang Gung Memorial Hospital, Keelung. Between February 2012 and March 2013, 30 peritonitis patients were recruited, who, following surgery, were hospitalized and diagnosed with sepsis. Participants were evaluated for sepsis using the 2013 sepsis guidelines (4). All participants survived and recovered from peritonitis by the end of this study. Individuals excluded from our study were below the age of 18 years, not subjected to surgery, in a very critical condition, or in a life-threatening condition. This study was approved by the Multicenter Research and Ethics Committee of Chang Gung Medical Foundation Institutional Review Board (100-2020A3) and Taipei City Hospital Institutional Review Board (TCHIRB-1010520-E). All patients were enrolled after signing and dating an approved informed consent. Patient clinical information was collected according to the approved Institutional Review Board procedures.

Experimental Design

We enrolled a prospective cohort of patients who were diagnosed with acute peritonitis and would receive emergency surgery. From each participant, 5-10 mL of whole blood was phlebotomized at three time points: one day before operation (pre-operation), during the surgery (surgery), and one day before discharge (recovery). To separate the serum, the whole blood samples were centrifuged within 24 h of withdrawal; fractions were stored at -80°C.

Data Collection

In addition to collecting blood samples, clinical data of each participant were recorded for future analysis. The clinical data included age, gender, medical history, laboratory results and clinical symptoms. Routine biochemical and clinical data were determined by the hospital clinical laboratory. Glutamine and selenium were detected in biological laboratories.

Serum Glutamine Concentration Measurement

Serum glutamine was measured using a commercial kit (EnzyChrom Glutamine Assay Kit, catalog number: EGLN-100; BioAssay Systems, Hayward, CA, USA) at the National Yang-Ming University Laboratory. The minimum volume of serum was 20 µl per sample per assay. The test was replicated three times for each sample. A DU 800 UV/Visible Spectrophotometer (Beckman Coulter, Indianapolis, IN, USA) was used to determine absorbance at 565 nm of each sample.

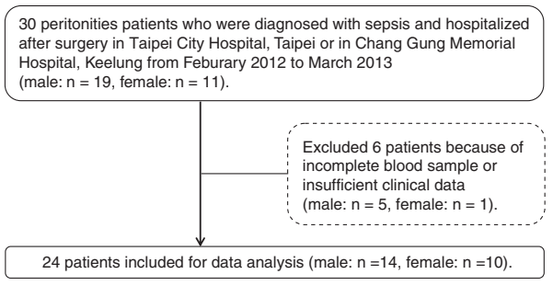

Fig. 1. The selection procedure for study participants.

Serum Selenium Concentration Measurement

Serum selenium was measured using an inductively coupled plasma mass, ICP-MS, Xseries II (Thermo, Waltham, MA, USA). The following instrumental parameters were used for ICP-MS analyses. The extraction voltage was -678 V, the forward power was 1200 W, the rf power was 1 W, and the focus voltage was 12.5 V. The nebulizer gas flow rate was 0.88 L/min, and the CCT1 gas (7% H_2/ 93% He) flow rate was 6.7 mL/min. Dwell times were 10 ms for selenium; 10 replicates per sample were conducted with 100 sweeps per replicate.

Statistical Analysis

Serum glutamine and selenium concentrations at three different time points were compared: pre-operation, surgery day and recovery day. All data are reported as the mean ± standard deviation (SD), or as percentages of the population. For statistical analyses, Student *t*-tests calculated with the R software package were used. $P < 0.05$ was considered to indicate statistical significance.

Results

Our study enrolled 30 patients beginning in February of 2012 to March 2013. Six patients were excluded due to incomplete sample collection or insufficient clinical data. Twenty-four patients were included in our final data analysis (Fig. 1). Age, gender, body mass index (BMI) and the Charlson comorbidity index (CCI) are shown in Table 1. All participants were administered antibiotics during hospitalization. Days of antibiotic use ranged from 1.08 to 12.6, and the average was 4.7 days. Nineteen patients were on a regular diet. Five patients were administered nutritional supplements; the minimum days were 3, the maximum days were 9. Two of these patients were administered Kabiven, which contains 1.2 g/L glutamine, for 5 or 8 days. The other patients received a 10% amino acid infusion combined with

Table 1. Characteristics of enrolled patients.

