Nutrition and Cancer

ISSN: 0163-5581 (Print) 1532-7914 (Online) Journal homepage: www.tandfonline.com/journals/hnuc20

Effects of Malnutrition on Neutrophil/ Mononuclear Cell Apoptotic Functions in Children with Acute Lymphoblastic Leukemia

Fatma Betul Cakir, Su Gülsün Berrak, Gonul Aydogan, Aysin Tulunay, Cetin Timur, Cengiz Canpolat & Emel Eksioglu Demiralp

To cite this article: Fatma Betul Cakir, Su Gülsün Berrak, Gonul Aydogan, Aysin Tulunay, Cetin Timur, Cengiz Canpolat & Emel Eksioglu Demiralp (2017) Effects of Malnutrition on Neutrophil/ Mononuclear Cell Apoptotic Functions in Children with Acute Lymphoblastic Leukemia, Nutrition and Cancer, 69:3, 402-407, DOI: 10.1080/01635581.2017.1267778

To link to this article: https://doi.org/10.1080/01635581.2017.1267778

Published online: 20 Jan 2017.

NUTRITION AND CANCER
2017, VOL. 69, NO. 3, 402–407
http://dx.doi.org/10.1080/01635581.2017.1267778

Taylor & Francis
Taylor & Francis Group

Effects of Malnutrition on Neutrophil/Mononuclear Cell Apoptotic Functions in Children with Acute Lymphoblastic Leukemia

Fatma Betul Cakir[a], Su Gülsün Berrak[b], Gonul Aydogan[c], Aysin Tulunay[d], Cetin Timur[e], Cengiz Canpolat[f], and Emel Eksioglu Demiralp[d]

[a]Bezmialem Vakif University, Pediatric Hematology-Oncology, Istanbul, Turkey; [b]The Children's Hospital at Montefiore, The Pediatric Hospital for Albert Einstein College of Medicine and Refuah Health Center, NewYork, NY, USA; [c]Istanbul Kanuni Sultan Suleyman Government Education and Research Hospital, Pediatric Hematology-Oncology, Istanbul, Turkey; [d]Marmara University Medical Center, Internal Medicine Hematology-Immunology Department, Istanbul, Turkey; [e]Istanbul Medeniyet University, Pediatric Hematology-Oncology, Istanbul, Turkey; [f]Acibadem University School of Medicine, Pediatric Hematology-Oncology, Istanbul, Turkey

ABSTRACT

Background: Recent studies claim that apoptosis may explain immune dysfunction observed in malnutrition. *Objective*: The objective of this study was to determine the effect of malnutrition on apoptotic functions of phagocytic cells in acute lymphoblastic leukemia (ALL). Materials and Methods: Twenty-eight ALL patients (13 with malnutrition) and thirty controls were enrolled. Neutrophil and mononuclear cell apoptosis of ALL patients and the control group were studied on admission before chemotherapy and repeated at a minimum of three months after induction of chemotherapy or when the nutritional status of leukemic children improved. *Results*: The apoptotic functions of both ALL groups on admission were significantly lower than those of the control group. The apoptotic functions were lower in ALL patients with malnutrition than those in ALL patients without malnutrition, but this was not statistically significant. The repeated apoptotic functions of both ALL groups were increased to similar values with the control group. This increase was found to be statistically significant. *Conclusions*: The apoptotic functions in ALL patients were not found to be affected by malnutrition. However, after dietary intervention, increased apoptotic functions in both ALL patient groups deserve mentioning. Dietary intervention should always be recommended as malnutrition or cachexia leads to multiple complications. Enhanced apoptosis might originate also from remission state of cancer.

ARTICLE HISTORY
Received 28 December 2015
Accepted 17 November 2016

Introduction

Apoptosis is a well-recognized, programmed cell death. It plays an important role as a common homeostatic property of all tissues, including tumor tissue (1). Recently, it has been reported that apoptotic cells of the immune system can actively regulate the immune response, and extensive apoptosis may disturb the host defense and suppress the immune system (1–5). Apoptosis of phagocytic cells is a very dynamic and complex issue, both indicating positive signals that activate mononuclear phagocytes to migrate toward apoptotic cells, and also negative signals that inhibit granulocyte migration (1). On the other hand, malnutrition is a common cause of secondary immune deficiency still having unresolved mechanisms. It is an important risk factor for infection, sepsis, and death (6–9) and compromises immune response by reducing cell-mediated immunity, phagocyte function, secretory antibody response (6,10), and antibody affinity and by affecting the complement system and cytokine production (6,11). Hence, apoptosis may explain immune dysfunction observed in states of under nutrition and malnutrition (1,6,7). Given the central role of immune system in malnutrition and involvement of apoptosis in regulation of the immune system, we aimed to investigate the effect of malnutrition on apoptosis of the cells making up the immune system in patients with acute lymphoblastic leukemia (ALL).

