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Total Parenteral Nutrition and Nutritional Assessment in Leukaemic Children Undergoing Bone Marrow Transplantation

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The aggressive radiotherapy and chemotherapy used in conditioning regimens for children with leukaemia undergoing bone marrow transplantation (BMT) cause a severe catabolic state. Total parenteral nutrition (TPN) is indispensable in the management of these patients. 25 children with leukaemia undergoing BMT were studied to evaluate the efficacy of TPN and the value of anthropometric parameters and biochemical variables (albumin, retinol-binding protein and prealbumin) in monitoring nutritional status in the critical post-BMT phase. The complications of TPN were mainly metabolic, generally mild and easily controlled. The hyperalimentation solution and infusion line were not responsible for infection in any patient. The marked variations in anthropometric parameters and albumin expected in such patients were not observed in our children due to the nutritional support given. Prealbumin and retinol-binding protein showed statistically significant, positive variations ($P < 0.01$), thus proving sensitive indices of the response to nutritional repletion.
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INTRODUCTION

BONE MARROW TRANSPLANTATION (BMT) has improved the survival of a significant percentage of children with leukaemia [1]. The use of aggressive chemotherapy and radiotherapy in pre-BMT conditioning regimens makes total parenteral nutrition (TPN) essential to avoid complications such as protein energy malnutrition (PEM) in the early post-BMT phase and the consequent increase of morbidity.

At present, nutritional status and the efficacy of nutritional support are usually evaluated by a series of anthropometric and biochemical indices [2, 3]. Weight variations may not be useful as nutritional indices in the short-term evaluation [4, 5]. The literature underlines the value of prealbumin (PA) [6] and retinol-binding protein (RBP) [7] as reliable biochemical indices of early and even subclinical alterations of nutritional status [4, 8–12]. Though literature is replete with information concerning biochemical evaluation during TPN, few data have been published on PA and RBP in severely immunosuppressed children [13, 14].

The first aim of this study was to assess the nutritional benefits and risks of TPN in critical children with severe metabolic stress and at high risk for malnutrition. In this setting we evaluated the role of PA and RBP as nutritional indices.

PATIENTS AND METHODS

Patients

25 patients (16 males, 9 females, aged 3 years 7 months–17 years 10 months, median 10 years 7 months) who received a BMT (allogeneic in 23 cases, autologous in 2) at our centre from September 1986 and September 1989 were considered eligible for this study. Clinical and haematological features of our patients are listed in Table 1.

Pre-BMT conditioning differed according to type and phase of disease, and included high-dose chemotherapy and fractionated total body irradiation (FTBI) (Table 2). All patients received immunosuppressive treatment with intravenous cyclosporin at standard dosage for graft-versus-host disease (GVHD) prophylaxis starting 1 day before BMT. As intestinal decontaminating treatment each patient was given colimycin and neomycin orally. In all cases prophylaxis of viral infection was also administered, consisting of high-dose intravenous immunoglobulins (Sandoglobulin) weekly and intravenous acyclovir daily starting 6 days before BMT and continuing until 30 days after BMT. Bacterial infections and fungal complications were treated with multiple broad-spectrum antibiotics (ceftazidime, amikacin, teicoplanin, imipenem) and amphotericin B, respectively. Acute GVHD was treated by increasing the cyclosporin doses and adding steroid therapy. TPN was given to all the children from the beginning of the conditioning regimen, 7 days before BMT. According to their clinical situation, patients were allowed to consume desired foods orally.

TPN schedule

TPN solutions were infused by central venous catheter (CVC) (Hickman or Broviac). The maximum infusion rate was 120 ml/h and was regulated by peristaltic pumps (Abbott-Shaw model 3). The line and bags were prepared by the nursing team under a sterile vertical laminar air flow equipment (Steril CTH 48). Each infusion line was tested for possible contamination by surveillance cultures at the end of the preparation and at the end of the infusion.