Event	Characteristics
Age (years)	56.8 ± 21.9
Male (%)	14 (58.3)
BMI	24.7 ± 3.9
CCI	2.5 ± 2.7
Antibiotic used	
Number (%)	24 (100)
Hours	113.1 ± 64.3
Nutritional supplement	
Numbers (%)	5 (20.8)
Hours	138.2 ± 62.2
Nutrition products	
Amino acid + SMOF	3 (12.5)
Kabiven	2 (8.3)

In the table, mean ± SD data are shown unless stated otherwise; BMI, body mass index; CCI, Charlson Comorbidity Index; parenteral lipid emulsion (SMOF).

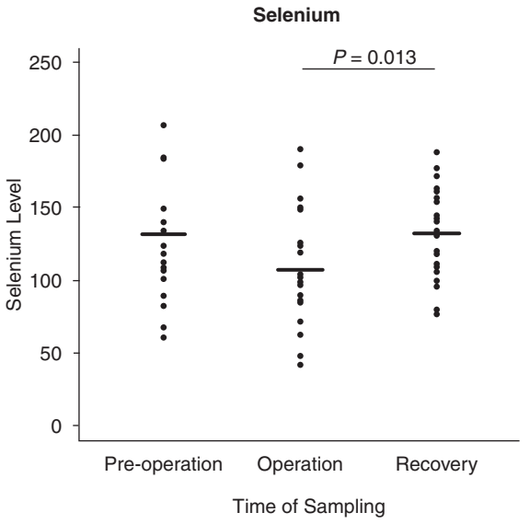

Fig. 3. Serum selenium concentrations at different time points.

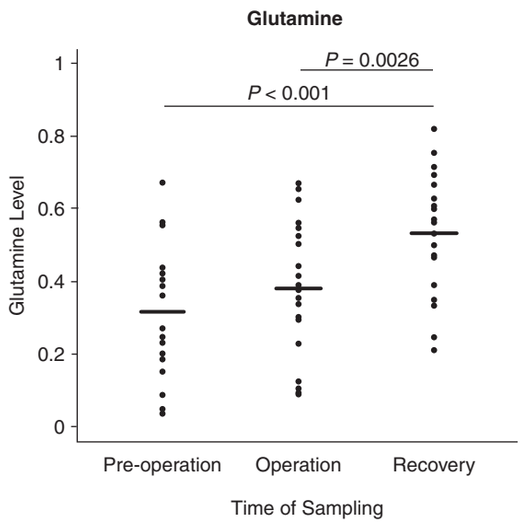

Fig. 2. Serum glutamine concentrations at different time points.

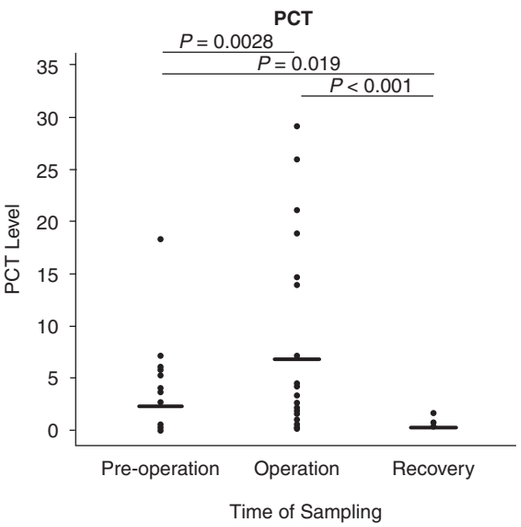

Fig. 4. Serum PCT level at different time points.

parenteral lipid emulsion (SMOF) for 3, 5 and 9 days, respectively (Table 1).

The patients' serum glutamine concentrations are shown in Fig. 2. The mean pre-operation glutamine concentration was 0.317 ± 0.168 mM, and the mean surgery glutamine concentration was 0.381 ± 0.167 mM. These means were not significantly different (P = 0.204). However, there were significant differences between pre-operation day and recovery day (0.317 ± 0.168 *vs.* 0.532 ± 0.155, P < 0.001). There was also a significant difference between the mean surgery and recovery glutamine concentrations (0.381 ± 0.167 *vs*. 0.532 ± 0.155, P = 0.0026).

