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Reduced Plasma Amino Acid Levels During Allogeneic Hematopoietic Stem Cell Transplantation Are Associated with Systemic Inflammation and Treatment-Related Complications

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A B S T R A C T

Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) are challenged by cytotoxic effects of the conditioning regimen, resulting in tissue damage, systemic inflammation, and increased metabolic demands for amino acids to regenerate damaged tissues, reconstitute hematopoietic cells, and establish antioxidant defenses. To date, few studies have addressed the role of plasma amino acid (PAA) levels during transplantation, and it remains unknown if amino acid deficiency can aggravate treatment-related morbidity. We determined plasma levels of the 23 human amino acids in 80 HSCT recipients (age 1.1 to 55.4 years) before conditioning and on days +7 and +21 post-transplant along with C-reactive protein (CRP) and IL-6 levels on day +7. Significant changes were observed in plasma concentrations of several human amino acids during HSCT. On day +7, numerous amino acids were inversely correlated with both CRP and IL-6, including glutamic acid, serine, alanine, glutamine, arginine, cysteine, glycine, histidine, lysine, tryptophan, threonine, taurine, proline, and methionine (r = -.22 to -.66; all $P < .05$). Patients who developed sinusoidal obstruction syndrome (SOS) had significantly lower mean total PAA levels compared with patients without SOS (2013 ng/L [95% confidence interval (CI), 1709 to 2318 ng/L] versus 2706 ng/L [95% CI, 2261 to 3150 ng/L]; P = .006), along with lower individual levels of glutamic acid, serine, arginine, glycine, lysine, valine, tryptophan, threonine, and proline on day +7 (all $P < .05$). Patients with severe acute graft-versus-host disease had a lower mean total PAA level (1922 ng/L [95% CI, 1738 to 2106 ng/L] versus 2649 ng/L [95% CI, 2244 to 3055 ng/L]; P = .014) and lower levels of serine, glutamine, cysteine, glycine, lysine, and threonine on day +7 (all $P < .05$). These results indicate a relationship between low concentrations of certain amino acids and the risk of treatment-related complications.

INTRODUCTION

Although allogeneic myeloablative hematopoietic stem cell transplantation (HSCT) has improved survival in patients with severe hematologic diseases, this treatment is challenged by severe adverse events due to immunosuppression, acute graft-versus-host disease (aGVHD), and direct cytotoxicity of the conditioning regimen [1].

Cytotoxic effects of the conditioning reach a maximum during the first 3 weeks after transplantation, and the resulting release of antigens from necrotic tissue, bacterial translocation from the gastrointestinal tract, and release of proinflammatory cytokines result in a condition of metabolic stress comparable to critical illnesses such as sepsis and trauma. This condition is accompanied by an increased metabolic rate and accelerated catabolism, proteolysis, and gluconeogenesis [2–6]. During this phase, the metabolic demand for amino acids involved in tissue repair, regeneration of the hematopoietic cells, antioxidant defenses, and production of acute phase reactants is highly elevated [5,7]. The synthesis of acute phase reactants and antioxidants by the liver is thought to play a role in balancing proinflammatory and anti-inflammatory mechanisms by inducing negative feedback on the inflammatory response and limiting oxidative damage, thereby favoring regenerative

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processes while limiting the degree of systemic inflammation, which has been associated with increased treatment-related morbidity and mortality after HSCT [4,8,9].

The majority of HSCT recipients suffer from varying degrees of mucositis, resulting in limited food intake and malnutrition, which is further intensified by significant catabolism of muscle tissue to meet the increased demand for amino acids in production of antioxidants and acute phase reactants [5,10].

It has previously been suggested that an insufficient intake of amino acids could adversely affect the production of antioxidants (e.g. glutathione), thereby compromising antioxidant defenses and inducing an increase in the inflammatory response [7,11–13].

To date, few studies have addressed amino acid levels during HSCT, and whether amino acid deficiency may aggravate treatment-related morbidity remains unknown. In the present study, we aimed to determine free plasma amino acid (PAA) levels before and after transplantation and to investigate associations with systemic inflammation and treatment-related complications. We hypothesized that low PAA levels during HSCT could be associated with high proinflammatory activity and related complications, including sinusoidal obstruction syndrome (SOS) and aGVHD.

METHODS

Study Population

We prospectively recruited 80 patients (age 1.1 to 55.4 years) undergoing their first allogeneic HSCT at Rigshospitalet in Copenhagen between June 2010 and January 2013. All patients received pretransplantation myeloablative conditioning based on total body irradiation (TBI) combined with chemotherapy (n = 45) or chemotherapy alone (n = 35). The study was approved by the local Ethics Committee (reference H-1-2010-009) and conducted in accordance with the Declaration of Helsinki. Written and oral informed consent was obtained for all patients. Clinical characteristics of the patients are listed in Table 1 and have been published previously [14,15].