The infusion solution was prepared in ethylvinylacetate bags (Terumo) with 10%, 30% or 50% glucose solution, a 20% lipid solution (Intralipid, Pierrel) and an aminoacid solution (Freamine III, Baxter), so that final solutions at increasing

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Table 1. Patients' characteristics

Patient (sex, age†)	Disease	Phase	BMT	Conditioning schedule*	Take days	TPN (days)
1 (F, 17.1)	ALL	2nd CR	allo	A	10	25
2 (M, 13.9)	ALL	3rd CR	allo	C	14	21
3 (F, 6.9)	ALL	2nd CR	auto	C	12	24
4 (M, 4.2)	ALL	2nd CR	allo	B	13	25
5 (F, 11.7)	ALL	2nd CR	allo	A	16	27
6 (M, 3.7)	ALL	2nd CR	allo	A	16	26
7 (M, 5.7)	ALL	2nd CR	allo	A	20	28
8 (M, 6)	ALL	2nd CR	allo	A	12	30
9 (F, 7)	ALL	3rd CR	allo	A	14	40
10 (M, 11.9)	ALL	3rd CR	allo	A	13	26
11 (F, 12)	ALL	3rd CR	allo	A	12	26
12 (F, 15)	ALL	2nd CR	allo	A	15	26
13 (F, 11.8)	ALL-T	1st CR	allo	B	12	23
14 (M, 5.4)	ALL-B	1st CR	auto	C	—	20
15 (M, 8.3)	AnLL M1	1st CR	allo	B	14	30
16 (M, 10)	AnLL M3	Relapse	allo	D	17	30
17 (M, 12.1)	AnLL M3	1st CR	allo	B	13	26
18 (M, 12.5)	AnLL M4	1st CR	allo	D	22	35
19 (F, 5.1)	AnLL M5	1st CR	allo	D	19	29
20 (M, 10.7)	AnLL M5	1st CR	allo	C	15	23
21 (M, 5.5)	Biphen.AL	1st CR	allo	C	18	23
22 (M, 15)	CML Ph$^+$	CP	allo	B	12	26
23 (F, 8)	CML Ph$^+$	CP	allo	D	18	33
24 (M, 6.1)	NHL	1st CR	allo	B	12	25
25 (M, 11.2)	RAEB	RAEB	allo	B	—	25

*See Table 2. †Age in years.
ALL = acute lymphoblastic leukaemia, CR = complete remission, Allo = allogeneic, Auto = autologous, AnLL = acute non-lymphoblastic leukaemia, M1–5 = FAB classification, Biphen.AL = biphenotypic acute leukaemia, CML Ph$^+$ = chronic myelogenous leukaemia Philadelphia$^+$, CP = chronic phase, NHL = non-Hodgkin lymphoma, RAEB = refractory anaemia with excess of blasts.

concentrations of 10%, 12.5% and 15% were obtained. Electrolytes, vitamins and trace elements (Baxter) were added to the hyperalimentation solution based on the clinical and metabolic requirements of each patient. The amount of calories and water supplied was based on the patient's needs and metabolic stress.

The caloric requirement was calculated according to age as follows: 80–100 kcal per kg daily for children aged 0–18 months, 50–75 kcal per kg daily for those aged 18 months–7 years, and 45–65 kcal per kg daily for those over 7 years. The protein calculated requirements were 2.5–3 g per kg daily for children aged 0–18 months and 1.5–2.5 g per kg daily for those aged over 7 years. The ensured nitrogen:caloric (g/kcal) ratio was 1:150.

The mean daily caloric intake was determined as percent of the estimated requirements because differences in patients' age render other presentations of data useless.

Assessment of nutritional status

Nutritional status was evaluated by anthropometric (weight, mid-arm circumference, triceps skinfold) and biochemical variables (albumin, PA, RBP) at the start, on day 15 and at the end of TPN. Weight was determined using a scale with + 10 g accuracy. TSF was always measured by the same investigator with a Harpenden caliper (British Indicators, St Albans). PA was measured with an automatic immunonephelometer (Beckman). RBP was determined by simple radial immunodiffusion [15]. Anthropometric and biochemical data are shown as means, though patients differ for age and size. In fact our interest was in the evaluation of differences at different times in the same study population.

