Glutamine-enriched parenteral nutrition after autologous peripheral blood stem cell transplantation: effects on immune reconstitution and mucositis

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Background and Objectives. Glutamine (gln), a non-essential amino acid, has recently received increasing attention because it becomes essential during stress and catabolic states: glutamine seems to modulate immune function and to promote faster intestinal healing after chemotherapy. We designed two consecutive randomized clinical trials to evaluate the role of glutamine-enriched parenteral nutrition (GEPN) in patients with hematologic malignancies submitted to high dose chemotherapy and autologous peripheral blood stem cell transplantation (aPBSCT) or immunoselected CD34$^+$ aPBSCT.

Design and Methods. In study1, the Gln group (12 patients) received total parenteral nutrition (TPN) enriched with glutamine 20 g from day +1 after aPBSCT, while the placebo group (15 patients) received TPN lacking in glutamine (placebo). In study2, the Gln group (10 patients) received TPN enriched with glutamine 13.46 g from day +1, while the placebo group (11 patients) received a placebo.

Results. In the first study, a lymphocyte count >0.5×10^9/L was achieved on day 16.5 in the Gln group and on day 29 in the placebo group (p=0.005); in the second study, the lymphocyte count >0.5×10^9/L was achieved on day 18 in the Gln group and on day 29 in the placebo group (p=0.009). Lymphocyte subset analysis showed an increase of CD3$^+$ and CD4$^+$ and normalization of the CD16$^+$CD56$^+$ subset. Furthermore patients receiving GEPN showed a decrease in the mucositis severity peak calculated by the DMS (daily mucositis score: sum of the daily score of signs and symptoms) (p=0.047).

Interpretation and Conclusions. GEPN is safe and effective and improves lymphocyte recovery after aPBSCT; further studies are needed to assess the clinical benefits of such an approach in order to justify its economic impact.

Key words: glutamine, PBSCT, immune system, mucositis.

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Glutamine, a non-essential amino acid, has recently received increasing attention because it becomes essential during stress and catabolic conditions, including stem cell transplantation. This amino acid is the most abundant amino acid in plasma and skeletal muscle: it represents 25% of the free amino acid's pool in the extracellular fluids and 60% in the skeletal muscle.[1] Because of its important role in the regulation of acid-base homeostasis,[3] it is also used for renal ammoniogenesis;[2] its amide nitrogen is utilized in support of purine and pyrimidine synthesis.[4,5] Furthermore, it is a regulator of protein turnover.[6-8] There is biochemical and physiologic evidence that glutamine supports the function of the intestinal mucosal system. It has a metabolic role, including energy generation and amino acid synthesis, and a regulatory role, through the activation of genes involved in cell cycle progression in the mucosal cell.[9] Glutamine is also utilized at high rates by cells of the immune system such as lymphocytes and macrophages.[10] During catabolic conditions (surgery, trauma, chemotherapy, radiotherapy, stem cell transplantation) there is a progressive depletion of glutamine as a consequence of both a considerably increased requirement by the gastrointestinal tract, kidney, and lymphocytes and a reduction of the endogenous production of glutamine.[11] We designed two randomized clinical trials in order to evaluate the role of two different formulas of glutamine-enriched parenteral nutrition in patients undergoing high dose chemotherapy and autologous peripheral blood stem cell transplantation (aPBSCT) or immunoselected CD34$^+$ aPBSCT for hematologic malignancies.

Design and Methods

Patients

Patients submitted to selected or unselected aPBSCT at our Hematology Department from October 1998 to August 1999 were enrolled in the first randomized study. Twenty-seven consecutive patients were randomized into two groups: the study1-Gln group was formed of 12 patients who received Glamin™ (Fresenius Kabi) 1000 mL/die (a parenteral amino acid solution also containing free glutamine 20 g) from day +1 after aPBSCT, while the study1-placebo group, formed of 15 patients, received a placebo. The patients' characteristics are shown in Table 1. In September 1999, the switch to a commercial premixed nutrition bag, changing the characteristics of the TPN, and the availability of a new

Table 1. Characteristics of patients enrolled in study 1.

	Glutamine group	*Placebo group*
N. of patients	12	15
Sex (F/M)	5/7	5/10
Age (years)	37.5 (17-66)	47 (18-56)
Disease (n. of patients)	NHL 5	NHL 5
	AML 4	AML 1
	MM 1	MM 6
	Osteosarcoma 1	CLL 1
	HD 1	HD 2
Status at transplant	CR 5	CR 6
	PR 6	PR 6
	PD 1	PD 3
Conditioning regimen	BuCy2 4	BuCy2 2
	TT-HDMel 1	TT-HDMel 1
	BEAM 1	BEAM 2
	BuMel 3	BuMel 6
	Carboplatinum-VP16 1	NovMel 1
	BAVC 1	HDMel 2
	TT-Mel-Bu 1	TT-Mel-Bu 1
Graft type	$CD34^+$ APBSCT 4	$CD34^+$ APBSCT 6
	APBSCT 8	APBSCT 9
G-CSF	4	8
N. of $CD34^+$ cells $\times 10^6$/kg infused	5.935 (2.55-21.6)	5.1 (2.9-57)