Quantification of PAAs, Urea, and Creatinine

Blood samples used for amino acid analysis were collected at 3 time points during HSCT: before initiation of the conditioning regimen (baseline) and on days +7 and +21 post-transplantation. Blood was collected in lithium-heparin anticoagulated tubes and centrifuged shortly after collection, after which plasma was isolated and stored at -80 °C within 2 hours after sampling. Free plasma concentrations of the 23 human amino acids were determined by reverse-phase high-performance liquid chromatography of their phenyl isothiocyanate derivatives (PicoTag, Waters, Woburn, MA), as reported previously [16]. Total PAA level was calculated as the sum of all individual amino acid levels. Plasma levels of urea and creatinine were measured daily during hospitalization as part of the general clinical routine. An elevated urea/creatinine ratio can be caused by various conditions, including prerenal azotemia, gastrointestinal hemorrhage, and a severe catabolic state [17,18]. In our study, urea/creatinine ratio served as a measure of protein catabolism by calculating levels during transplantation as percentages of pretransplantation levels. To account for age- and sex-related differences in the normal ranges of creatinine and urea, we used the preconditioning value (day -7) as a reference and calculated values for the subsequent days as percent of baseline, making it possible to pool all patients in the analyses.

Inflammatory Parameters

EDTA anticoagulated blood was collected on day +7, and plasma was isolated and analyzed for IL-6 using the Human Th1/Th2/Th17 Cytometric Bead Array Kit and a FACSCalibur flow cytometer (Becton Dickinson A/S, Albertslund, Denmark). This time point was selected based on previous observations of peaking IL-6 levels at this stage [19]. The limit of detection was 2.5 pg/mL. Levels of C-reactive protein (CRP) were measured daily during hospitalization using the Modular P module (Roche, Basel, Switzerland).

Parenteral Nutrition and Use of Glucocorticoids

We extracted the number of days on parenteral nutrition (PN) for each patient during the course of HSCT. Data on the administration of glucocorticoids during HSCT were collected and converted to prednisolone equivalents/kg.

Screening for SOS

Patients were retrospectively evaluated for occurrence of SOS using the modified Seattle criteria [20,21]. For a diagnosis of SOS, 2 of the following 3 criteria must be present within the first 20 days after transplantation: >2% weight gain from baseline, serum bilirubin >34 μmol/L, and hepatomegaly with right upper quadrant pain. Patients were weighed daily during hospitalization, and total bilirubin levels were measured using Modular P module (Roche, Basel, Switzerland).

Table 1
Patient and Transplantation Characteristics

Characteristic	Value
All patients, N (%)	80 (100)
Male sex, n (%)	48 (60)
Age at transplantation, yr, median (range)	
Recipients	17.0 (1.1-55.4)
Donors	30.0 (.0-60.8)
Disease at transplantation, n (%)	
Acute lymphoblastic leukemia	25 (31)
Acute myelogenous leukemia	19 (24)
Other leukemia	5 (6)
Myelodysplastic syndrome	10 (13)
Non-Hodgkin lymphoma	2 (2)
Multiple myeloma	1 (1)
Severe aplastic anemia	6 (8)
Immunodeficiency	7 (9)
Other nonmalignant disease	5 (6)
Donor type, n (%)	
HLA-identical siblings	18 (23)
HLA-matched unrelated donors	54 (67)
HLA-mismatched unrelated donors	8 (10)
Stem cell source, n (%)	
Bone marrow stem cells	57 (71)
Peripheral blood stem cells, G-CSF-mobilized	15 (19)
Umbilical cord blood stem cells	8 (10)
Conditioning regimen, n (%)	
TBI + cyclophosphamide or etoposide	45 (56)
Busulfan + cyclophosphamide	17 (22)
Cyclophosphamide + fludarabine	9 (11)
Other high-dose chemotherapy	9 (11)
Antithymocyte globulin as part of conditioning, n (%)	37 (46)
GVHD prophylaxis, n (%)	
Cyclosporine + methotrexate	69 (86)
Cyclosporine + corticosteroids	3 (4)
Cyclosporine alone	8 (10)
Cytomegalovirus IgG mismatch, n (%)	
Seronegative donor to seropositive recipient	24 (30)
Seropositive donor to seronegative recipient	9 (11)

Statistical Analyses

Mixed-model repeated-measures analyses were used for paired comparisons between baseline values of PAAs and the subsequent time points, as well as for analyses of changes in urea and creatinine levels. Correlation analyses were performed using Spearman's rank-order correlation analysis. Nonparametric statistics were applied in analyses where the inflammatory parameters were included, because these were not normally distributed. The Kruskal-Wallis nonparametric univariate test was used for comparison of continuous variables between groups owing to the small patient numbers in each subgroup. A 2-sided P value <.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Changes in PAA Levels During HSCT

Table 2 shows the development for each amino acid during the period. Total PAA level remained relatively unchanged during and after HSCT; the mean total PAA concentration was 2390 ng/L (95% confidence interval [CI], 2158 to 2622 ng/L) before conditioning, 2548 ng/L (95% CI, 2317 to 2779 ng/L) on