Serum selenium concentration patterns did not change in the same way as observed for glutamine. The mean pre-operation selenium concentration was higher than the mean surgery selenium concentration, but not significantly higher (130.68 ± 56.98 *vs.* 106.6 ± 36.39, P = 0.105) (Fig. 3). The mean surgery selenium concentration was 106.6 ± 36.39 ng/mL, which was significantly lower than the mean recovery selenium concentration (131.65 ± 28.79, P = 0.013). In addition, there were no significant differences in the mean selenium concentrations between pre-operation day and recovery day (130.68 ± 56.98 *vs.* 131.65 ± 28.79, P = 0.944).

Furthermore, we also measured the PCT and CRP levels of the participants at different disease

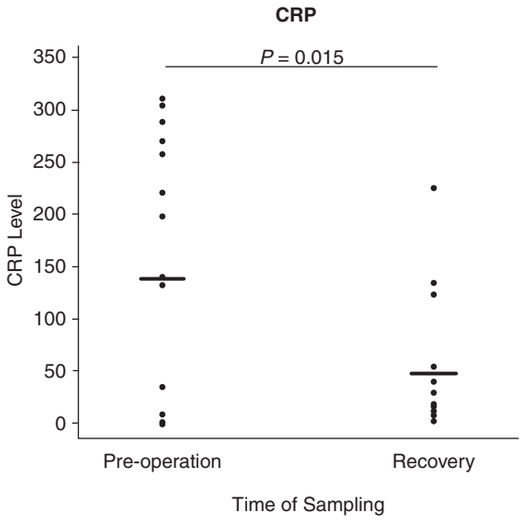

Fig. 5. Serum CRP level at different time-points.

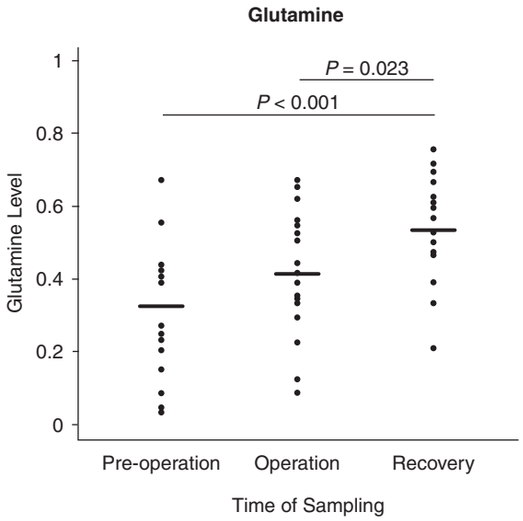

Fig. 6. Serum glutamine concentrations at different time points, excluding five patients who received nutritional support.

stages. The PCT concentration increased after surgery, but decreased when the patients recovered from illness (Fig. 4). The mean PCT concentrations of the pre-operation, surgery day and recovery day were 2.353 ± 4.063 ng/mL, 6.839 ± 8.589 ng/mL, and 0.292 ± 0.339 ng/mL, respectively (n = 24). The mean CRP level at the acute stage of sepsis was significantly higher than that during the recovery stage (139.05 ± 121.54 mg/dL *vs*. 48.58 ± 59.82 mg/dL, $P = 0.015$) (Fig. 5). However, this pattern was inconsistent across the patients. The CRP level on the recovery day was observed to be higher than the initial value in six of the sixteen patients (37.5%) (Fig. 5). In addition, the CRP concentration was still higher than the normal range (<1 mg/dL) even with rehabilitation (Fig. 5).

Nutritional supplements may be an important factor for affecting serum amino acid or electrolyte levels of patients. Five patients in this study received nutritional supplements, including carbohydrates, amino acids and lipids. Amino acids are general components of nutritional supplements in contrast to selenium, which is a micronutrient. Therefore, the nutritional supply might have affected our results, especially with regard to the glutamine level of the participants. To clarify this aspect, the glutamine level was analyzed again after exclusion of these five patients (Fig. 6). The same pattern was observed as in the previous result (Fig. 2). The data suggested that the serum glutamine level of septic peritonitis patients rebounded regardless of the general nutritional supply.

