

Original Article

Total parenteral nutrition enriched with glutamine and ω -3 fatty acid in adult patients undergoing autologous stem cell transplantation

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ABSTRACT: Background: Malnutrition is associated with an increase in infections. Artificial nutrition provides supportive care in hematological disease. Chemotherapy has harmful consequences on the functional integrity of the gastrointestinal tract, and patients develop oroesophageal mucositis and gastrointestinal toxicity, with decreased oral intake and nutrient absorption. Glutamine is a non-essential amino acid, but in an extreme metabolic condition, when its consumption exceeds its synthesis, such as during stem cell transplantation, it becomes "a conditionally essential amino acid". On the other hand, the fish oil-derived n-3 fatty acids play an important role in modulating inflammatory and immune response, by regulating prostaglandins, thromboxane A₂, and leukotriene synthesis. The aims of our study were to demonstrate a synergic effect between the antiinflammatory effects of eicosapentaenoic acid and the stimulatory effects of glutamine on enterocytes, and to evaluate the impact of antiinflammatory properties of n-3 fatty acids on principal clinical end points.

Patients and methods: We enrolled 45 patients with oncohematological diseases who underwent autostem cell transplantation; we divided our patients into 3 groups (comparable with respect to age, diagnosis, pretreatment, and conditioning regimens): 16 patients (group A) received standard glutamine-free total parenteral nutrition; 13 (group B), total parenteral nutrition containing glutamine 0.25 g/kg per day; and 16 (group C), total parenteral nutrition containing glutamine 0.25 g/kg per day plus Omegaven 1.5 mL/kg per day. We evaluated the 3 groups on the basis of length of hospital stay, days of total parenteral nutrition, duration of febrile neutropenia, mean duration of antibiotic therapy, use of antifungal drugs, polymorphonuclear neutrophil engraftment, grade of mucositis, diarrhea, and emesis.

Conclusions: We demonstrated that the use of glutamine- and eicosapentaenoic acid-enriched total parenteral nutrition (group C) is significantly associated with short duration of hospital stay (Dunnett T3 test, $\alpha = 0.05$), short duration of febrile neutropenia and antibiotic therapy (Bonferroni test), reduced days of total parenteral nutrition, and infrequent use of antifungal drugs, compared with groups A and B. (*Nutritional Therapy & Metabolism* 2009; 27: 39-45)

KEY WORDS: Immunonutrition, Autologous stem cell transplantation, Total parenteral nutrition, Mucositis

INTRODUCTION

Parenteral nutrition carries out an important role in supporting the cure of many pathologies and, in particular, hematological disorders, sustaining the patient for the management of complex therapeutic programs such as high-dose chemotherapy or hematopoietic stem cell

(HSC) transplantation. In patients undergoing an HSC transplant, an adequate nutritional state is deemed crucial regardless of the presence or not of a basic malnutrition state recorded at hospitalization. HSC transplantation represents a valid therapeutic option in the treatment of numerous neoplastic and nonneoplastic hematological disorders. There are 2 different types of trans-

plant: autologous or allogeneic. The first is performed with HSCs previously collected from the same patient and is indicated in the treatment of chronic pathologies such as myelomas and some types of lymphomas, in a phase of the disease in which the bone marrow is not compromised, or in acute leukemias in which a compatible familial donor is not available. An allogeneic transplant is performed using HSCs collected from a donor, familial or not related. The possible sources of HSCs are the bone marrow and the peripheral and placental blood.

A fundamental stage in an HSC transplant, either autologous or allogeneic, is the conditioning regimen that always precedes the transplant procedure. High doses of antineoplastic drugs, sometimes also accompanied by total body irradiation, result in complications and adverse effects, either direct, due to the administration of drug, or indirect, usually secondary to a decrease in immune defenses. Some of these complications are limited to single allogeneic transplants, such as the graft-versus-host disease (GVHD); others are common in both types of transplant.

Cases of bacteremia, clinically manifest fungemia, sepsis, or infections, most of which are opportunistic, are frequent. Furthermore, the incidence of mucositis of the gastrointestinal tract is elevated. Cases of hepatic veno-occlusive disease (VOD), on the other hand, are rare.