*NHL: non-Hodgkin's lymphoma; AML: acute myeloid leukemia; MM: multiple myeloma; HD: Hodgkin's disease; CLL: chronic lymphocytic leukemia; PR: partial remission; CR: complete remission; PD: progressive disease; PBSCT: peripheral blood stem cell transplantation; CD 34+: immunoselected peripheral blood stem cell transplantation; *:for conditioning regimen abbreviations, see Table 3.*

Table 2. Characteristics of patients enrolled in study 2.

	Glutamine group	*Placebo group*
N. of patients	10	11
Sex (F/M)	5/5	3/8
Age (years)	31.5 (22-61)	49 (27-61)
Disease (n. of patients)	NHL 3	NHL 4
	MM 4	MM 4
	Osteosarcoma 1	AML 2
	HD 2	HD 1
Status at transplant	CR 1	CR 3
	PR 7	PR 5
	PD 2	PD 3
Conditioning regimen	BuCy2 1	BuCy2 2
	HDMel 3	HDMel 1
	BEAM 3	BEAM 1
	BuMel 1	BuMel 7
	Carboplatinum-VP16 1	
	TT-Mel-Bu 1	
Graft type	$CD34^+$ APBSCT 6	$CD34^+$ APBSCT 4
	APBSCT 4	APBSCT 7
G-CSF	7	5
N. of $CD34^+$ cells $\times 10^6$/kg infused	3.18 (2.01-10.21)	4.36 (1.06-21.2)

For abbreviations, see Tables 1 and 3.

glutamine solution, promoted us to design a second study which included 21 consecutive patients, randomized into two groups: the study2-Gln group was formed of 10 patients who received Dipeptiven™(Fresenius Kabi) 100 mL/die (a parenteral solution containing glutamine 13.46 g) from day +1 after aPBSCT, while the study2-placebo group comprised 11 patients who received a placebo. The patients' characteristics are shown in Table 2. Informed consent was obtained from all patients or guardians. The protocol was approved by the Institutional Review Board at Universita' Cattolica Sacro Cuore. Both study groups were comparable for age, sex and diagnosis.

Transplant procedure

Conditioning regimens and CD 34^+/kg cell dose infused were comparable between groups and full details on conditioning regimens are shown in Table 3. In the first study the conditioning regimen was busulfan-melphalan in 9 patients, busulfan-cyclophosphamide in 6 patients, BEAM in 3 patients, high-dose melphalan in 2 patients, thiotepa high-dose melphalan in 2 patients and in 2 patients this regimen was combined with busulfan. Carboplatinum and etoposide was used in 1 patient, mitoxantrone and melphalan in 1 patient, and, finally,

Table 3. Conditioning regimens.

BuMel	Busulfan 4 mg/kg on day –5 through -2; Melphalan 90 mg/m^{2}on day –1
BuCy2	Busulfan 4 mg/kg on day –7 through –4; Cyclophosphamide 60 mg/kg on day –3 and –2
BEAM	BCNU 300 mg/m^2 on day –6 ; Aracytin 200 mg/m^2 on day –5 through –2; Etoposide 200 mg/m^2 on day –5 through –2; Melphalan 140 mg/m^2 on day –1
HDMel	Melphalan 100 mg/m^2 on day –3 and –2
TTHDMel	Thiotepa 10 mg/kg on day –6; Melphalan 140 mg/m^2 on day –2
TT-MelBu	Thiotepa 10 mg/kg on day –6; Melphalan 140 mg/m^2 on day –2; Busulfan 4 mg/kg on day –8 through –6
Carbo-VP16	Etoposide 200 mg/m^2 on day -7through –4; Carboplatinum 375 mg/m2 on day –7 through –4
BAVC	BCNU 800 mg/m^2 on day –6 ; Amsacrine 150 mg/m^2 on day –5 through –3; Etoposide 150 mg/m^2 on day-5 through –3; Cytosine Arabinoside 300 mg/m^2 on day –5 through –3
NovMel	Mitoxantrone 60 mg/m^2 on day –5; Melphalan 180 mg/m^2 on day –2

BCNU, amsacrine, etoposide and ara-C in 1 patient.

On day 0, 17 patients received aPBSCT, 10 patients received immunoselected CD34+ aPBSCT (4 in the study1-Gln group and 6 in the study1-placebo group). The CD34+ cell selection was performed with a CeprateSC System (Cellpro, Bothell, WA, USA) for 3 patients and with a CliniMACS System (Miltenyi Biotech GmbH, Bergish-Gladbach, Germany) for the other 7 patients. From day +1, 12 patients (4 in the study1-Gln group and 8 in the study1-placebo group) received granulocyte colony-stimulating factor (G-CSF) (lenograstim, rHuG-CSF, Chugai-Rhône-Poulenc Rorer) subcutaneously (sc) at a standard dose of 263 μg/die, until a polymorphonuclear cell (PMN) count > 0.5×10^9/L was reached and maintained for 3 consecutive days.