Clinical monitoring of patients during TPN

Blood cells count and chemistries were determined in all patients at different intervals (daily, biweekly or weekly) according to the clinical course after BMT. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea and creatinine were studied in particular because of their known relation with the plasma levels of rapid turnover proteins [16].

All clinical complications were recorded accurately by a physician involved in the study.

Statistical analysis

Variations of the nutritional parameters were compared using Student's *t* test for paired observations [17].

RESULTS

BMT and TPN related problems

The mean (S.D.) duration of TPN was 26.9 (4.4) days (median 26 days) and the daily caloric intake was 82.3 (12.9)% (median 78%) of the estimated requirement for each patient.

All the patients tolerated TPN support well. Metabolic and mechanical complications occurred in only a few of them, and were mild, of short duration, and not life-threatening. Mild hyperglycaemia (140–200 mg/dl) with or without glycosuria was observed in 5 patients and always resolved by slowing the infusion rate and decreasing the concentration of the hyperalimentation solution. 1 patient presented reversible non-ketotic hyperosmolar dehydration due to excess of TPN infusion following damage to the peristaltic pump.

22 of the patients (88%) had fever before the engraftment. Marrow aplasia (polymorphonuclear cells $< 0.05 \times 10^9$/l) had a mean (S.D.) duration of 17.5 (6.5) days (range 11–35, median 15). Fever developed 5 (4) days (range 0–17, median 5) after

Table 2. Conditioning schedules

Schedule A	
Vincristine	1.5 mg/m^2 intravenously, day -8
Vincristine	0.5 mg/m^2/24 h intravenously, days -8,-7,-6,-5,-4
Cyclophosphamide	60 mg/kg intravenously, days -3,-2
Fractioned total body irradiation (FTBI)	2 Gy twice daily, days -7,-6,-5
Schedule B	
Cyclophosphamide	60 mg/kg intravenously, days -6,-5
FTBI	2 Gy twice daily, days -3,-2,-1
Schedule C	
Cytarabine	2 g/m^2 intravenously twice daily, days -9,-8
Cyclophosphamide	60 mg/kg intravenously, days -6,-5
FTBI	2 Gy twice daily, days -3,-2,-1
Schedule D	
Busulphan	1 mg/kg orally four times daily, days -9.-8,-7,-6
Cyclophosphamide	50 mg/kg intravenously, days -5,-4,-3,-2

BMT. A total of 24 febrile episodes occurred. Fever (> 38°C) lasted 4.5 (3.5) days (range 0–14, median 3.5). The febrile episodes were classified as one sepsis due to *Pseudomonas aeruginosa*, one enterocolitis due to *Clostridium difficilis*, one mucositis due to *Candida albicans*, one urinary tract infection due to *Escherichia coli*, one oesophagitis of probable viral origin, and 19 fevers of unknown origin. Blood cultures of CVC were always negative and peripheral vein blood cultures were positive in only one case (the patient with *Ps aeruginosa* sepsis). The febrile episodes could not be attributed to infection of the CVC or of the TPN line.

20 patients presented with an acute GVHD grade I, limited to the skin, in 11 cases; grade II in 6 (skin and gut in 4; skin and liver in 1; skin, gut and liver in 1); and grade III in 1 (skin and gut). The classification of GVHD was according to standard agreements [18].

21 of the patients (84%) had mucositis due to chemotherapy and total body irradiation (mild in 10, moderate in 4, severe in 7) at varying sites and in varying associations (stomatitis, oesophagitis, gastritis, enteritis).

In 23 patients the mean (S.D.) time for a take was 14 (3) days (median 14). Engraftment was not successful in the other 2 who both died, 1 of rejection and multiorgan failure, and 1 of respiratory failure in the course of *Ps aeruginosa* infection with pulmonary localisation. Another 1 had venoocclusive disease of the liver and died of lung haemorrhage.