Parenteral nutrition has been transformed through the years from a simple support therapy to an "additional" and fundamental therapy for patients undergoing an HSC transplant, due to its role in reducing both the incidence and severity of some of the complications previously described. Currently, the most widespread indication for the administration of parenteral nutrition comprises malnutrition, defined as a functional and structural alteration of the organism secondary to an imbalance between indispensable nutritional requirements, consumption, and use. In particular for patients with hematological disorders who undergo HSC transplants, it is essential to use nutritional support in the following situations:

1. negative nitrogen balance, which, according to many, should be the only indication for administration of parenteral nutrition;
2. increase in energetic requirement equal to approximately 130%-150% of the normal requirement;
3. altered glucose tolerance, due to the possible administration of drugs such as steroids and cyclosporine and to the onset of infections, with detrimental effects on the pancreas;
4. increase of cholesterol and triglyceride concentrations, in particular in patients being treated for chronic GVHD;
5. decreased intake and absorption of water-soluble vita-

mins and increase in antioxidant system requirements such as for tocopherol and beta-carotene, which are a direct consequence of the high-dose chemotherapy and radiation endured by patients during the conditioning regimen;

6. possible micronutrient deficiency due to malabsorption and increased demand for bone marrow reconstitution.

For all of these reasons, nutritional support in transplanted patients is administered regardless of the basic nutritional state, with the aim of preventing malnutrition; the latter can be secondary to the conditioning regimens, or caused by increased needs due to cytoreductive therapy, or due to presence of infections or sepsis or to other complications constituting a "stress" condition in the organism.

Glutamine in parenteral nutrition

Glutamine (GLN) is the most common amino acid in the organism. In normal conditions, GLN is a non-essential amino acid, since it can be produced in a sufficient amount able to satisfy the organism's needs. It covers various important functions:

1. it is the main energetic substrate of the jejunal mucosa;
2. it stimulates the cell proliferation of the ileus and the colon crypts;
3. it supplies substrates, with cysteine and glycine, for the synthesis of glutathione (GSH);
4. it reduces the release of proinflammatory cytokines such as IL-6 and 8 in the intestinal mucosa;
5. it supplies energy;
6. it is the progenitor for the synthesis of purine, pyrimidine, and its nucleotides;
7. it is the fundamental substrate for renal ammoniogenesis;
8. it is a nitrogen carrier between the various tissues;
9. it regulates the release of IL-2 from activated T lymphocytes.

Fatty acids in parenteral nutrition

Epidemiology studies have shown a correlation between the fat held in food and the appearance of cardiovascular diseases, and have shown a preventative role for polyunsaturated fatty acids. Recently, a better understanding has been reached of the mechanism of action and physiology of ω -3 fatty acids and of its derived eicosanoids, recognizing, above all, their antiinflammatory effects.

Among the fatty acid progenitors, there are linoleic acid from which ω -6 fatty acids γ -linolenic acid and arachidonic acid are derived, and α -linolenic acid from

which the ω -3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) are derived. Linoleic acid and α -linolenic acid are essential fatty acids and carry out important functions in the synthesis of cell membranes, influencing inflammatory reactions and immunological resistance. ω -3 fatty acid inhibits the release of proinflammatory arachidonic acid metabolites (thromboxane A_2 and leukotriene B_4) or of other cytokines such as TNF- α , IL-1, and IL-6. With the administration of ω -3 fatty acid, vasoconstriction and platelet aggregation are reduced, with an increase in PGI $_2$ and PGI $_3$ vasodilator effects; moreover, there is an increased release of nitrogen monoxide which supports an enhanced organ perfusion.

There are not many studies of ω -3. Only after the discovery of the protective effect for cardiovascular pathologies have investigations intensified. In hematology, one of the first studies was a perspective one made in 1998 by a group of researchers from Seattle, Washington, in the United States (8). Their objective was to estimate bacteremia, fungemia, and infections within the first 100 days after an HSC transplant. Five hundred and twelve patients were enrolled, homogeneous for age, weight, height, and antiinfective treatment, all affected by hematological neoplasia and undergoing autologous or allogeneic HSC transplant. They were divided into 2 groups: the first group (253 patients) received parenteral standard nutrition with 25%-30% lipids, while the second group (259 patients) received a low-lipid dose of 6%-8%. The results showed that there were no substantial differences between the 2 groups with regard to the development of infections, GVHD, maintenance of a normal glucose level, and in the achievement of engraftment. The study instead provided significant results concerning the importance of linoleic acid and ω -3, identifying, when these are absent, linoleic acid.