Table 2
PAA Levels During HSCT

Amino Acid	Preconditioning Level, ng/L, mean (95% CI)	Level on Day +7, ng/L, mean (95% CI)	*P* Value	Level on Day +21, ng/L, mean (95% CI)	*P* Value
Aspartate	4 (3-5)	2 (1-3)	**.03**	2 (1-3)	**.03**
Glutamic acid	57 (43-70)	59 (46-73)	.78	66 (52-81)	.31
Serine	100 (83-117)	118 (102-135)	.10	87 (69-104)	.28
Alanine	300 (253-347)	282 (236-329)	.60	266 (218-315)	.33
Glutamine	528 (498-558)	473 (444-503)	**.003**	560 (528-590)	.10
Arginine	42 (23-61)	70 (51-89)	**.04**	51 (31-71)	.51
Asparagine	38 (35-42)	24 (21-28)	<**.0001**	32 (29-36)	**.005**
Citrulline	14 (12-15)	3 (2-5)	<**.0001**	8 (7-10)	<**.0001**
Cysteine	29 (25-32)	30 (27-34)	.37	43 (40-46)	<**.0001**
Glycine	187 (153-222)	219 (184-253)	.19	218 (183-254)	.21
Histidine	49 (43-55)	47 (42-53)	.66	42 (37-48)	.07
Isoleucine	55 (46-64)	58 (49-67)	.64	48 (39-57)	.23
Leucine	102 (88-116)	138 (124-151)	**.0002**	98 (84-112)	.70
Lysine	132 (117-147)	138 (123-153)	.56	136 (120-151)	.76
Valine	180 (163-197)	252 (236-269)	<**.0001**	185 (168-202)	.63
Tryptophan	38 (35-42)	47 (44-51)	**.0001**	40 (37-44)	.39
Threonine	96 (84-108)	107 (95-119)	.19	110 (98-122)	.09
Taurine	34 (30-38)	38 (35-42)	.07	39 (35-42)	**.05**
Tyrosine	65 (60-70)	54 (49-59)	**.0002**	54 (49-60)	**.0005**
Proline	212 (180-244)	200 (169-232)	.60	172 (140-205)	.08
Phenylalanine	47 (38-56)	94 (85-103)	<**.0001**	66 (57-75)	**.003**
Ornitine	62 (56-67)	61 (56-66)	.89	55 (50-60)	.06
Methionine	20 (13-27)	30 (23-37)	**.04**	26 (19-33)	.22
Total	2390 (2158-2622)	2548 (2317-2779)	.34	2412 (2172-2652)	.90

Significant *P* values are in bold type.

day +7, and 2412 ng/L (95% CI, 2172 to 2652 ng/L) on day +21. For the individual amino acids, significant decreases from preconditioning level to day +7 were found for tyrosine, aspartate, glutamine, asparagine, and citrulline, and all values remained low on day +21 with the exception of glutamine, which returned to preconditioning level.

Increases were observed for leucine, valine, tryptophan, methionine, arginine, and phenylalanine from preconditioning to day +7, and of these, only phenylalanine remained elevated on day +21. In addition, increases were observed for taurine and cysteine on day +21 compared with preconditioning values.

Changes in Creatinine and Urea During HSCT

We analyzed creatine and urea levels to identify any change in urea/creatine ratio indicating increased amino acid metabolism. Before conditioning, 2 patients had slightly elevated renal parameters, with a maximum creatinine level of 110 μmol/L.

To account for age- and sex-related differences in the normal ranges of creatinine and urea, we used the baseline value (on day -7) as a reference and calculated values for the subsequent days as percentages of preconditioning levels. Changes in creatinine and urea levels and urea/creatinine ratio from baseline to day +21 are shown in Figure 1.

We found significant increases from preconditioning levels in both urea and urea/creatinine ratio from day 0 onward (all $P < .05$). Peak urea levels were found on day +21 (mean, 200%; 95% CI, 171% to 229%; $P < .0001$), whereas the peak urea/creatinine ratio was seen on day +11 (207%; 95% CI, 182% to 132%; $P < .0001$) and remained elevated to day +21. Creatinine levels was significantly elevated from baseline only between days +19 and +21.

PN and Use of Glucocorticoids

During the course of transplantation, 73 patients (91%) received PN, for a median of 16 days (interquartile range [IQR], 12 to 21), from day -7 to day +21. No associations were found between total PAA levels on days +7 and +21 and cumulative days on PN.

Twenty-six patients (33%) received glucocorticoids between day -7 and day +21. All patients started treatment after day 0, and only 4 patients were treated before day +7. The median cumulative dosage of prednisolone during this period was 0 mg/kg (Range .0 to 27.38 mg/kg). The use of PN and glucocorticoids was distributed equally in pediatric and adult patients.

Associations with Patient Characteristics

Total PAA level before conditioning were independent of patient age, sex, and diagnosis. The mean levels were significantly higher in children (defined as age <16 years) compared with adult patients on day +7 (2727 ng/L [95% CI, 2343 to 3110 ng/dL] versus 2382 ng/L [95% CI, 1789 to 2975 ng/dL]; $P = .002$) and on day +21 (2654 ng/L [95% CI, 2377 to 2931 ng/dL] versus 2177 ng/L [95% CI, 2043 to 2311 ng/dL]; $P = .0007$). No differences in PAA levels during HSCT were observed between patients who received TBI and those who did not receive TBI or between patients with different donor types, donor matches, stem cell sources, or diagnoses (malignant versus benign diseases).