In the second study the conditioning regimen was busulfan-cyclophosphamide in 3 patients, thiotepa-busulfan-melphalan in 1 patient, busulfan-melphalan in 8 patients, carboplatinum-etoposide in 1 patient, high-dose melphalan in 4 patients, and BEAM in 4 patients.

On day 0, 11 patients were submitted to aPBSCT, 10 patients to immunoselected CD 34+ aPBSCT (6 in the study2-Gln group and 4 in the study2-placebo group); the immunoselection was carried out using the CliniMACS device in all patients. From day +1, 12 patients (7 in the study2-Gln group and 5 in the study2-placebo group) received G-CSF sc at a standard dose of 263 μg/die, until a PMN count ≥ 0.5×10^9/L was reached and maintained for 3 consecutive days.

Supportive care

Prophylactic treatment for *Pneumocystis carinii* pneumonia included one trimethoprim-sulfamethoxazole double-strength tablet every 12 hr until day –1 and later when a stable engraftment was achieved; oral ciprofloxacin (500 mg every 12 hr) from day –7 until stable granulocyte recovery; acyclovir (500 mg/m^2) from day –7 to day +100; oral amphotericin B suspension. Broad-spectrum antibiotics, generally including amikacin (7.5 mg/kg every 12 hr), ceftazidime (2 g every 8 hr), vancomycin (15 mg/kg every 12 hr) or teicoplanin (12 mg/kg every 24 hr) were used for sustained temperature elevations of ≥ 38°C. If the fever persisted (> 72 hours from the start of antibiotics), amphotericin B (0.5-1.0 mg/kg every 24 hr) was administered. When the hemoglobin concentration was < 8 g/dL, packed RBC were infused and when the platelet count was < 15×10^9/L, single donor units of platelets were transfused. All blood products were irradiated with 15 Gy.

Amino acid suspension

Because of free glutamine's instability during heat sterilization and prolonged storage and limited solubility (35 g/L at 20°C), free glutamine cannot be used in the routine clinical setting. Synthetic glutamine dipeptides, however, are stable under heat sterilization and highly soluble; these properties qualify the dipeptides as suitable constituents of nutritional preparations.[12] The first dipeptide available was *glycil-glutamine*,[13] characterized by a solubility of 154 g/L (H_2O at 20°C); then , in 1999, a new dipeptide, *alanyl-glutamine* characterized by a solubility of 568 g/L (H_2O at 20°C), became available.[12]

Thus, in the first study we used a complete, well-balanced amino acid solution (*Glamin™*), containing 18 essential and non-essential amino acids (details are reported in Table 4). A final volume of 1000 mL (containing 30.27 g of stable glycil-L-glutamine: 10.27g glycine and 20 g glutamine) of this solution were administered daily. In the second study a 20% solution of the glutamine containing dipeptide alanyl-glutamine (*Dipeptiven™*) was utilized at the daily dose of 100 mL/die (containing 20 g of alanyl-glutamine: 8.20 g L-alanine and 13.46 g *L-glutamine*).

Table 4. Components of TPN used in study 1.

	Glutamine	*Placebo*
Leucine	7.9 g	7.7 g
Isoleucine	5.6 g	5.9 g
Valine	7.3 g	5.6 g
Methionine	5.6 g	4.5 g
Lysine	9 g	8.7 g
Threonine	5.6 g	3.4 g
Phenylalanine	5.85 g	4.8 g
Tryptophan	1.9 g	1.3 g
Histidine	6.8 g	2.4 g
Arginine	11.3 g	8.1 g
Proline	6.8 g	9.5 g
Alanine	16 g	6 g
Glycine	11.21 g	11.9 g
Serine	4.5 g	5 g
Tyrosine	2.28 g	–
Glutamic acid	5.6 g	–
Aspartic acid	3.4 g	–
Cysteine	–	0.18 g
Glutamine	20 g	–
Phospholipids	56 g	56 g
33% glucose solution	1000 mL	1000 mL
Hydrosoluble vitamins	165.6 mg	165.6 mg
Liposoluble vitamins	1.44 mg	1.44 mg

Parenteral formulas

In the first study, the Gln group received daily parenteral nutrition composed of *Intralipid™ 10%* (Fresenius Kabi, Uppsala, Sweden) at a dose of 500 mL, *Glamin™* 1000 mL, 33% *glucose solution* 1000 mL, and *hydrosoluble* and *liposoluble vitamins*. The

Table 5. Components of TPN used in study 2.