Renal and hepatic function

In 22 patients (88%) renal function tests were normal for all the post-BMT course, with slight fluctuations, always in the normal range, due to iatrogenic events.

17 patients presented no significant variations of liver function tests. 5 others (20%) had moderately elevated transaminases [mean (S.D.) serum ALT 203 (137) U/ml, serum AST 116 (79) U/ml] at the start of TPN but the values returned to normal in a mean of 7 days; the elevation was attributable to mild toxicity due to aggressive radiotherapy and chemotherapy. 3 patients manifested important renal and hepatic impairment caused by BMT related toxicity that proved fatal. Thus in 22 of the 25 patients there was no hepatic or renal damage that could impair the synthesis and excretion of the rapid turnover proteins.

Anthropometric variables and albumin

All the patients considered were well and in good nutritional condition when they underwent BMT. Before the transplant 22 of them had a weight for height of over the 25th percentile, and albumin was more than 3 g/dl in all cases.

The anthropometric variables studied, weight, mid-arm circumference (MAC) and triceps skinfold (TSF), did not vary significantly from the start to the end of TPN. The mean (S.D.) values of weight at the start, at 15 days and at the end of TPN and the mean difference between values at the start and end of TPN were, respectively, 33.13 (12.82), 33.62 (12.01), 33.74 (12.40) and 0.63 (1.32) kg. The respective values for MAC were 20.70 (3.20), 21.00 (3.96), 21.14 (3.70) and 0.45 (1.37) cm, and the TSF values were 11.30 (4.89), 11.32 (4.16), 11.50 (4.95) and 0.60 (2.33) mm. The standard deviations in weight, MAC, and TSF are due to the different age and size of population rather than to abnormal changes in individual patients.

Albumin did not present statistically significant variations. The mean (S.D.) values at the start, on day 15 and at the end of TPN and the difference between values at the start and end of TPN were 3.94 (0.35), 3.76 (0.28), 3.78 (0.32) and −0.20 (0.40) mg/dl, respectively.

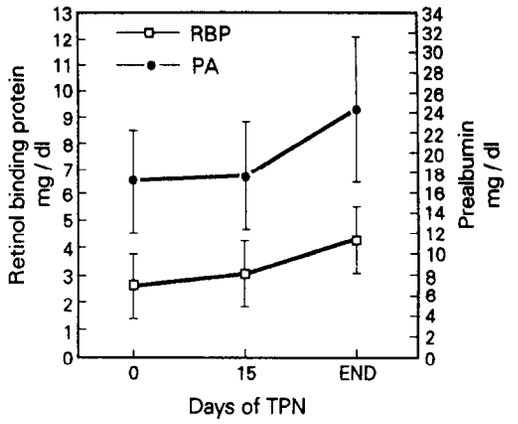

Fig. 1. Mean values of prealbumin (PA) and retinol-binding protein (RBP) at the beginning (0), on day 15 (15) and at the end (END) of TPN.

Prealbumin and retinol-binding protein

The mean (S.D.) PA levels in the 25 patients at the start, on day 15 and at the end of TPN (Fig. 1) were 18.26 (6.94), 17.53 (5.96) and 23.79 (7.42) mg/dl. The difference at day 15 and at the end of TPN with respect to the start were, respectively, −0.11 (7.18) and +7.07 (7.10) mg/dl. Mean RBP concentrations at the same times were 2.77 (1.12), 3.06 (1.28) and 4.18 (1.59) mg/dl; the differences in RBP at day 15 and at the end of TPN were +0.27 (1.43) and +1.40 (2.1) mg/dl.