Systemic Inflammation

CRP levels before the start of conditioning were within the normal range (median, 3.6 pg/mL; IQR, 1.4 to 10.0 pg/mL). CRP peaked at approximately 1 week after transplantation, with a median value of 55.2 pg/mL (IQR, 13.0 to 17.0 pg/mL) on day +7, followed by a gradual decline to a median of 6.6 pg/mL (IQR, 2.0

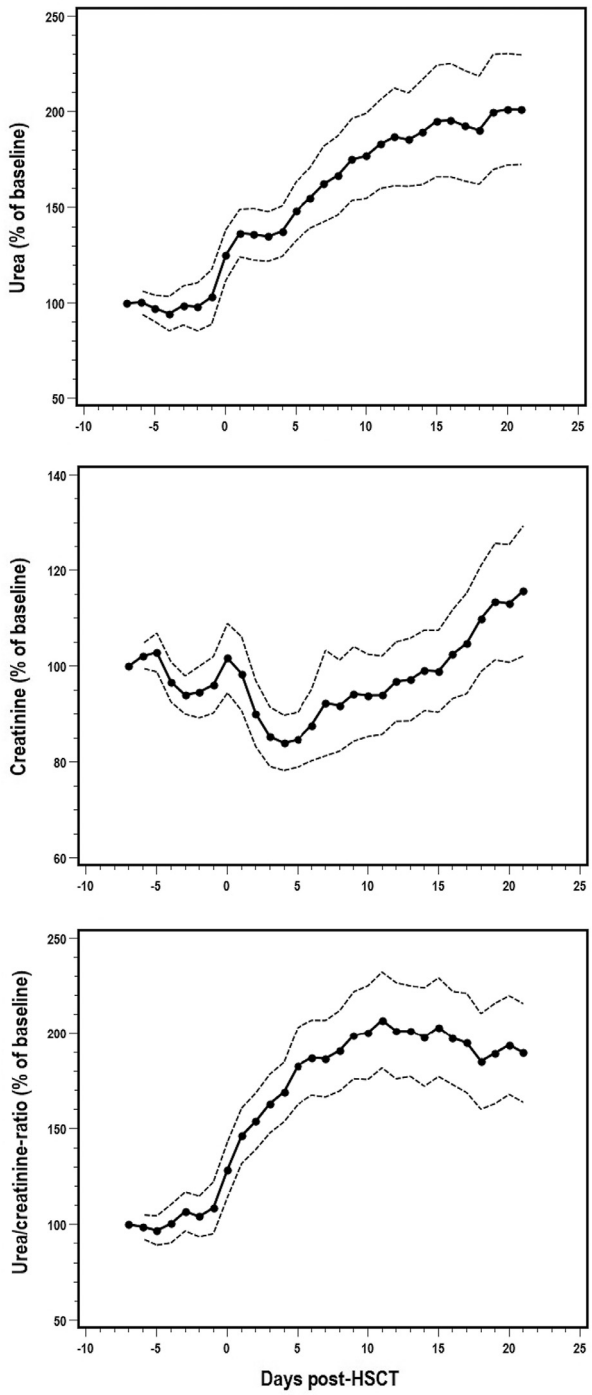

Figure 1. Mean plasma levels of urea and creatinine and the urea/creatinine ratio during the course of HSCT from baseline (before the start of conditioning) to day +21 post-transplantation, reported as percentage of preconditioning levels. Day 0 refers to the day of transplantation. The dotted line represents 95% CI.

to 23.2 pg/mL) on day +21. In addition, the median IL-6 level on day +7 was elevated to 35.2 pg/mL (IQR, 12.5 to 105.6) pg/mL) (reference value, <5 pg/mL in healthy controls).

We investigated associations between PAA levels and markers of inflammation and found that total PAA levels on day +7 were negatively correlated with both CRP (r = -.52; $P < .0001$) and IL-6 (r = -.62; $P < .0001$) levels on day +7 post-transplantation. In addition, several individual amino acids were inversely correlated with both CRP and IL-6, with the strongest correlations for serine, glutamine, glycine, arginine, and threonine (all $P < .001$) (Figure 2), followed by glutamic acid, alanine, cysteine, histidine, tryptophan, taurine, proline, and methionine (all $P < .05$). On day +21, negative correlations were found between CRP and total PAA as well as for serine, alanine, glutamine, asparagine, glycine, isoleucine, leucine, lysine, and threonine, whereas a positive association was found between phenylalanine and CRP (Table 3).

In children, but not in adults, we found a significant positive correlation between days on PN and levels of the inflammatory parameters IL-6 (r = .41; P = .008) and CRP (r = .49; P = .002) at day +7.