	Glutamine	*Placebo*
Leucine	4 g	4 g
Isoleucine	2.8 g	2.8 g
Valine	3.7 g	3.7 g
Methionine	2.8 g	2.8 g
Lysine	4.5 g	4.5 g
Threonine	2.8 g	2.8 g
Phenylalanine	4 g	4 g
Tryptophan	0.95 g	0.95 g
Histidine	3.4 g	3.4 g
Arginine	5.6 g	5.6 g
Proline	3.4 g	3.4 g
Alanine	16.2 g	8 g
Glycine	4 g	4 g
Serine	2.2 g	2.2 g
Tyrosine	0.12 g	0.12 g
Glutamic acid	2.8 g	2.8 g
Aspartic acid	1.7 g	1.7 g
Cysteine	0.28 g	0.28 g
Glutamine	13.46 g	–
Phospholipids	106 g	106 g
Glucose	150 g	150 g
Hydrosoluble vitamins	165.6 mg	165.6 mg
Liposoluble vitamins	1.44 mg	1.44 mg

placebo group received daily parenteral nutrition composed of *Intralipid ™* 10% 500 mL, *Freamine™* 8.5% (Fresenius Kabi, Uppsala, Sweden) 1000 mL, 33% *glucose solution* 1000 mL, and *hydrosoluble* and *liposoluble* vitamins (the characteristics of the TPN are shown in Table 4). From 1999, we used a premixed balanced commercial parenteral nutrition bag adding only the glutamine solution. Thus, patients in the study2-Gln group received a daily parenteral nutrition composed of *Kabimix™* 1830 (Fresenius Kabi, Uppsala, Sweden), *hydrosoluble* and *liposoluble vitamins and Dipeptiven™* (Fresenius Kabi, Uppsala, Sweden) 100 mL (according to the manufacturer's instructions), while the study2-placebo group received daily parenteral nutrition composed of *Kabimix™*1830 and *hydrosoluble* and *liposoluble vitamins* (the characteristics of the TPN are shown in Table 5).

Endpoints

During both studies we evaluated clinical outcomes (time to neutrophil recovery, time to lymphocyte recovery, time to platelet recovery, duration of hospitalization, days of non-prophylactic antibiotics, number of days with body temperature > 38°C, incidence of sepsis, virus infection and or disease, reticulocyte recovery (> 1%), and number of RBC and platelet units infused. Furthermore, in order to evaluate the effect of glutamine on the immune system, we analyzed the reconstitution of the lymphocyte subsets in detail (at baseline, and days 15, 30, 60, 90, and 120 after transplant) by flow cytometry.[14] The other major endpoint was the assessment of the severity of mucositis during the transplant protocol. The costs of glutamine-enriched parenteral nutrition were also evaluated. Isonitrogenic TPN was not provided since nitrogen balance was not included as an endpoint in this study based on previously published isonitrogenic studies.[15]

Oral mucositis: daily mucositis score

In order to score oral mucositis, we adopted the daily mucositis score (DMS).[16] The DMS is a daily score obtained from summing single scores (from 0 to 3) assigned to five major signs and symptoms of mucositis (lesions, erythema, edema, difficulty and pain during swallowing). The DMS was recorded daily from day 0 to discharge by one of us (SDM) who was blind to the treatment with glutamine. We considered the daily course, the peak and the duration of mucositis.

Statistical analysis

The Mann-Whitney *U*-test was used to analyze continuous factors. We adopted the Kruskal-Wallis test for multiple comparisons and Dunn's test for *post hoc* evaluation. The χ^2 test was chosen for the analysis of the categorical factors. Statistical significance was defined as $p<0.05$.

Results

First study: comparisons between the glutamine and placebo groups

Data are summarized in Tables 6A and 6B. Patients who received glutamine supplementation achieved a lymphocyte count >500/μL faster than patients who received placebo (day 16.5 vs day 29: $p=0.005$). A significant decrease of mucositis peak was observed in patients in the glutamine group (3.5 vs 5: $p=0.044$). Lymphocyte subset analysis on day +30 after transplant showed that patients who were given GEPN had a greater increase in the $CD4^+$ subset (300 vs 115 cells/μL: $p=0.014$) and normalization of the $CD16^+CD56^+$ subset (156 vs 319 cells/μL: $p=0.02$). On day +60 the CD4/CD8 ratio was significantly higher in patients receiving GEPN (0.35 vs 0.25: $p=0.025$). There were no other significant differences between the two groups concerning other analyzed outcomes. Costs analysis revealed a six-fold increase in costs of daily parenteral nutrition enriched with glutamine (Table 9A).

Second study: comparisons between the glutamine and placebo groups

Data are summarized in Tables 7A and 7B. Patients receiving GEPN achieved 500 lymphocytes/μL faster than those receiving placebo (day 18 vs day 29: $p=0.009$). Lymphocyte subset analy-

Table 6A. Results of study 1.