Considering the 22 patients without significant hepatic or renal alterations the PA vlaues at the start, on day 15 and at the end of TPN were 17.15 (4.52), 17.69 (6.26) and 24.40 (7.17) mg/dl. The difference at day 15 and at the end of TPN with respect to the start was +0.53 (6.59) and +7.47 (7.11) mg/dl. For RBP the respective mean values were 2.60 (0.72), 3.06 (1.37), 4.29 (1.58), +0.45 (1.34) and +1.70 (1.61) mg/dl.

PA and RBP levels fell markedly in 1 of the 3 patients excluded from the above analysis because of severe preterminal status and in the other 2 PA presented a trend similar to that of the other patients whereas RBP increased concomitantly with renal impairment.

In the whole series and in the group without compromised hepatic and renal function, PA and RBP both varied significantly at the end of TPN with respect to the start ($P < 0.01$); the variations observed on day 15 of TPN were not statistically significant.

DISCUSSION

A child undergoing BMT is at severe risk of malnutrition, and TPN is mandatory in these patients. In our series the aggressive chemotherapy and radiotherapy [19, 20] of the conditioning regimens and the duration of malabsorption resulting from gastrointestinal lesions [18] could cause a weight loss due to acute catabolism and worsening of the other anthropometric parameters, as reported by other authors [21]. The amount of calories supplied to our patients by TPN was on average about 82% of the estimated requirements, which must be considered as a valid result since TPN is frequently interrupted in patients undergoing BMT because the infusion lines have to be used for numerous other treatments that are not always compatible with the hyperalimentation solution.

The complications of TPN in our experience were prevalently metabolic, generally mild and easily controlled. At least in the period of administration of TPN no febrile episode was attributable to infection of the CVC or infusion line. Only 28% of the patients studied presented severe mucositis. Whether TPN contributes to diminish gastrointestinal toxicity is still not well established, but in our opinion is an intriguing hypothesis. In a previous work we reported, in patients with high risk acute leukaemias treated with similar intensive chemotherapeutic schedules, a lower rate and severity of mucositis in the group supported by NPT versus the control group [22].

We must underline that our BMT recipients were in good nutritional condition from the beginning of the study and the anthropometric parameters (weight, MAC, TSF) did not vary significantly during the crucial phase of wasting after BMT. This result is certainly attributible to TPN.

However other authors consider that anthropometric parameters are of little use in evaluating the efficacy of TPN since variations may be masked by alterations of water balance [4]. These authors observed that, in patients undergoing BMT, a decrease of body cell mass, associated with slight variations of lean mass and fat mass, was not accompanied by variations of body weight.

Variations of body composition could be measured better by the isotopic dilution technique [23] but its routine application is not always clinically feasible in the case of critically ill patients.

Like the anthropometric parameters, albumin did not vary significantly in our patients. Albumin variations correlate with body cell mass and lean mass, but are of little use to assess nutritional status if malnutrition is of short duration, of recent onset, or subclinical. This is due to the long half-life of this protein and its notable total mass [4, 8].

Another protein used to evaluate nutritional status, transferrin [24] is certainly a more reliable index than albumin since it has a shorter half-life (9 days), but it was not considered in the present study because of the wide distribution of its values in malnutrition and the characteristics of our patients who generally received multiple transfusions.

Numerous authors have proposed PA and RBP as biochemical markers to monitor nutritional status in not transplanted patients [6–11]. The half-lives of RBP and PA are estimated to be respectively 12 hours and 2 days. The plasma values of PA and RBP correlate inversely proportionally with any hepatic damage and in particular with total bilirubin and serum AST concentrations [16]. RBP levels generally increase in the presence of renal damage, as does the RBP:PA molar ratio (1:1) which instead is not modified in liver disease [16, 25]. Furthermore, PA levels fall significantly following stress and inflammation [26].