A subsequent randomized study performed in 2001 in Osaka, Japan (9), assessed the role of ω -3 in the development of systemic inflammatory response syndrome (SIRS). Sixteen patients affected by hematological disorders and who had undergone an allogeneic HSC transplant were enrolled and divided into 2 homogeneous groups in terms of clinical characteristics. The first group ($n = 7$) received 1.8 g/day of ω -3 orally, beginning 3 weeks before transplant and ending 180 days after. The second group ($n = 9$) did not receive the additional ω -3. The purpose of the study was to assess the role of these fatty acids with regard to the reduction of infections, GVHD, and endothelial damage. The outcome demonstrated that among the group of patients treated with ω -3, a significant reduction resulted in the production of leukotriene B_4 , thromboxane A_2 , and PGI $_3$ ($p < 0.001$) and also of TNF- α , IFN- γ , and IL-10 ($p <$

0.005). Furthermore, a higher survival rate ($p < 0.01$) was also reported, as well as the development of GVHD. Serum proinflammatory cytokine concentrations and development of infections were lower. Among the groups who received additional ω -3, a reduction in platelet aggregation was also reported. In platelets, arachidonic acid is metabolized by cyclooxygenase in thromboxane A_2 , which is a powerful mediator of platelet aggregation. In endothelial cells, instead, the same enzymatic pathway produces PGI $_2$, which inhibits platelet aggregation. EPA is metabolized either by the platelets or the endothelial cells in thromboxane A_3 and PGI $_3$, and both inhibit platelet aggregation. All of this has very important effects on the microcycle restraining ischemic diseases and organ damage.

Very similar results were reported in a study in 2005, comprising all studies completed regarding the use of ω -3, although they are not numerous. For patients with hematological disorders who underwent an HSC transplant, an important role of ω -3 has emerged in reducing the incidence and severity of some complications such as infections, GVHD, and SIRS. Furthermore positive correlations with improvement in quality of life, faster hematological remission, reduction of mortality and hospitalization were reported (9).

Preliminary results of a retrospective multicenter study on the use of glutamine and fatty acids in patients who underwent autologous hematopoietic stem cell transplantation

The preliminary results of the study performed in collaboration with the Clinical Nutritional Service and the HSC Transplantation Center of the hematology division of the Policlinico Tor Vergata are detailed below. The objective was to assess the impact of total parenteral nutrition (TPN) on the process of autologous transplantation of HSCs. In particular we have compared 3 groups of patients homogeneous for their clinical characteristics and have administered 3 different types of parenteral nutrition to them: A, standard; B, enriched with GLN, and C, enriched with GLN and ω -3.

Rationale of the study

Toxicity is frequently high following high-dose chemotherapy with the use of a gastroenteric apparatus, and extends beyond compromising adequate nutrient intake and absorption; it can also cause severe gastroenteritis, which can lead to morbidity and mortality of patients with hematological disorders in a short time. After HSC transplant, oral mucositis occurs in 60% to 100% of cases due to the cytotoxic effect that antineoplastic drugs

have on epithelial cells. Generally, it is characterized by pain, dysphagia, edema, ulceration, pseudomembrane, and xerostomia, and normal nutrition becomes difficult or even impossible. Data on intestinal mucositis are more difficult to interpret since the symptoms (nausea and vomiting) can also be due to the beginning of other infectious complications. Therefore, the differential diagnosis is supported today by the survey of ultrasound instrumentation, taking into consideration that a mucosa thickness of > 4 mm suggests mucositis, even in the absence of microbiological isolation.

Furthermore, in these patients, an immune dysfunction appears to be present characterized by a biphasic response to stimuli of various natures. Initially, there is an excessive cellular activation with large amounts of proinflammatory cytokines which can lead to even lethal organ damage. Later, an offsetting response occurs, usually associated with the production of large amounts of antiinflammatory molecules. It is thought that a suitable preparation of nutrients can exert modulatory effects on both the hyperinflammatory phase (referred to as SIRS) and the compensatory phase (referred to as the compensatory antiinflammatory response).

On the basis of these considerations, our study assessed the impact of parenteral nutrition in 3 different formulations, on the clinical performance of patients undergoing HSC transplants.