End Point	Glutamine group	Placebo group	Mann-Whitney U-test
N. of CD 34+ cells×10^6/kg infused	5.935 (2.55-21.6)	5.1 (2.9-57)	$p = 0.98$
Days to PMN >0.5×10^9/L	12 (10-21)	12 (9-21)	$p = 0.69$
Days to Plts >20×10^9/L	12 (7-30)	12 (7-21)	$p = 0.9$
Days to lymphocytes >0.5×10^9/L	16.5 (10-27)	29 (12-50)	$p = 0.005$
Days of antibiotic therapy	11 (5-15)	9.5 (4-22)	$p = 0.66$
Days of body temp. >38°C	2 (0-17)	2 (0-10)	$p = 0.6$
Days of hospitalization	27.5 (23-70)	25 (16-37)	$p = 0.22$
DMS peak	3.5 (2-9)	5 (3-12)	$p = 0.044$
Mucositis duration	10.5 (6-15)	13 (11-15)	$p = 0.1$

PMN: polymorphonuclear granulocytes; Plts: platelets; DMS: daily mucositis score.

Table 6B. Results of lymphocyte subset analyses.

Subset*	day	Glutamine group	Placebo group	Mann-Whitney U-test	Normal range
CD 4+/μL	+30	300 (86-882)	115 (47-360)	p=0.014	670-950
CD 16+CD 56+/μL	+30	156 (10-980)	319 (81-681)	p=0.02	70-190
Ratio	+60	0.35 (0.2-1.6)	0.25 (0.1-0.5)	p=0.025	1.1-1.8

**only statistically significant differences are reported.*

sis on day +15 and +30 after transplant showed that patients who were given GEPN had a greater increase of the CD8+ subset than those receiving placebo (day +15: 258.5 vs 50.5 cells/μL: p=0.041; day +30: 460 vs 266 cells/μL: p=0.038) and on day +60 had normalization of the CD16+CD56+ subset (205 vs 297.5 cells/μL: p=0.029). Patients receiving GEPN had a faster PMN recovery (>500 and >1000 PMN/μL). This was probably due to a casual imbalance in the number of patients receiving immunoselected CD34+ and, consequently G-CSF support, between the glutamine and placebo groups. There were no other significant differences between the two groups in other analyzed outcomes. Cost analysis revealed a two fold increase in the costs of daily parenteral nutrition enriched with glutamine (Table 9B).

In order to check the consistency of our results, we pooled the data from patients receiving GEPN in both studies and compared them to the pooled data from the patients receiving placebo. These pooled data are summarized in Tables 8A and 8B. Patients receiving glutamine supplementation achieved a lymphocyte count >500/μL faster than patients receiving placebo (day 17 vs day 29:

Table 7A. Results of study 2.

End Point	Glutamine group	Placebo group	Mann-Whitney U-test
N. of CD 34+ cells ×10^6/kg infused	3.18 (2.01-10.21)	4.36 (1.06-21.2)	$p = 0.59$
Days to PMN >0.5×10^9/L	10 (8-19)	13 (11-22)	$p = 0.001$
Days to Plts >20×10^9/L	13 (8-33)	13 (10-22)	$p = 0.57$
Days to lymphocytes >0.5×10^9/L	18 (12-22)	29 (12-60)	$p = 0.009$
Days of antibiotic therapy	8.5 (0-6)	10 (5-15)	$p = 0.64$
Days of body temp. >38°C	0.5 (0-6)	2 (0-5)	$p = 0.38$
Days of hospitalization	29 (23-40)	27 (23-39)	$p = 0.45$
DMS peak	7 (4-8)	8 (5-11)	$p = 0.12$
Mucositis duration	13 (10-21)	13 (10-22)	$p = 0.91$

Table 7B. Results of lymphocyte subset analyses.

Subset*	day	Glutamine group	Placebo group	Mann-Whitney U test	Normal range
CD 8+/μL	+15	258.5 (48-1006)	50.5 (13-112)	p=0.041	505-695
CD 8+/μL	+30	460 (306-1314)	266 (156-469)	p=0.038	505-695
CD 16+CD 56+/μL	+60	205 (10-300)	297.5 (221-800)	p=0.029	70-190

**only statistically significant differences are reported.*

Table 8A. Overall comparison of glutamine vs placebo: (study1 + study2).

End Point	Glutamine group	Placebo group	Mann-Whitney U-test
N. of CD 34+ cells×10^6/kg infused	4.305 (2.01-21.6)	5.025 (1.06-57)	$p = 0.5$
Days to PMN >0.5×10^9/L	11 (8-21)	12.5 (9-22)	$p = 0.08$
Days to Plts >20×10^9/L	13 (7-33)	13 (7-22)	$p = 0.84$
Days to lymphocytes >0.5×10^9/L	17 (10-27)	29 (12-60)	$p < 0.0001$
Days of antibiotic therapy	9.5 (0-16)	10 (4-22)	$p = 0.77$
Days of body temp. >38°C	1.5 (0-17)	2 (0-10)	$p = 0.85$
Days of hospitalization	28 (23-70)	27 (23-39)	$p = 0.15$
DMS peak	6 (2-9)	7 (3-12)	$p = 0.047$
Mucositis duration	11 (6-21)	13 (10-22)	$p = 0.17$

Table 8B. Results of lymphocyte subset analyses.