Furthermore, neutrophils release anti-inflammatory peptides, alpha-defensins, following engagement of their apoptosis programme. Further anti-inflammatory molecules released directly from apoptotic cells seem likely and await elucidation.

Materials and Methods

This prospective study is approved by the local ethics committee of the Marmara University and funded by the

CONTACT Fatma Betul Cakir fbetulcakir@gmail.com Bezmialem Vakif University Department of Pediatrics, Pediatric Hematology- Oncology, Adnan Menderes Bulvarı, Vatan Street, 34093, Fatih, Istanbul, Turkey.

Marmara University Academic Research and Projections Commission. Signed parental informed consent was obtained.

The newly diagnosed ALL patients were enrolled from three different centers. The age and sex-matched control group were chosen from healthy children without chronic diseases attending outpatient clinics. All children had physical examinations carried out including height, weight, ideal weight (IW), triceps skinfold thickness (TSFT), upper arm muscle circumference (UAMC) and calculation of body mass index (BMI) at initial presentation by the same physician (Dr. FBC). IW and BMI of each patient and healthy control were compared with the national standards (12). IW measurements below 90% and scores below the 5th percentile for BMI values were considered to be in malnutrition limits. TSFT and UAMC were compared with the standards developed by A. Roberto Frisancho (13). TSFT measurements below the 5th percentile and UAMC measurements below the 15th percentile were evaluated as malnourished. ALL patients were identified to have malnutrition if two of the four anthropometric measurements (IW, BMI, TSFT, and UAMC) were within malnutrition limits. At the same time, blood was taken for specific biochemical analyses including albumin, prealbumin and transferrin and for apoptosis tests before induction chemotherapy from both patient groups; blood was also obtained from control group for apoptosis tests as the test control values. Serum was isolated for biochemical analyses and stored at –40°C until all sera are collected at the end of the study. Apoptosis tests were studied at the same day on childrens' inclusion to the study before induction chemotherapy. ALL patients with malnutrition received oral diet counseling with calorie-rich foods and also commercially available concentrated sources of carbohydrates, proteins, or fats. Their daily estimated energy requirements were calculated according to ASPEN guidelines that are, briefly; ages 1–7 years need 120 kcal/kg/day, ages 7–12 years need 75 kcal/kg/day, and ages above 12 years need 60 kcal/kg/day (14). During the remission induction period, steroids were administered as the main chemotherapeutic agent to ALL patients. Steroids increased some of the children's appetites. Increased food intake made these patients gain weight earlier. The anthropometric measurements were performed periodically in all patients for at least three months after the induction of chemotherapy until their nutritional status improved. When the nutritional status improved, control blood was taken for specific biochemical analyses from both patient groups and for apoptosis tests from both patient groups and control group.

Isolation of White Blood Cells

White blood cells from venous blood of the patients were isolated by an ammonium chloride-based method. Briefly, 3 ml of blood was mixed with 30 ml of erythrocyte lysing solution (0.1 ml EDTA; 155 mM NH_4Cl; 10 mM $KHCO_3$) and was incubated at room temperature for 15 min. Following centrifugation at 600 *g* for 10 min, supernatant was discarded, and the cell pellet was washed with phosphate-buffered solution gel (PBS-gel; PBS containing 0.1% gelatin) and cell count was adjusted to $3–4 \times 10^6$.