Discussion

One important limitation of the current clinical biomarkers of sepsis, namely CRP and PCT, is that they may be elevated by noninfectious causes of inflammation such as trauma, surgery or autoimmune disease (12, 28-31). Although the average value of the patient's CRP level was high at the acute stage and low at one day before discharge, the value was much higher than normal range (Fig. 5). This indicates that the CRP level is affected by many factors, and not only by septic severity. Moreover, approximately 37.5% of the total patients' CRP level was increased before discharge. This means that the CRP level is not suitable as a marker in septic patients because of its inconsistency. PCT levels were decreased after recovery, but were affected by surgery (32), consistent with data of this study. The PCT level was high after surgery but was reduced to the normal range on the recovery day (Fig. 4), which indicates that surgery may affect the PCT level. We consider that PCT could be used to assess the disease condition of a patient, but the effect of surgery should be accounted for. In contrast, plasma glutamine levels are reportedly steady before and after surgery (33). Consistent with our results, glutamine levels were not affected by surgery, and were increased at recovery day (Fig. 2). Because the serum glutamine concentration increased with improvement of the patient's condition and was undisturbed by surgery, we suggest that glutamine is a suitable marker for assessing and monitoring the condition of septic patients.

Another issue is whether glutamine or selenium

supplementation in sepsis patients is necessary. Previous studies are divided over whether to administer supplemental glutamine to septic patients (19, 34-37). However, a large recent trial found that glutamine supplementation might be harmful, and recommended against giving glutamine to critically ill patients (38). The body maintains a free pool of plasma glutamine through muscle protein degradation and *de novo* synthesis. Glutamine may be produced by peripheral tissues such as muscles, and plasma glutamine levels may increase in diseased states. Glutamine supplementation is unnecessary in septic patients except in severely malnourished individuals, because muscle tissues release glutamine to compensate for plasma glutamine deficiency (37). This conclusion is consistent with our results. The serum glutamine levels of the participants rebounded without exogenous glutamine supply. Hence, we consider that glutamine supplementation is not necessary.

However, selenium supplementation in sepsis was recommended by previous studies (27, 39, 40). Because this study showed that the serum selenium levels on the recovery day were not increased compared to the value at pre-operation day, the selenium level did not obviously reflect the condition of the septic patients. Two potential explanations for this observation could be considered. Firstly, selenium levels of individuals recovering from sepsis may increase too slowly for the increase to have been apparent on the recovery days specific to the present study. Secondly, selenium is a trace element and a compensatory mechanism for selenium deficiency other than exogenous supply might not exist. This may explain why selenium supplementation is necessary for septic patients. In addition, the mean selenium level on the operation day was lower than the mean selenium level on the pre-operation and recovery day. This indicates that similar to the PCT, surgery might affect the serum selenium level.

The limitations of this study include its small sample size, limited number of time points evaluated, and different recovery days for different patients. Furthermore, serum glutamine levels, but not muscle glutamine levels, were measured. Serum selenium level decrease were not entirely consistent with changes observed in selenium-related protein activity.

Conclusions

Glutamine levels are decreased in the acute stage and rebound when septic peritonitis patients are in recovery. The altered patterns of glutamine levels are more consistent than the altered CRP levels in most patients. In contrast to PCT, glutamine is not affected by surgery. We suggest that glutamine could be developed as an effective biomarker for assessing the disease condition of septic patients recovering from peritonitis surgery. Selenium is unsuitable as an indicator of sepsis assessment because the concentration of serum selenium does not increase when the patient's condition improves. In addition to the commonly used clinical indicators, we recommend including glutamine as a biomarker for sepsis severity assessment.

Acknowledgments

This study was sponsored by Chang Gung Memorial Hospital Research Projects Grant (CMRPG2G0041, CMRPG2G0071, CMRPG2G0081, CMRGP2G0311 and CRRPG2F0011), and Ministry of Science and Technology Grant (104-2311-B-182A-002-MY3).

Conflict of Interest

The authors hereby declare research grants and patent licensing arrangements related to the subject matter of this article.