Subset*	day	Glutamine group	Placebo group	Mann-Whitney U test	Normal range
CD 4+/μL	+15	106 (11-775)	59 (6-286)	$p = 0.016$	670-950
CD 4+/μL	+30	191 (78-882)	116 (47-360)	$p = 0.026$	670-950
CD 8+/μL	+60	467 (136-3727)	955 (207-2617)	$p = 0.04$	505-695
CD 3+/μL	+30	586 (169-2794)	352 (66-2161)	$p = 0.049$	1185-1540
CD 16+CD 56+/μL	+60	205 (10-394)	336 (71-874)	$p = 0.021$	70-190

**Only statistically significant differences are reported.*

Table 9A. Costs of daily total parenteral nutrition in study 1.

	Glutamine group	*Placebo group*
Intralipid™ 10% 500 mL	€ 5.01	€ 5.01
33% glucose solution 1000 mL	€ 0.56	€ 0.56
Freamine™ 8,5% 1000 mL	–	€ 3.05
Glamin™ 1000 mL	€ 62.63	–
Vitamins	€ 3.37	€ 3.37
Total	€ 71.57	€ 11.99

Table 9B. Costs of daily total parenteral nutrition in study 2.

	Glutamine group	*Placebo group*
Kabimix™ 1830 2580 mL	€ 38.03	€ 38.03
Dipeptiven™ 100 mL	€ 37.56	–
Vitamins	€ 3.37	€ 3.37
Total	€ 78.96	€ 41.4

$p<0.0001$) (Figure 1). When the DMS was considered, patients receiving GEPN had a significantly lower mucositis score on days +10 (2 vs 5: $p=0.046$), +11 (2 vs 5: $p=0.016$), +12 (1.5 vs 3.5: $p=0.041$) (Figure 2). The peak of mucositis was also significantly lower in patients receiving GEPN (6 vs 7: $p=0.047$). Lymphocyte subsets analysis on day +15 and +30 after transplant showed that, compared to patients receiving placebo, patients being given GEPN had a higher number of $CD4^+$ cells (day +15: 106 vs 59 cells/μL: $p=0.016$; day +30: 191 vs 116 cells/μL: $p=0.026$). On day +60, the GEPN recipients had fewer $CD8^+$ cells (467 vs 955 cells/μL: $p=0.04$), and normalization of the $CD16^+$ $CD56^+$ subset (205 vs 336 cells/μL: $p=0.021$). On day +30 the GEPN recipients had higher numbers of $CD3^+$ cells (586 vs 352 cells/μL: $p=0.049$) (Figures 3A, 3B, 3C, 3D). There were no other significant differences between the two groups concerning other analyzed outcomes.

Discussion

During the last few years many clinical trials have pointed out the importance of glutamine in nutritional support. Evidence for the role of glutamine in parenteral nutrition was first provided by Ziegler *et al.* in patients affected by catabolic illnesses[17] or undergoing aggressive treatment. In fact glutamine, 0.57 g/kg body weight per day, reduced fluid retention and minimized expansion of the extracellular

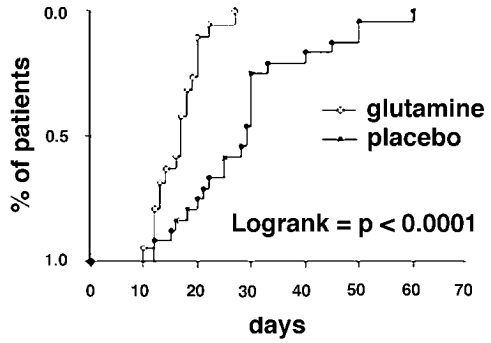

Figure 1. Time to lymphocyte recovery > 500/μL.

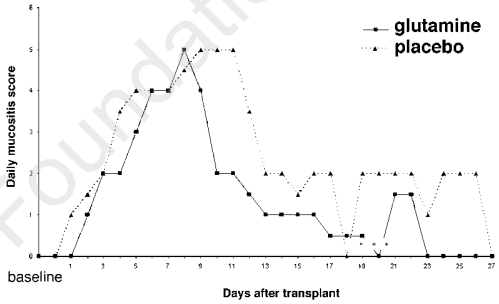

Figure 2. Daily mucositis score: based on Donnelly JP *et al.*[15] **Statistically significant difference.*

fluid compartment.[18] Furthermore, in patients submitted to allogeneic bone marrow transplantation, those receiving parenteral glutamine rather than standard parenteral nutrition, had improved nitrogen balance, a reduced incidence of clinical infection, lower rates of microbial colonization and a shorter stay in hospital.[15] Ziegler also demonstrated a higher percentage of blood lymphocytes, and higher $CD3^+$, $CD4^+$ and $CD8^+$ T-lymphocytes counts in patients submitted to allogeneic bone marrow transplantation (BMT) who received GEPN than in the controls.[19] On the other hand, other authors did not find differences between control groups and groups receiving GEPN after autologous or allogeneic BMT when infection, sepsis, mucositis, diarrhea and GVHD incidence were analyzed; they only showed reductions in time spent in hospital and fluid retention.[20]