Evaluation of Apoptosis and Cell Necrosis

Apoptosis was induced in cells using phorbol myristate acetate (PMA, Sigma-Aldrich, Taufkirchen, Germany) as previously described and optimized (15). Briefly, two tubes were prepared for each apoptosis experiment, and 1×10^6 cells/ml were dispensed into the tubes. One of these was induced for apoptosis using 100 ng/ml of PMA, at 37°C for 3 h, while the other was incubated at the same temperature without stimulation as a control. Fluorogenic caspase-3 substrate, PhiPhiLux (PhiPhiluxG1D2, Cat. No. 235430 Calbiochem®, EMD Biosciences, Inc. Merck KGaA, Darmstadt, Germany) was used to stain and then evaluate apoptotic events in mononuclear cells and neutrophils. Following washing with PBS-gel, cells were labeled with 50 μL PhiPhiLux at 37°C in the dark for 1 h. Cells were washed again with PBS-gel. During evaluation by flow cytometry, 20 mg/ml of propidium iodide was added to the tubes to reveal the late stages of apoptotic cell death.

For analysis, mononuclear cells and neutrophils were separately gated according to their granularity and size, on forward scatter versus side scatter plots. Early apoptosis, late apoptosis, and cell necrosis were evaluated on fluorescence 1 (FL1 for PhiPhiLux) versus fluorescence 3 (FL3 for propidium iodide) plots. While the percentage of cells stained with PhiPhiLux only was evaluated as early apoptosis, the percentage of cells stained with both PhiPhiLux and propidium iodide was evaluated as late apoptosis and the percentage of cells stained with propidium iodide only was evaluated as necrotic cells.

Statistical Analysis

Two independent groups were compared with Pearson chi-square test for categorical variables. Comparisons between two independent groups were made by Fisher exact test if the number of one independent group was below five. Student *t*-test and Mann-Whitney U test were used for comparisons of continuous variables between two independent groups. Dependent groups were compared with Wilcoxon signed-ranks test for

continuous variables. Significance level was accepted with $P < 0.05$. Early neutrophil apoptosis test is one of the primary tests in this study and probably has the largest sample width. For 12 percent rise in early neutrophil apoptosis (standard deviation as 15), it is planned to have 15 cases per group to yield 80% power with 95% confidence interval. SPSS 17.0 program was used in order to evaluate the statistical tests.

Results

Demographic Data

Thirteen ALL patients (9 girls, 4 boys) with malnutrition, 15 ALL patients (10 girls, 5 boys) without malnutrition aged between 11 months and 16 years, and 30 healthy controls were enrolled into the study. Age between patients and healthy controls is allowed to be matched to ±3 months (Table 1). Except for the UAMC measurement that has a borderline statistical significance, IW, BMI, and TSFT measurements of ALL patients with malnutrition were found to be significantly lower than those of both ALL patients without malnutrition and healthy controls at admission to the study (Table 1). Except for the transferrin value, albumin and prealbumin values of ALL patients with malnutrition were found to be significantly lower than those of ALL patients without malnutrition at admission to the study (Table 2). These values of ALL patients with malnutrition were shown to reach those values of ALL patients without malnutrition when their nutritional status improved (Table 2).

Effect of Malnutrition on Apoptotic Functions

The measurements of neutrophil apoptotic and necrotic neutrophil functions of ALL patients with malnutrition were lower than ALL patients without malnutrition before induction of chemotherapy but could not reach a statistical significance (Table 3).

The measurements of mononuclear cell apoptotic and necrotic mononuclear cell functions of ALL patients with malnutrition were lower than those of ALL patients without malnutrition before induction of chemotherapy but could not reach a statistical significance (Table 3).

The measurements of neutrophil apoptotic and necrotic neutrophil functions of ALL patients with and without malnutrition before induction of chemotherapy were significantly lower than those of the control group (Table 3). The measurements of mononuclear cell apoptotic and necrotic mononuclear cell functions of ALL patients with and without malnutrition before induction of chemotherapy were significantly lower than those of the control group (Table 3).

Effect of Nutritional Supplementation on ALL patients with Malnutrition on Apoptotic Functions

The average length of time between the baseline apoptotic measures and last measure of the patients is ±3,6 months. This is also the duration for the nutritional status of ALL children to improve.

The repeated neutrophil and mononuclear cell apoptotic functions of ALL patients with and without malnutrition before induction of chemotherapy were shown to increase to the values of those of the control group three months after the induction of chemotherapy (Table 4). This increase was found to be statistically significant for all neutrophil and mononuclear cell apoptotic functions ($P < 0.01$, Wilcoxon signed-rank test; Table 5).