Treatment-Related Inflammatory Complications

Eighteen patients developed SOS, with a median onset at day +9 (IQR, days +5 to +10). These patients are described in Supplementary Table S1. The patients who developed SOS had a significantly lower total mean PAA level on day +7 compared with those without SOS (2013 ng/L [95% CI, 1709 to 2318 ng/L] versus 2706 ng/L [95% CI, 2261 to 3150 ng/L]; P = .006). No significant associations between SOS status and total PAA levels were found at other time points. Regarding the individual amino acids, patients with SOS had lower levels of glutamic acid (P = .028), serine (P = .013), arginine (P = .015), glycine (P = .004), lysine (P = .0008), valine (P = .048), tryptophan (P = .0007), threonine (P = .003), and proline (P = .031) on day +7 compared with patients without SOS (Figure 3). No associations were found between SOS and the cumulative days on PN or use of glucocorticoids.

Eleven patients developed grade III-IV aGVHD, with a median onset on day +16 (IQR, days +11 to +29). Of these, 6 patients had severe gastrointestinal aGVHD. Patients developing severe aGVHD had lower mean total PAA levels on day +7 (1922 ng/L [95% CI, 1738 to 2106 ng/dL] versus 2649 ng/L [95% CI, 2244 to 3055 ng/dL]; P = .014), as well as lower levels of serine (P = .004), glutamine (P = .029), cysteine (P = .021), glycine (P = .034), lysine (P = .047), and threonine (P = .010) on day +7, but these associations with aGVHD were not observed at other time points. Patients with severe aGVHD received significantly higher doses of glucocorticoids (mean, .0 mg/kg [95% CI, .0 to 1.9 mg/kg] versus 8.9 mg/kg [95% CI, .0 to 20.0 mg/kg]; $P < .0001$). No association was found between aGVHD and cumulative days on PN.

We evaluated the aforementioned significant associations in multivariate analyses. Regarding SOS, we adjusted for recipient age and the use of TBI, cyclophosphamide and busulfan as part of conditioning. In these analyses, higher total PAA levels on day +7 remained associated with a decreased risk of SOS (odds ratio, .88; 95% CI, .76 to 1.00 per 100 ng/L increase in total PAA level; P = .05). Associations also remained significant for some individual amino acids, including lysine (P = .004), valine (P = .043), tryptophan (P = .004), and threonine (P = .012).

For aGVHD, we adjusted for recipient age, donor type (HLA-matched sibling, HLA-matched unrelated, or HLA-mismatched unrelated donor), use of antithymocyte globulin, and TBI. No significant association was found between total PAA level and the risk of aGVHD (odds ratio, .88; 95% CI, .74 to 1.04) per 100 ng/L increase in total PAA level (P = .13).

DISCUSSION

In this study, we investigated associations between PAA levels and systemic inflammation and treatment-related complications during the early phase after transplantation. We found that low levels of several human amino acids are associated with elevated levels of proinflammatory markers, as well as with the development of SOS and severe aGVHD.