References

1. Alhazzani, W., Jacobi, J., Sindi, A., Hartog, C., Reinhart, K., Kokkoris, S., Gerlach, H., Andrews, P., Drabek, T., Manzanares, W., Cook, D.J. and Jaeschke, R.Z. The effect of selenium therapy on mortality in patients with sepsis syndrome: a systematic review and meta-analysis of randomized controlled trials. *Crit. Care Med*. 41: 1555-1564, 2013.
2. Andrews, P.J., Avenell, A., Noble, D.W., Campbell, M.K., Croal, B.L., Simpson, W.G., Vale, L.D., Battison, C.G., Jenkinson, D.J. and Cook, J.A. Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. *BMJ* 342: d1542, 2011.
3. Bloos, F. and Reinhart, K. Rapid diagnosis of sepsis. *Virulence* 5: 154-160, 2014.
4. Bollhalder, L., Pfeil, A.M., Tomonaga, Y. and Schwenkglenks, M. A systematic literature review and meta-analysis of randomized clinical trials of parenteral glutamine supplementation. *Clin. Nutr*. 32: 213-223, 2013.
5. Cetinbas, F., Yelken, B. and 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* 25: 661.e1-661.e6, 2010.
6. Chen, Y.C., Chang, S.C., Pu, C. and Tang, G.J. The impact of nationwide education program on clinical practice in sepsis care and mortality of severe sepsis: a population-based study in Taiwan. *PLoS One* 8: e77414, 2013.
7. Dellinger, R.P., Levy, M.M., Rhodes, A., Annane, D., Gerlach, H., Opal, S.M., Sevransky, J.E., Sprung, C.L., Douglas, I.S., Jaeschke, R., Osborn, T.M., Nunnally, M.E., Townsend, S.R., Reinhart, K., Kleinpell, R.M., Angus, D.C., Deutschman, C.S., Machado, F.R., Rubenfeld, G.D., Webb, S., Beale, R.J., Vincent, J.L. and Moreno, R. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock. *Intensive Care Med*. 39: 165-228, 2013.
8. Dos Santos, R., Viana, M.L., Generoso, S.V., Arantes, R.E., Davisson Correia, M.I. and Cardoso, V.N. Glutamine supplementation decreases intestinal permeability and preserves gut mucosa