MATERIALS AND METHODS

This study was carried out in 48 patients affected by oncohematological pathologies who underwent autologous HSC transplants. The patients were enrolled from 2003 and 2005 at the HSC transplantation centers of the Policlinico of Rome "Tor Vergata". Written informed consent was obtained from all patients or their legal representatives. We have analyzed results obtained from the use of 3 different formulations of TPN. In particular: 16 patients (group A) received standard parenteral nutrition, 13 patients (group B) received TPN enriched with GLN (Dipeptiven; Fresenius) at a dosage of 0.25 g/kg per day, 19 patients (group C) received supplementation with both GLN, at a dosage analogous to the previous one, and ω -3 (Omegaven; Fresenius) at a dosage of 1.5 mL/kg per day. The 3 groups were comparable in terms of diagnosis and pre-transplant conditioning regimen. Their ages were between 18 and 67 years. The patients enrolled showed preserved renal and hepatic function and had negative medical history for dyslipidemia, cardiovascular diseases, and coagulopathies. Parenteral nutrition was

isocaloric, with standard nitrogen contribution, and started the day after HSC infusion. Weaning occurred when hematological recovery was recognized, measured by polymorphonuclear neutrophil (PMN) counts $> 500/\mu\text{L}$ for 3 consecutive days, or when the patient was able to guarantee at least 50% oral caloric intake.

The means of the 3 groups have been compared by using the analysis of variance to a classification criterion. The Bonferroni multiple comparison test has been applied to compare each pair of group's mean. A value of $p < 0.05$ was considered significant.

Objectives of the study

The principal aim of our study was to compare the effectiveness of 3 different parenteral nutrition formulations (standard, with GLN, and with both GLN and ω -3) in groups of patients with homogeneous clinical characteristics. The comparison was carried out to assess the following parameters:

1. duration of hospital stay;
2. duration of TPN administration;
3. number of days with fever during neutropenia;
4. time necessary for engraftment (PMN $> 500/\mu\text{L}$ for 3 consecutive days);
5. duration of the antibiotic therapy administration and use of antifungal drugs; and
6. assessment of the grade of mucositis according to the World Health Organization (WHO) scale.

RESULTS

Analyzing the duration of the hospital stay, we obtained different mean values among the 3 groups ($p < 0.01$). In fact, in group A (standard TPN) the mean value was 24.63 days (± 7.7); in group B (TPN+GLN), the mean value is 24.53 days (± 6.8); and in group C (TPN + GLN + ω -3), 14.68 days (± 3.2). Trying to establish the pairwise statistical significance, we noted that the mean time of hospital stay of group C (14.68 ± 3.2 days) was lower, both with respect to group A ($p < 0.01$) and to group B ($p = 0.01$).

In evaluating the days of TPN administration, even here a remarkable difference among the 3 groups ($p = 0.001$) was registered. The mean value reported in group A was 14 days (± 4.6); in group B, 13.38 days (± 2.9); and in group C, 9.95 days (± 1.8). In this case, it is evident that the mean value for group C was lower than that for group A ($p = 0.011$) and group B ($p = 0.004$).

Even when considering the mean number of days with fever during neutropenia, the values reported

TABLE I - LENGTH OF HOSPITAL STAY (DAYS)

Group	Patients	Mean	Range
A	16	24.63	15-43
B	13	24.54	14-38
C	19	14.68	10-23

TABLE II - DURATION OF FEBRILE NEUTROPENIA (DAYS)

Group	Patients with febrile neutropenia	Mean	Range
A	16/16	4.81	1-16
B	13/13	7.69	1-14
C	16/19	3.06	1-7

TABLE III - NUMBER DAYS OF TOTAL PARENTERAL NUTRITION

Group	Patients	Mean	Range
A	16	14	8-23
B	13	13.38	10-21
C	19	9.95	6-13

TABLE IV - DAYS OF ANTIBIOTIC THERAPY

Group	Patients receiving TAB	Mean	Range
A	16/16	10.13	5-27
B	13/13	12.85	6-21
C	17/19	7.47	4-15

TAB = Antibiotic therapy

TABLE V - USE OF ANTIFUNGAL DRUGS

Group	Patients with TAF (%)
A	5/16 (31.3)
B	5/13 (38.5)
C	1/19 (5.3)