Discussion

In this study, it is hypothesized that malnutrition may disturb macrophage and neutrophil apoptosis and

Table 1. Demographic features of ALL patient groups and the control group at admission before induction chemotherapy.

	ALL with malnutrition (n:13)	Comparison of ALL with and without malnutrition *P*-values	ALL without malnutrition (n:15)	Comparison of ALL without malnutrition and control group *P*-values	Control group (n:30)	Comparison of ALL with malnutrition and control group *P*-values
Gender: Male n(%)	4 (31)		5 (33)		11(37)	
Female n(%)	9 (69)	0.6*	10 (67)	0.82*	19(63)	0.7*
Age (months) (Mean ± SD)	95 ± 53.6	0.38**	80.1 ± 58.2	0.71**	84.7 ± 54.4	0.61**
Ideal weight (%) (Mean ± SD)	84.19 ± 4.88	<0.01***	100.86 ± 10.1	0.96***	100.76 ± 5.02	<0.01***
Upper arm muscle circumference (cm) (Mean ± SD)	13.23 ± 3.21	0.06**	15.13 ± 2.93	0.37**	15.2 ± 3.7	0.07**
Triceps skinfold thickness (cm) (Mean ± SD)	7.63 ± 1.46	<0.01**	11.96 ± 2.12	0.76**	11.2 ± 2.1	<0.01**
Body mass index (Weight/ Height^2)	14.57 ± 1.63	<0.01**	18.11 ± 2.81	0.13**	17.3 ± 2.3	<0.01**

ALL: Acute Lymphoblastic Leukemia, *P-values calculated from Pearson chi-square test, **P-values calculated from Mann-Whitney U test, ***P-values calculated from simple *t*-test, SD: standard deviation.

Table 2. Biochemical values of ALL patient groups at admission before induction chemotherapy and the control values repeated when the nutritional status improved.

	ALL with malnutrition Mean ± SD (Day 0)	Comparison of ALL with and without malnutrition *P*-values	ALL without malnutrition Mean ± SD (Day 0)	ALL with malnutrition Mean ± SD (control at least 3 months later)	Comparison of ALL with and without malnutrition *P*-values	ALL without malnutrition Mean ± SD (control at least 3 months later)
Albumin	3.26 ± 0.23	<0.01*	3.78 ± 0.22	4.23 ± 0.44	0.78*	4.18 ± 0.31
Prealbumin	11.3 ± 2.88	<0.01*	15.1 ± 3.31	16.9 ± 4.69	0.07*	18.1 ± 3.52
Transferrin	1.69 ± 0.27	0.09*	1.88 ± 0.32	2.16 ± 0.21	0.59*	2.01 ± 0.44

*P- values calculated from Mann-Whitney U test.
SD: standard deviation.

contribute to the immunosuppressed state. ALL, like many other cancers, exhibits loss of control in normal proliferation, differentiation, and also apoptosis (16,17). As expected, we demonstrated significantly lower neutrophil and mononuclear cell apoptotic measurements in ALL patient groups than the healthy controls.

Apoptotic cell death may have a role in suppressing the immune system (1–7). Voll et al., Chen et al. and Byrne et al. showed that the presence of apoptotic cells during monocyte and macrophage activation increases the secretion of anti-inflammatory cytokine interleukin 10 (IL-10), transforms growth factor-β, and inhibits proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-1, and IL-12; thus, the cellular immune system deactivates itself and other cells (2–4). Reiter and Krammer also found that apoptotic tumor cells in the environment decreases the competence of macrophages in the clearance of tumor cells and thus enhances the growth of tumor cells (5). Recently, de Oliveira et al. investigated the effect of protein malnutrition on the synthesis of IL-1a, IL-1b, IL-6, and IL-12 by peritoneal macrophages in rats, evaluating the NF-jB signaling pathway that is activated by TNF-α via TNF-receptor. It is revealed that macrophages from malnourished animals could neither activate NF-jB nor induce the production of IL-1b and IL-12 after TNF-α stimulation in comparison with cells from control animals (6,7). Several other studies described a marked deficit in TNF-α, IL-1, and IL-6 production in malnutrition (6,7,18–20), whereas others have reported that starvation increases TNF-α production (21,22). It is generally observed that in malnutrition states, when low levels of TNF-receptor are present, individuals are more susceptible to infection. A reduction in the stabilization of circulating TNF-α may contribute to this possibility (7). Hence, reduced levels of TNF-α is associated with download of all the biologic processes including inflammation, growth, differentiation, and also apoptosis (6,7). On the other hand, Ortiz et al. established that severe malnutrition is significantly associated with enhanced apoptosis in thymocytes of experimentally malnourished rats compared with well-nourished rats (21). They reported that malnutrition-induced apoptosis could be related to alterations of the microenvironment of the thymus and/or to an

Table 3. Neutrophil and mononuclear cell apoptosis measurements of ALL patient groups and the control group at admission before induction chemotherapy.