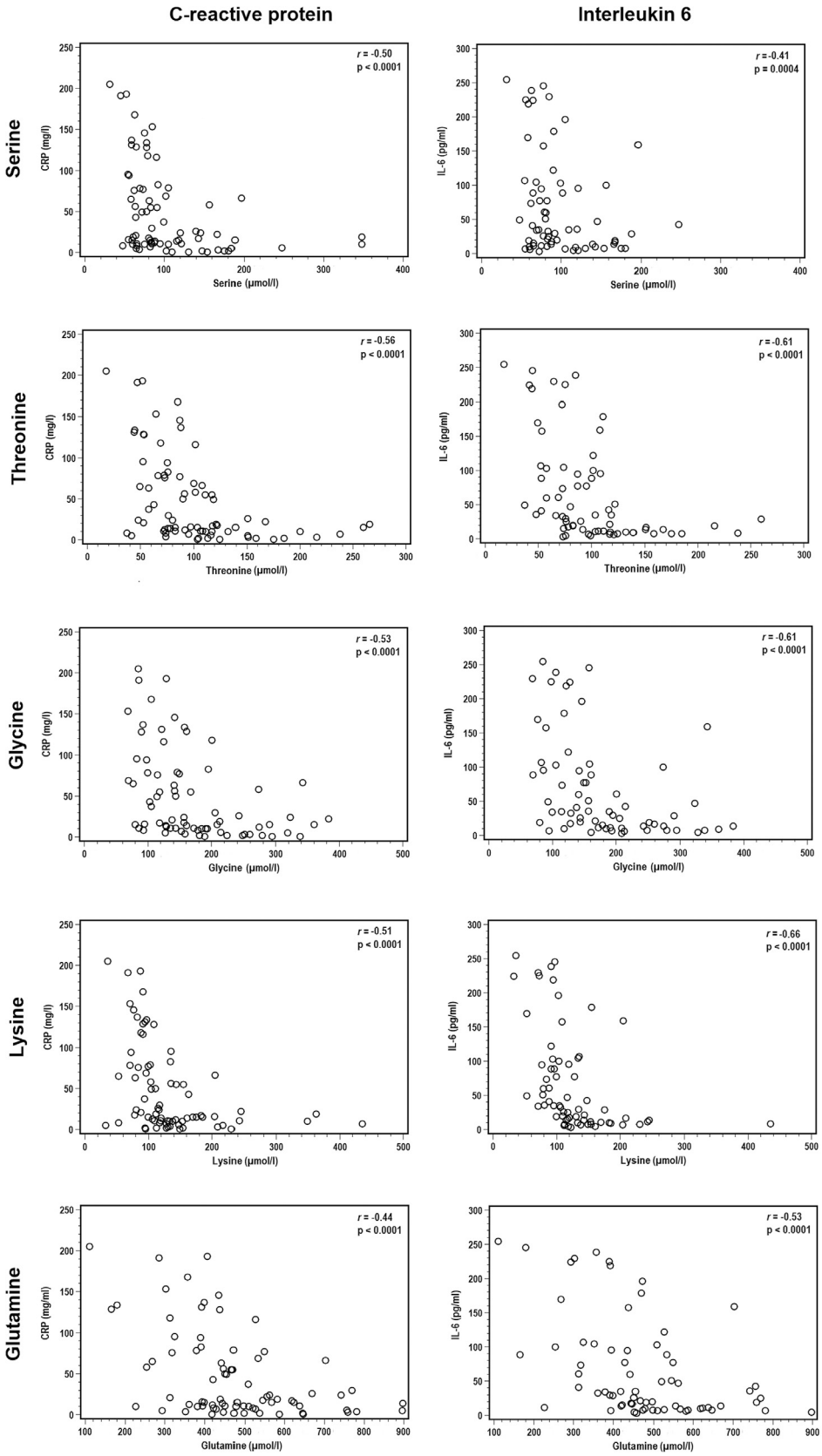

Figure 2. Spearman's rank-order correlation between PAA and inflammatory parameters. Plasma levels of serine, threonine, glycine, lysine, and glutamine on day +7 post-transplantation were correlated with same-day levels of CRP (*left*) and IL-6 (*right*).

Table 3
Spearman's Rank-Order Correlation Analyses Between PAA Levels and Same-Day Inflammatory Parameters

Amino acid	CRP day +7		IL-6 day +7		CRP day +21	
	r	*P* Value	*r*	*P* Value	*r*	P Value
Aspartate	-.15	.202	-.07	.573	-.10	.412
Glutamic acid	-.22	**.050**	-.27	**.025**	-.17	.166
Serine	-.50	<**.0001**	-.41	**.0004**	-.36	**.003**
Alanine	-.33	**.003**	-.44	**.0001**	-.24	**.050**
Glutamine	-.44	<**.0001**	-.53	<**.0001**	-.53	<**.0001**
Arginine	-.46	<**.0001**	-.56	<**.0001**	-.17	.160
Asparagine	-.03	.780	-.30	**.012**	-.31	**.010**
Citrulline	-.15	.204	-.12	.316	-.30	**.013**
Cysteine	-.27	**.016**	-.24	**.043**	-.19	.131
Glycine	-.53	<**.0001**	-.61	<**.0001**	-.43	**.0003**
Histidine	-.41	**.0002**	-.46	<**.0001**	-.23	.061
Isoleucine	-.09	.452	-.19	.122	-.38	**.002**
Leucine	-.09	.444	-.11	.356	-.26	**.030**
Lysine	-.51	<**.0001**	-.66	<**.0001**	-.38	**.002**
Valine	-.31	**.006**	-.21	**.079**	-.21	.082
Tryptophan	-.34	**.002**	-.30	**.013**	.01	.939
Threonine	-.56	<**.0001**	-.61	<**.0001**	-.40	**.0007**
Taurine	-.42	**.0001**	-.32	**.007**	-.07	.581
Tyrosine	.05	.646	-.02	.897	.06	.606
Proline	-.43	<**.0001**	-.42	**.0003**	-.15	.235
Phenylalanine	.06	.588	.22	.060	.46	<**.0001**
Ornitine	-.08	.470	-.05	.668	-.19	.115
Methionine	-.33	**.004**	-.29	**.013**	-.17	.173
Total	-.52	<**.0001**	-.62	<**.0001**	-.44	**.0002**

Significant *P* values are in bold type.

To date, few studies have investigated amino acid levels during HSCT, and those studies are limited by small patient samples, of 10 and 11 patients, respectively [22,23]. To our knowledge, associations between amino acids and systemic inflammation and related early complications have not been investigated previously. Although these studies investigated PAA levels at different time points (i.e. on day 0 and day +14, respectively), we can confirm previous findings of significant decreases in asparagine, histidine, and glutamine levels, as well as increases in leucine, phenylalanine, valine, and methionine early after transplantation. Our findings of elevated arginine, taurine, cysteine, and tyrosine levels are in contrast to these previous studies, and we were not able to confirm the previously reported changes in plasma levels of serine, alanine, ornithine, isoleucine, and threonine.