In our 22 patients without significant alterations of hepatic or renal function the trend demonstrated by PA was different from that of RBP. PA remained almost stable until day 15 of TPN (day 7 after transplant). Then it showed a statistically significant rise ($P < 0.01$). In other series of severely malnourished subjects or patients with various gastrointestinal diseases PA generally showed a positive response to nutritional repletion within 4–7 days of the start of TPN [9, 6]. The latent period observed in our patients is explained by the severe metabolic stress and inflammation resulting from the conditioning regimen, with maximum clinical expression generally in the first week after BMT, that is, from day 7 to day 15 of TPN. Despite the high stress induced by conditioning therapy in this first period, PA did not lower significantly. The recovery of PA coincided not only with the continuation of nutritional support but also with the resolution of stress, the progressive return of the organism's capacity to repair tissues and counteract infections that followed marrow take, and the pharmacological control of any intercurrent inflammatory events such as GVHD. The efficacy of TPN in correcting severe catabolism was documented by the absence of significant fall of PA in the most critical phases after BMT and by the trend of RBP. This protein rose constantly, at first slower and then faster and significantly during the whole period of nutritional support. In the other 3 patients variations of rapid turnover proteins were expected in view of the organ disease present. 2 of these children, 1 with rejection and multiorgan failure and 1 with *Ps aeruginosa* sepsis, presented a marked increase of RBP together with renal function worsening. In the third case, owing to veno-occlusive disease, the parallel fall of PA and RBP was associated with the severe liver disfunction.

Rapid turnover protein variations, can be considered useful indices in monitoring nutritional status and the response to TPN in children receiving a BMT. Evaluations of PA and RBP levels must however take into account the clinical condition and biohumoral findings of each patient since they may be influenced by various factors (stress, inflammation, liver disease, kidney disease).

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Detection of Tyrosine Hydroxylase mRNA and Minimal Neuroblastoma Cells by the Reverse Transcription–Polymerase Chain Reaction

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To facilitate the diagnosis of bone marrow metastasis in neuroblastoma, we have developed a method of amplifying and detecting the tyrosine hydroxylase (TH) mRNA sequence in bone marrow cells using a combination of reverse transcription and the polymerase chain reaction (RT/PCR). By this method, the sequence of TH was detected clearly in the neuroblastoma tissues of all 6 patients and not detected in the bone marrow cells of any of the 9 negative control children. In a reconstitution experiment, 1 neuroblastoma cell per 100 000 normal bone marrow cells could be detected, thus indicating the great sensitivity of this method. Based on these results, this technique may be of value in the diagnosis and treatment follow-up of bone marrow metastasis of neuroblastoma.
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INTRODUCTION

NEUROBLASTOMA IS one of the most common malignant solid tumours of childhood. It originates in the neural crest, grows rapidly into a huge mass and frequently metastasises to bone marrow. Since disseminated neuroblastoma still carries a poor prognosis, massive therapy followed by allogeneic or autologous bone marrow transplantation has become the current modality of consolidation treatment in such patients. It is important to detect tumour cells in the bone marrow of neuroblastoma patients in order to diagnose the disease, monitor its course during remission or relapse, and to confirm that pretherapy bone marrow stored for autologous transplantation is tumour-free when harvested from a patient. The present means of diagnosing bone marrow metastasis in neuroblastoma include histological, biochemical, and immunohistological analysis. Morphological distinction between tumour cells and primitive lymphoblasts can be difficult, and when only a small proportion of the cells are aberrant, it can be difficult to arrive at a diagnosis by these methods.

Neuroblastoma is characterised by the secretion of catecholamines in approximately 95% of patients [1]. Therefore, it is expected that almost all neuroblastomas produce tyrosine hydroxylase (TH), the first enzyme in the pathway of catecholamine biosynthesis. Expression of the TH gene is regulated in a tissue-specific manner during neonatal development and differentiation [2, 3]. Therefore, if TH mRNA is detected in the bone marrow cells of neuroblastoma patients, we can regard them as having bone marrow metastasis. In this report we exploited the combined method of reverse transcription and polymerase chain reaction (RT/PCR) to detect the mRNA of TH in bone marrow cells and demonstrated that this was a potential tool for detecting minimal bone marrow metastasis in neuroblastoma.

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