	ALL with malnutrition Mean ± SD (Day 0)	Comparison of Day 0 measurements of ALL with and without malnutrition *P*-values*	ALL without malnutrition Mean ± SD (Day 0)	Comparison of Day 0 measurements of ALL without malnutrition and control group *P*-values*	Control group Mean ± SD	Comparison of Day 0 measurements of ALL with malnutrition and control group *P*-values*
Neutrophil necrosis† (%)	10.56 ± 5.38	0.78	11.5 ± 5.64	<0.01	21.87 ± 14.36	<0.01
Early neutrophil apoptosis‡ (%)	3.86 ± 2.86	0.27	5.79 ± 4.66	<0.01	19.96 ± 14.19	<0.01
Late neutrophil apoptosis§ (%)	20.54 ± 10.03	0.96	20.62 ± 10.81	<0.01	41.23 ± 20.79	<0.01
Mononuclear cell necrosis† (%)	3.96 ± 2.29	0.92	5.31 ± 4.94	<0.01	11.34 ± 9.8	<0.01
Early mononuclear cell apoptosis‡ (%)	5.6 ± 3.44	0.85	6.37 ± 4.87	<0.01	13.2 ± 10.53	<0.01
Late mononuclear cellapoptosis§ (%)	2.83 ± 1.59	0.65	3.66 ± 2.54	<0.01	12.22 ± 10.55	<0.01

ALL, Acute Lymphoblastic Leukemia, SD, Standard Deviation,
*P-values calculated with Mann-Whitney U test.
†percentage of cells that are stained by propidium iodide only.
‡measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3.
§measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3 and propidium iodide.

Table 4. Neutrophil and mononuclear cell apoptosis measurements of ALL patient groups and the control group repeated when the nutritional status improved.

	ALL with malnutrition Mean ± SD	Comparison of measurements of ALL with and without malnutrition *P*-values*	ALL without malnutrition Mean ± SD	Comparison of measurements of ALL without malnutrition and control group *P*-values*	Control group Mean ± SD	Comparison of measurements of ALL with malnutrition and control group *P*-values*
Neutrophil necrosis† (%)	22.06 ± 9.24	0.71	22.56 ± 7.32	0.46	21.87 ± 14.36	0.61
Early neutrophil apoptosis‡ (%)	19.23 ± 9.39	0.92	19.38 ± 11.29	0.86	19.96 ± 14.19	0.64
Late neutrophil apoptosis§ (%)	41.84 ± 9.55	1	42.04 ± 18.26	0.8	41.23 ± 20.79	0.8
Mononuclear cell necrosis† (%)	11.78 ± 7.07	0.31	9.74 ± 7.21	0.76	11.34 ± 9.8	0.44
Early mononuclear cell apoptosis ‡ (%)	8.82 ± 4.44	0.78	10.06 ± 5.36	0.47	13.2 ± 10.53	0.36
Late mononuclear cell apoptosis§ (%)	12.2 ± 9.86	0.58	10.99 ± 8.66	0.99	12.22 ± 10.55	0.47

ALL, Acute Lymphoblastic Leukemia; SD, Standard deviation,
*P-values calculated with Mann-Whitney U test.
†Percentage of cells that are stained by propidium iodide only.
‡Measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3.
§Measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3 and propidium iodide.