Another variable was introduced by the use of the enteral route of glutamine administration (30 g/die). The rationale for oral administration was that glutamine is a primary fuel for the enterocyte and gut-

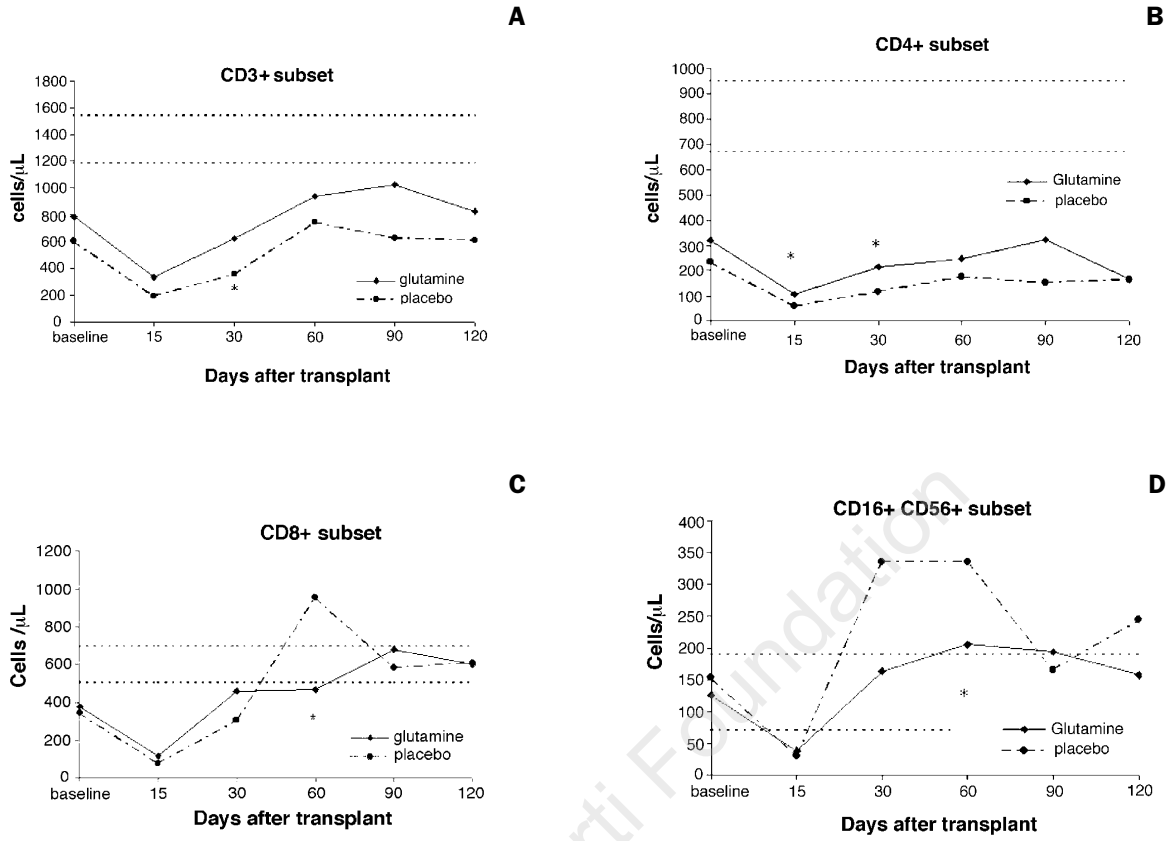

Figure 3A. Dotted lines represent reference ranges determined in a cohort of 20 normal control subjects, *statistically significant difference. 3B. Dotted lines represent reference ranges determined in a cohort of 20 normal control subjects,*statistically significant difference. 3C. Dotted lines represent reference ranges determined in a cohort of 20 normal control subjects,*statistically significant difference. 3D. Dotted lines represent reference ranges determined in a cohort of 20 normal control subjects, *statistically significant difference.

associated lymphoid tissue. When oral glutamine was administered to patients submitted to autologous or allogeneic transplantation, no statistically significant differences in the number of days of parenteral nutrition, number of days required until resumption of oral intake, duration of hospitalization, number of days and highest grade of mucositis, and quantity and number of days of diarrhea were found.[21] When the administration of glutamine was combined (oral or parenteral, according to patients' compliance) there were no significant differences in hospital stay, days of total parenteral nutrition, neutrophil recovery, sepsis, mucositis, or diarrhea.[22]