Myeloablative conditioning regimens expose patients to high-dose chemotherapy with or without TBI, resulting in

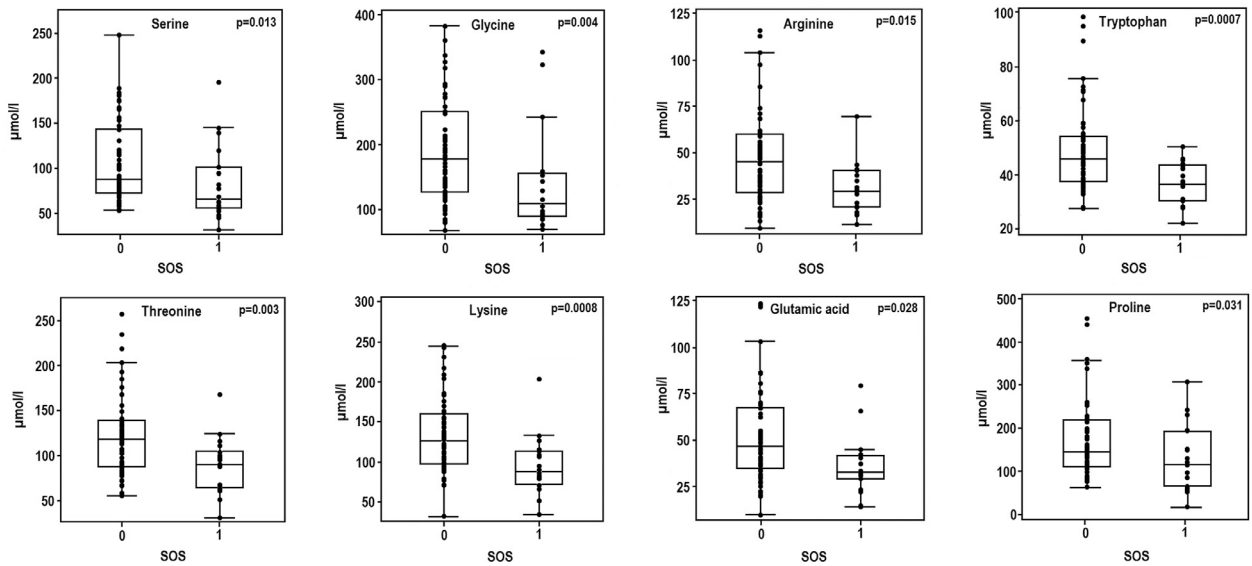

Figure 3. Amino acid levels according to a diagnosis of SOS. Associations between plasma levels of serine, glycine, arginine, tryptophan, threonine, lysine, glutamic acid, and proline on day +7 and occurrence of SOS.

severe systemic toxicity [2,24]. Antigens released from necrotic tissue and endotoxins translocated through disrupted epithelial barriers are responsible for the systemic inflammatory response seen in the early post-transplantation period with the release of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 [25–27]. This response stimulates the synthesis of acute phase reactants and endogenous antioxidants, which act as mediators of the immune system with various physiological effects, including inhibition of pathogens, negative feedback on the proinflammatory response, and regulation of the coagulation system. This serves the purpose of eliminating pathological actors while preventing a harmful systemic inflammatory response [28,29]. Dissemination of the inflammation and a dysregulated host response may increase morbidity and mortality in a wide range of critical illnesses [30].

Diseases with inflammatory reactions result in changes in the endogenous metabolism, with an increased metabolic demand for amino acids due to proliferation of immune cells, cytokine and antibody production, as well as regenerative mechanisms and synthesis of acute phase reactants and antioxidants [5,7,31]. When this demand is not met by an increase in supply, the body may gain amino acids from its "reservoir" through muscle catabolism [32–34]. In the short term, this might be considered an adaptive response, but the progressive loss of muscle may become problematic, and a high extent of reduced muscle mass in critical illness is related to poor survival and slow recovery. Because the composition of amino acids in muscle tissue differs somewhat from the profile needed in the production of proinflammatory and anti-inflammatory components, the amino acid turnover is greatly increased [7].

In the present study, we found an overall increase in urea level and the urea/creatinine ratio, indicating increased amino acid turnover in our patient population. Furthermore, we found increases in plasma leucine and valine levels, supporting the theory of significant muscle protein breakdown. Supporting these findings, a previous study that investigated protein turnover in HSCT found significant increases in leucine levels and decreases in nitrogen balance, in line with our findings of increased amino acid turnover [35].

Generally, metabolic stress in a critically ill patient results in an overall catabolic state with peripheral insulin resistance and decreased synthesis of anabolic hormones, such as insulin, insulin-like growth factor 1, and testosterone [36–38]. This may leave the patient in a state of anabolic resistance, in which muscle protein synthesis may be impaired despite a sufficient nutrient intake, contributing to the decreased muscle mass [5]. This has been demonstrated in patients with severe burns or sepsis, in whom supplementation of amino acids did not result in increased muscle protein synthesis or improved whole-body net protein balance [39–41].

In the setting of HSCT, the daily recommended intake of energy resources and proteins is rarely met. Patients suffer from varying degrees of mucositis and its clinical manifestations of abdominal pain, anorexia, nausea and vomiting, resulting in limited food intake. In many cases, enteral nutrition or PN supplementation is required [42]. These patients are further challenged by a damaged intestinal barrier, rendering them even more susceptible to stress metabolism and progressive loss of muscle tissue [43].