obstruction of early thymocyte maturation. Rivadeneira et al. also reported enhanced apoptosis of macrophages in rats with protein-calorie malnutrition compared to rats fed with a normal protein diet (22). El-hodhod et al. showed enhanced lymphocyte apoptosis in children with protein energy malnutrition and also found that these changes were successfully reversible by proper nutritional rehabilitation (23). However, contrary to these findings, Luo et al. established significantly disturbed oral epithelial cell apoptosis and proliferation rates in malnourished patients with gastrointestinal cancer (24). They showed increased apoptosis rates after 3 days of nutritional support. Likewise, we found lower apoptotic functions in ALL patients with malnutrition compared with ALL patients without malnutrition, but this finding did not reach statistical significance. Larger sample sizes of ALL patients with and without malnutrition might show significant results in determining the means of nutrition. Small sample size of the patient groups is perhaps the most important limitation of our study. Malnutrition might delay apoptotic functions of neutrophils and mononuclear cells as de Oliveira et al. detected in the mouse model of protein energy malnutrition. The conflicting results of these studies and thus limited

Table 5. The comparison of neutrophil and mononuclear cell apoptosis measurements of ALL patient groups at admission before induction chemotherapy and when the nutritional status improved.

	ALL with malnutrition Mean ± SD (Day 0)	Comparison of measurements at Day 0 and when the nutrional status improved in ALL with malnutrition *P*-values*	ALL with malnutrition Mean ± SD (at improved nutritional status)	ALL without malnutrition Mean ± SD (Day 0) *P*-values*	Comparison of measurements at Day 0 and when the nutrional status improved in ALL without malnutrition *P*-values*	ALL without malnutrition Mean ± SD (at improved nutritional status)
Neutrophil necrosis† (%)	10.56 ± 5.38	<0.01	22.06 ± 9.24	11.5 ± 5.64	<0.01	22.56 ± 7.32
Early neutrophil apoptosis‡ (%)	3.86 ± 2.86	<0.01	19.23 ± 9.39	5.79 ± 4.66	<0.01	19.38 ± .64
Late neutrophil apoptosis§ (%)	20.54 ± 10.03	<0.01	41.84 ± 9.55	20.62 ± 10.81	<0.01	42.04 ± 18.26
Mononuclear cell necrosis† (%)	3.96 ± 2.29	<0.01	11.78 ± 7.07	5.31 ± 4.94	<0.01	9.74 ± 7.21
Early mononuclear cell apoptosis‡ (%)	5.6 ± 3.44	<0.01	8.82 ± 4.44	6.37 ± 4.87	<0.01	10.06 ± 5.36
Late mononuclear cellapoptosis§ (%)	2.83 ± 1.59	<0.01	12.2 ± 9.86	3.66 ± 2.54	<0.01	10.99 ± 8.66

ALL, Acute Lymphoblastic Leukemia; SD, Standard deviation,
*P-values calculated with Wilcoxon Signed Ranks test.
†percentage of cells that are stained by propidium iodide only.
‡measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3.
§measured by percent fluorescence of Phi-Philux that is cleaved by active caspase-3 and propidium iodide.

understanding of the relationship between malnutrition and apoptosis indicate the necessity for further studies in this issue. The most important finding in our study is that after dietary intervention, we detected increased apoptotic functions in both ALL patient groups. Dietary intervention, in the cancer setting, should always be recommended as malnutrition or cachexia leads to multiple complications like febrile neutropenia, mucositis, infection, and so treatment delay. Our study in this respect is original and emphasizes the impact of nutritional intervention on cancer. However, improved apoptosis might originate also from remission state of cancer. We cannot speculate on whether the cancer state or malnutrition is more important in determining apoptosis. Further studies with larger samples of patient groups and more evidence about complex mechanisms of apoptosis are needed to understand the overt effect of malnutrition on apoptotic functions.