These conflicting results led us to design a randomized trial to evaluate the role of GEPN in improving immune function and in reducing mucositis severity in patients affected by hematologic malignancies submitted to aPBSCT. We chose the parenteral route of administration in order to bypass erratic bioavailability and variable compliance of oral administration in patients with feeding difficulty. The autologous setting of the PBSCT abrogated issues of graft-versus-host disease and related immunosuppression as confounding factors in lymphocyte recovery. We used *Glamin™* until the switch to commercial premixed nutrition bags, and then used *Dipeptiven™*, a new solution containing glutamine, in a second consecutive randomized trial. The difference in the amount of glutamine administered in the two studies (20 g vs 13.46 g) also allowed us to verify whether there was a dose-related effect.[17] Both studies showed that glutamine strongly improved lymphocyte reconstitution. Patients receiving GEPN (either 20g or 13.46g formula) achieved a total lymphocyte

count of 500 cell/μL significantly sooner than patients receiving placebo. These data were further substantiated by lymphocyte subset analyses; these changes included, in particular: a significantly earlier (day 15-30) increase of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes; furthermore, the NK subset, $CD16^+CD56^+$, was significantly lower on day +60 in patients receiving GEPN.

Our data further confirm those obtained *in vivo* by Ziegler[19] on lymphocyte recovery and $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes, in 20 patients submitted to allogeneic BMT, receiving 0.57g/kg/die of parenteral glutamine: the different behavior of NK and $CD8^+$ subsets on day +60 could reasonably be due to substantial differences in the settings (allogeneic bone marrow transplant vs autologous peripheral blood stem cell transplantation), in the glutamine dose and in the timing and number of samples analyzed (day ≅+30 vs day +120) by flow cytometry in the study reported by Ziegler.[19]

The results of the current study are consistent with data obtained from *in vitro* studies showing that glutamine is involved in lymphocyte proliferation, cell cycle progression, the glutathione pathway and, consequently, maintenance of an appropriate intracellular redox state[23] in lymphocytes.

Therefore, on the basis of the lymphocyte subset analyses, GEPN seems to promote specific immunity through rapid $CD4^+$ subset reconstitution; on the other hand, it limits the NK subset overshoot after aPBSCT. The increase of the NK subset in the early post-transplant phase has not been associated with a significant enhancement of activity against tumor or infection.[24]

The effect of glutamine on mucositis is still controversial. Previous studies found no benefit[15,20,25] while, more recently, Anderson *et al.* observed a decreased frequency and duration of mouth pain, assessed both by self-report and by opiate use, after oral glutamine in a randomized study in patients undergoing aPBSCT.[26,27] Our data revealed that GEPN significantly reduced the mucositis peak and tended to produce a more rapid resolution of the mucositis (Figure 2). This effect seems to be dose-related. In fact, patients who received the larger amount of glutamine (20 g/die) showed a significantly lower mucositis peak than patients who received placebo, whereas patients who received the smaller amount of glutamine (13.46 g/die) only showed a trend towards a decreased mucositis peak. The statistically significant difference was confirmed when patients receiving glutamine were compared to patients receiving placebo.

In conclusion, in our study parenteral glutamine supplementation resulted in a significant improvement of immune recovery after aPBSCT, demonstrated by both a rapid absolute lymphocyte recovery and the pattern of the lymphocyte subsets. A significant effect on mucositis was also documented and this effect was dose-related. Improvement of mucositis might lead to a better compliance of patients to high dose chemotherapy, preserving the integrity of the physiological barrier and reducing local and subsequent systemic spread of infections.[28,29] However the beneficial effects of glutamine were, in this study, counterbalanced by a significant increase in costs of parenteral nutrition. Although glutamine should be considered as an adjunctive metabolic support, further studies are needed to define its role better.

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Pre-publication Report & Outcomes of Peer Review

Contributions

SS, NP, MP and SDM contributed to the study's conception and design; LL, PC and FS were involved in daily clinical care of the patients; SC, AF and SDM collected data; SR performed the flow cytometry analyses; NP analyzed the data; SS, GL and GDO revised the manucript for the final approval.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Jorge Sierra, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Sierra and the Editors. Manuscript received July 8, 2002; accepted December 19, 2002.

In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Glutamine is the most abundant amino acid in plasma and skeletal muscle. Glutamine supports the function of the intestinal mucosal system and is used at high rates by cells of the immune system. In catabolic conditions there is a depletion of glutamine due to decreased synthesis and increased utilization. Several studies have suggested a benefit of supplementing total parenteral nutrition (TPN) with glutamine in patients with catabolic status.

What this study adds

In two consecutive randomized studies, the authors demonstrated improved immune reconstitution and a less intense mucositis in recipients of autologous peripheral blood stem cell transplants supported with glutamine-enriched TPN compared to TPN alone.

Caveats

This is a small series of patients who received relatively heterogeneous conditioning regimens for transplantation. Supplementation with glutamine increased the costs of TPN 2- to 6-folds.