TAF = Antifungal drugs

TABLE VI - DAYS TO POLYMORPHONUCLEAR NEUTROPHIL ENGRAFTMENT

Group	Patients	Mean	Range
A	16	13.31	9-33
B	13	13.31	10-20
C	19	11.53	8-20

TABLE VII - GRADE OF MUCOSITIS

Group	< WHO grade 1 (%)	> WHO grade 1 (%)
A	1/16 (6.3)	15/16 (93.7)
B	1/13 (7.7)	12/13 (92.3)
C	16/19 (84.2)	3/19 (15.8)

TABLE VIII - GRADE OF DIARRHEA

Group	< WHO grade 1 (%)	> WHO grade 1 (%)
A	10/16 (62.5)	6/16 (37.5)
B	2/13 (15.4)	11/13 (84.6)
C	14/19 (73.7)	5/19 (26.3)

among the 3 groups ($p = 0.008$) are considerably different. In group A, the mean value was 4.81 days (± 4.5); in group B, 7.69 days (± 4.2); and in group C, 3 days (± 2.2). Performing the pairwise statistical analysis, we observed that the mean value of group C was lower than that of group B ($p = 0.06$).

Regarding time to engraftment, we found similar values among the 3 groups with no statistically significant difference ($p = 0.466$). For group A, we found a mean value of 13.31 days (± 7.2); for group B, 13.31 days (± 3.2), and for group C, 11.53 days (± 3).

Regarding antibiotic therapy, we have to consider that 2 of the patients of group C did not require it, and therefore the analysis was performed on 46 patients. The differences in mean duration of administration among the 3 groups was statistically significant ($p = 0.011$). In group A, the mean duration of antibiotic therapy was 10.13 days (± 5.7) days; in group B, 12.85 days (± 4.7); and in group C, 7.47 days (± 3.2). Comparing the groups, a statistically significant difference was observed between the mean value of group C and that of group B ($p = 0.008$). As for the use of antifungal drugs, these were given to 5 patients of group A (31.3%), 5 of group B (38.5%), and only 1 of group C (5.3%).

We used the WHO scale in evaluating the severity of mucositis and observed how many patients experienced mucositis with a grade higher than 1. The values we found were in group A, 15 patients (93.8%), in group B, 12 patients (92.3%), and in group C, only 3 patients (15.8%).

Regarding diarrhea, values higher than 1 according to the WHO scale were reported in 6 patients of group A (37.5%), in 11 patients of group B (84.6%), and only in 5 patients of group C (26.35%).

CONCLUSIONS

Our study has demonstrated the important role of the association of glutamine with ω -3 fatty acids with regard to the reduction of gravity of some common complications frequently evidenced in transplanted patients. In fact, in group C, which received contemporary administration, we discovered the following:

- a reduced hospital stay duration ($p < 0.01$);
- a reduced duration of TPN administration ($p = 0.001$);
- a reduced duration of days with fever during neutropenia ($p = 0.008$);
- a briefer antibiotic administration ($p = 0.0011$) and a reduced use of antifungal drugs (administered to 1 patient only);
- a reduced gravity of problems with mucositis, evaluated by the international WHO scale. According to this scale, in group C, we noted values less than 1 in 16 patients (84.2%). Also in the case of diarrhea, 14 of 19 patients (73.7%) reported a value of less than 1.

However, no correlations have been identified with regard to the durations to achieve engraftment, which were rather similar and without statistically significant differences.

From the study, it has emerged that the administration of glutamine alone does not have a determining role in conditioning the course of the transplant, while in association with ω -3, a precious synergy is obtained. We assume that this is, from a biological point of view, due to the various mechanisms of action of both. In fact, if, on the one hand, GLN exercises a stimulus on the repli-

cation of the enterocytes, then, on the other hand, ω -3 reduces the release of proinflammatory cytokines, with a minor number of SIRS cases, which represents the direct cause of the most serious transplant-related complications. Although the cohorts in this study were not large, the results obtained are clear and statistically remarkable both in the global comparisons (by the analysis of variance to a classification criterion) and in the multiple comparisons (by the Bonferroni test).

Moreover, the importance of our study is also supported by the few data in the literature both on fatty acids and on the effects that these have in association with glutamine.

In conclusion these results represent a valid base to consider the possibility of a prospective randomized multicenter study, supported by opportune biological assessment regarding cytokine dosages and lymphocyte counts, comparing the use of 2 different parenteral nutrition formulations: 1 with only glutamine added and 1 with glutamine and ω -3 fatty acids.

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