References

1. Gregory CD and Pound JD. Cell death in the neighbourhood: direct microenvironmental effects of apoptosis in normal and neoplastic tissues. *J Pathol* **223**,177–194, 2011.
2. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, et al.: Immunosuppressive effects of apoptotic cells. *Nature* **390**, 350–351, 1997.
3. Chen W, Frank ME, Jin W, and Wahl SM: TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. *Immunity* **14**, 715–725, 2001.
4. Byrne A and Reen DJ: Lipopolysaccharide induces rapid production of IL-10 by monocytes in the presence of apoptotic neutrophils. *J Immunol* **168**, 1968–1977, 2002.
5. Reiter I, Krammer B, and Schwamberger G: Cutting edge: differential effect of apoptotic versus necrotic tumor cells on macrophage antitumor activities. *J Immunol* **163**, 1730–1732, 1999.
6. de Oliveira GP, Silva JD, de Araujo CC, Prota LF, Abreu SC, et al.: Intravenous glutamine administration reduces lung and distal organ injury in malnourished rats with sepsis. *Shock* **41**, 222–232, 2014.
7. de Oliveira DC, Hastreiter AA, Mello AS, de Oliveira Beltran JS, Oliveira Santos EW, et al.: The effects of protein malnutrition on the TNF-RI and NF-κB expression via the TNF-α signaling pathway. *Cytokine* **69**, 218–225, 2014.
8. Schneider SM, Veyres P, Pivot X, Soummer AM, Jambou P, et al.: Malnutrition is an independent factor associated with nosocomial infections. *Br J Nutr* **92**, 105–111, 2004.
9. Lim SL, Ong KC, Chan YH, Loke WC, Ferguson M, and Daniels L: Malnutrition and its impact on cost of hospitalization, length of stay, readmission and 3-year mortality. *Clin Nutr* **31**, 345–350, 2012.
10. Abe M, Akbar F, Matsuura B, Horiike N, and Onji M: Defective antigen-presenting capacity of murine dendritic cells during starvation. *Nutrition* **19**, 265–269, 2003.
11. Fock RA, Blatt SL, Beutler B, Pereira J, Tsujita M, et al.: Study of lymphocyte subpopulations in bone marrow in a model of protein energy malnutrition. *Nutrition* **26**, 1021–1028, 2010.
12. Neyzi O, Gunoz H, Andrzej F, Bundak R, Gökçay G, et al.: Türk çocuklarında vücut ağırlığı, boy uzunluğu, baş çevresi ve vücut kitle indeksi referans değerleri. *Çocuk Sağlığı ve Hastalıkları Dergisi* **51**, 1–14, 2008.
13. Frisancho AR: Mid arm circumference size norms for assessment of nutritional status. *Am J Clin Nutr* **27**, 1052–1058, 1994.
14. Anonymous: Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. Section XIII: Specific guidelines for disease-Pediatrics. JPEN **26**, 1SA–138SA, 2002.
15. Takei H, Araki A, Watanabe H, Ichinose A, and Sendo F: Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis. *J Leukoc Biol* **59**, 229–240, 1996.
16. Campana D, Coustan-Smith E, Manabe A, Buschle M, Raimondi SC, et al.: Prolonged survival of B-lineage acute lymphoblastic leukemia cells is accompanied by overexpression of bcl-2 protein. *Blood* **81**, 1025–1031, 1993.
17. Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Slöejes AW, et al.: Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* **91**, 2991–2997, 1998.
18. Fock RA, Vinolo MA, de Moura Sá Rocha V, de Sá Rocha LC, and Borelli P: Protein energy malnutrition decreases the expression of TLR-4/MD-2 and CD14 receptors in peritoneal macrophages and reduces the synthesis of TNF-alpha in response to lipopolysaccharide (LPS) in mice. *Cytokine* **40**, 105–114, 2007.
19. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, et al.: Tumor necrosis factor-alpha is required in the protective immune response against mycobacterium tuberculosis in mice. *Immunity* **2**, 561–572, 1995.
20. Muñoz C, Arévalo M, López M, and Schlesinger L: Impaired interleukin-1 and tumor necrosis factor production in protein-calorie malnutrition. *Nutr Res* **14**, 347–352, 1994.
21. Ortiz R, Cortes L, Gonzales-Marquez H, Gomez JL, Gonzales C, et al.: Flow cytometric analysis of spontaneous and dexamethasone-induced apoptosis in thymocytes from severely malnourished rats. *Br J Nutr* **86**, 545–548, 2001.
22. Rivadeneira DE, Grobmyer SR, Naama HA, Mackrell PJ, Mestre JR, et al.: Malnutrition-induced macrophage apoptosis. *Surgery* **129**, 617–625, 2001.
23. El-Hodhod MAA, Nassar MF, Zaki MM, and Moustafa A: Apoptotic changes in lymphocytes of protein energy malnutrition patients. *Nutr Res* **25**, 21–29, 2005.
24. Luo X, Zhou Y, Tao D, Yu Y, Hu J, et al.: Usefulness of oral mucosal epithelial cell apoptosis rate in nutritional assessment. *Nutr* **22**, 1032–1038, 2006.