In this study, we found associations not only between high levels of systemic inflammation and low total PAA levels, but also between systemic inflammation and low levels of a wide range of individual amino acids, including serine, glutamine, glycine, lysine, arginine, threonine, glutamic acid, alanine, cysteine, histidine, tryptophan, taurine, proline, and methionine. Interestingly, many of these amino acids are involved in the synthesis of anti-inflammatory mediators. Endogenous antioxidants and acute phase reactants (e.g. glutathione, glutathione peroxidase, orosomucoid) contain large amounts of the sulfur-containing amino acids, including methionine, cysteine, and taurine, which all are inversely associated with CRP and IL-6 levels [7]. In addition, glycine, lysine, glutamic acid, glutamine, and serine also account for important components in antioxidant synthesis and likewise are negatively correlated with CRP and IL-6 levels [7].

Unfortunately, our study design cannot provide insight into the mechanisms behind these associations. It can be speculated that this is due either to a protective effect against systemic inflammation or significant consumption of these amino acids in patients with a high degree of systemic inflammation. Previous studies in rat models of systemic inflammation have suggested that low intake of the sulfur-containing amino acids may compromise antioxidant defenses and exert a proinflammatory influence, because incorporation into other proteins is favored over synthesis of glutathione in conditions with insufficient intake [12]. Furthermore, in studies investigating endogenous antioxidant levels in patients undergoing HSCT [22,44] and in an experimental HSCT animal model [45], decreased antioxidant concentrations have been found during the early post-transplantation phase, supporting our findings and the idea that these patients may lack sufficient anti-inflammatory response mechanisms to counteract chemotherapy- and radiation-induced tissue damage.

In this study, we also found associations between low PAA levels and the development of early complications SOS and aGVHD. The pathophysiology of these complications is closely related to systemic inflammation, suggesting that reduced PAA levels and the related increase in systemic inflammation may translate into these acute toxicities of treatment [3,24,46]. Glutamine and arginine have been suggested as protective agents and mediators of epithelial repair, and high levels of these amino acids theoretically could protect against SOS and aGVHD, because these complications are related to disruption of epithelial barriers [47].

Owing to the observational design of this study, we acknowledge several limitations to the study. Most importantly, we cannot definitively identify the mechanisms behind our findings, and we cannot exclude the possibility that the observed association between PAA levels and inflammatory parameters is due merely to increased consumption of these amino acids in patients with a higher degree of systemic inflammation. Furthermore, we do not have complete information about the patients' nutritional status, which could be a potential confounder by influencing PAA levels. No association was found between cumulative days on PN and PAA levels; however, the majority of the patients in our cohort received PN throughout the first 3 weeks post-HSCT. Theoretically, we speculate that patients with the most severe grade of mucositis (and thus the greatest inflammatory response) will be those most commonly in need of PN, and therefore, it is likely that this would lead to underestimation of the observed association between low PAA levels and high inflammatory parameters. We found significant associations between days on PN and inflammatory parameters in children, supporting this idea. Because only one-third of our patients received glucocorticoids, and because treatment was generally provided after day +7, we do not suspect that glucocorticoid treatment had any significant influence on our results.

We acknowledge the potential challenges of including both adult and pediatric patients in our study, and have adjusted

accordingly for variability in baseline characteristics between children and adults by using preconditioning levels as a reference to make, for example, the levels of urea and creatinine comparable. In the multivariate analyses, we included age as a parameter to compensate for potential confounding between adult and pediatric patients. The adult patients in our cohort were relatively young and did not suffer from any significant comorbidities besides their hematologic diagnosis.

Future studies are needed to confirm our results and investigate the underlying mechanisms behind our findings. More frequent measurements of PAA levels would be of great value, and measurements of nitrogen balance along with quantification of muscle breakdown and amino acid kinetics in these patients would contribute to understanding the catabolic changes during the early post-transplantation period. In addition, it would be ideal to standardize the content of amino acids in the diet to eliminate differences in intake. Furthermore, it would be of great interest to quantify levels of glutathione during transplantation to evaluate changes in antioxidant levels and their associations with proinflammatory markers. Investigation of PAA levels in other transplantation regimens, such as reduced-intensity conditioning HSCT, haploidentical HSCT, and autologous HSCT, would be pertinent because these are becoming more frequent.

In conclusion, our findings demonstrate significant changes in a number of human amino acids during HSCT, along with inverse relationships between PAA levels and systemic inflammation and treatment-related complications. Together with previous studies, these results suggest a complicated range of metabolic changes during periods of critical illness. We hypothesize that increased metabolic demands, anabolic resistance due to systemic inflammation, and accelerated catabolism, along with an insufficient supply of amino acids involved in anti-inflammatory mechanisms, may contribute to the morbidity and mortality associated with treatment. Further studies are needed to determine whether anabolic agents along with supplementation of amino acids and/or other nutrient resources could potentially improve outcomes in patients undergoing HSCT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.bbmt.2019.03.018.

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