integrity in an experimental mouse model. *JPEN J. Parenter. Enteral Nutr*. 34: 408-413, 2010.
9. Eberhard, O.K., Haubitz, M., Brunkhorst, F.M., Kliem, V., Koch, K.M. and Brunkhorst, R. Usefulness of procalcitonin for differentiation between activity of systemic autoimmune disease (systemic lupus erythematosus/systemic antineutrophil cytoplasmic antibody-associated vasculitis) and invasive bacterial infection. *Arthritis Rheum*. 40: 1250-1256, 1997.
10. Forceville, X., Vitoux, D., Gauzit, R., Combes, A., Lahilaire, P. and Chappuis, P. Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. *Crit. Care Med*. 26: 1536-1544, 1998.
11. Gabay, C. and Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* 340: 448-454, 1999.
12. Grimble, R.F. Immunonutrition. *Curr. Opin. Gastroenterol*. 21: 216-222, 2005.
13. Gstraunthaler, G., Landauer, F. and Pfaller, W. Ammoniagenesis in LLC-PK1 cultures: role of transamination. *Am. J. Physiol*. 263: C47-C54, 1992.
14. Health Promotion Administration, Ministry of Health and Welfare. 2012 Leading causes of death in Taiwan, 2013.
15. Heyland, D., Muscedere, J., Wischmeyer, P.E., Cook, D., Jones, G., Albert, M., Elke, G., Berger, M.M. and Day, A.G. A randomized trial of glutamine and antioxidants in critically ill patients. *N. Engl. J. Med*. 368: 1489-1497, 2013.
16. Huang, T.S., Huang, S.S., Shyu, Y.C., Lee, C.H., Jwo, S.C., Chen, P.J. and Chen, H.Y. A procalcitonin-based algorithm to guide antibiotic therapy in secondary peritonitis following emergency surgery: a prospective study with propensity score matching analysis. *PLoS One* 9: e90539, 2014.
17. Huang, T.S., Shyu, Y.C., Chen, H.Y., Lin, L.M., Lo, C.Y., Yuan, S.S. and Chen, P.J. Effect of parenteral selenium supplementation in critically ill patients: a systematic review and meta-analysis. *PLoS One* 8: e54431, 2013.
18. Jang, J.Y., Shim, H., Lee, S.H. and Lee, J.G. Serum selenium and zinc levels in critically ill surgical patients. *J. Crit. Care* 29: 317.e5-8, 2014.
19. Jung, B., Molinari, N., Nasri, M., Hajjej, Z., Chanques, G., Jean-Pierre, H., Panaro, F. and Jaber, S. Procalcitonin biomarker kinetics fails to predict treatment response in perioperative abdominal infection with septic shock. *Crit. Care* 17: R255, 2013.
20. Kim, H. Glutamine as an immunonutrient. *Yonsei Med. J.* 52: 892-897, 2011.
21. Levy, M.M., Artigas, A., Phillips, G.S., Rhodes, A., Beale, R., Osborn, T., Vincent, J.L., Townsend, S., Lemeshow, S. and Dellinger, R.P. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. *Lancet Infect. Dis*. 12: 919-924, 2012.
22. Manzanares, W., Langlois, P.L. and Heyland, D.K. Pharmaconutrition with selenium in critically ill patients: what do we know? *Nutr. Clin. Pract.* 30: 34-43, 2015.
23. Meisner, M., Adina, H. and Schmidt, J. Correlation of procalcitonin and C-reactive protein to inflammation, complications, and outcome during the intensive care unit course of multiple-trauma patients. *Crit. Care* 10: R1, 2006.
24. Meisner, M., Tschaikowsky, K., Hutzler, A., Schick, C. and Schuttler, J. Postoperative plasma concentrations of procalcitonin after different types of surgery. *Intensive Care Med.* 24: 680-684, 1998.
25. Novak, F., Heyland, D.K., Avenell, A., Drover, J.W. and Su, X. Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit. Care Med.* 30: 2022-2029, 2002.
26. Oudemans-Van Straaten, H.M., Bosman, R.J., Treskes, M., Van Der Spoel, H.J. and Zandstra, D.F. Plasma glutamine depletion and patient outcome in acute ICU admissions. *Intensive Care Med*. 27: 84-90, 2001.
27. Rayman, M.P. The importance of selenium to human health. *Lancet* 356: 233-241, 2000.
28. Rayman, M.P. Selenium and human health. *Lancet* 379: 1256-1268, 2012.
29. Reinhart, K. and Meisner, M. Biomarkers in the critically ill patient: procalcitonin. *Crit. Care Clin.* 27: 253-263, 2011.
30. Sakr, Y., Reinhart, K., Bloos, F., Marx, G., Russwurm, S., Bauer, M. and Brunkhorst, F. Time course and relationship between plasma selenium concentrations, systemic inflammatory response, sepsis, and multiorgan failure. *Brit. J. Anaesth*. 98: 775-784, 2007.
31. Simon, L., Gauvin, F., Amre, D.K., Saint-Louis, P. and Lacroix, J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin. Infect. Dis*. 39: 206-217, 2004.
32. Soeters, P.B. and Grecu, I. Have we enough glutamine and how does it work? A clinician's view. *Ann. Nutr. Metab*. 60: 17-26, 2012.
33. Sponholz, C., Sakr, Y., Reinhart, K. and Brunkhorst, F. Diagnostic value and prognostic implications of serum procalcitonin after cardiac surgery: a systematic review of the literature. *Crit. Care* 10: R145, 2006.
34. Su, L., Han, B., Liu, C., Liang, L., Jiang, Z., Deng, J., Yan, P., Jia, Y., Feng, D. and Xie, L. Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: a prospective cohort study. *BMC Infect. Dis*. 12: 157, 2012.
35. Tang, B.M., Eslick, G.D., Craig, J.C. and Mclean, A.S. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. *Lancet Infect. Dis*. 7: 210-217, 2007.
36. Van Acker, B.A., Hulsewe, K.W., Wagenmakers, A.J., Soeters, P.B. and Von Meyenfeldt, M.F. Glutamine appearance rate in plasma is not increased after gastrointestinal surgery in humans. *J. Nutr.* 130: 1566-1571, 2000.
37. Wacker, C., Prkno, A., Brunkhorst, F.M. and Schlattmann, P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *Lancet Infect. Dis.* 13: 426-435, 2013.
38. Wang, Y., Jiang, Z.M., Nolan, M.T., Jiang, H., Han, H.R., Yu, K., Li, H.L., Jie, B. and Liang, X.K. The impact of glutamine dipeptide-supplemented parenteral nutrition on outcomes of surgical patients: a meta-analysis of randomized clinical trials. *JPEN J. Parenter. Enteral Nutr*. 34: 521-529, 2010.
39. Wessner, B., Strasser, E.M., Spittler, A. and Roth, E. Effect of single and combined supply of glutamine, glycine, N-acetylcysteine, and R,S-alpha-lipoic acid on glutathione content of myelomonocytic cells. *Clin. Nutr.* 22: 515-522, 2003.
40. Wood, S.M., Beckham, C., Yosioka, A., Darban, H. and Watson, R.R. β-Carotene and selenium supplementation enhances immune response in aged humans. *Integr. Med.* 2: 85-92, 2000.