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Effects of oral amino acid supplementation on long-term-care-acquired infections in elderly patients

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ABSTRACT

The very high general infection rate (IRI) observed in our Geriatric Intensive Rehabilitation Center (GIRC) led us to investigate whether patient supplementation with essential amino acids (EAAs), modulators of immuno-competence, could reduce IRI. Eighty elderly patients admitted to our GIRC ($n = 40$; age 79.5 ± 7.71; male/female 14/26) or placebo ($n = 40$; age 82.13 ± 6.15; male/female 13/27) were allocated to an 8 g/day oral EAAs group and were surveyed for infections (>48 h from admission) over the first month of their hospital stay. The IRI was 67% for the entire population of patients, 82.5% (33/40 patients) in the placebo group and 52% (21/40 patients) in the EAA group ($p < 0.02$). When patients were divided into infection group (IG) and without-infection group (WIG), independently of post randomization allocation, the WIG had higher levels of serum albumin ($p < 0.001$), blood hemoglobin (Hb) concentration ($p = 0.01$), dietary protein ($p = 0.008$) calorie intakes ($p = 0.05$) but lower serum C-reactive protein (CRP) ($p < 0.001$). The factor of CRP > 0.8 mg/dl and Hb ≤ 12 in females, ≤13 in males was associated 4 times and 3.6 times risk of infection, respectively, by sex. EAAs supplementation may lower the risk of infection by 30% in the rehabilitative elderly population. CRP and blood hemoglobin levels can be considered risk markers of future infection.
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1. Introduction

Infections acquired during hospitalization, particularly in long term-care population, are a major source of morbidity and mortality (Gerberding, 2002; Burke, 2003). The incidence rate of infections in our GIRC is very high (about 80%; unpublished data) suggesting that the adoption of recommended prevention measures (Boyce et al., 2002; Creedon, 2006) as well as of oral heath/hygiene assistance are not enough to substantially reduce GIRC-acquired infection and there may be alterations in the patients' immunological status on admission. Presently, admitted patients are at a high risk of reduced immuno-competence because of their origin (85% from acute medical and surgical wards) (Strausbaugh et al., 1993), post acute inflammatory response (Desborough, 2000), peri-operative transfusion (Nielsen, 1995), and stay (Cullen et al., 1994) and finally poor nutrition (Martindale and Cresci, 2005). All these conditions may add to the age factor in lowering patients' immuno-competence (Crossley and Peterson, 2000).

As the burden of GIRC-acquired infections is considerable in terms of mortality (16.6% in GIRC) and health-care costs, the possibility of improving or maintaining the patients' immunological capacity could aid preventive measures in reducing infections.

We hypothesized that oral supplementation of EAAs could reduce the incidence rate of GIRC-acquired infections. The

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rationale for using EAAs is based on the following considerations: First, several amino acids including leucine, glutamine, arginine, glycine and methionine have cellular and immune regulation properties which favor cellular energy generation and act as a building block supplier to the synthesis of cell properties and nucleic bases (Roth, 2007). Thus, EAAs could maintain or improve a patient's immunological capacity. Second, body EAA shortage can occur following acute catabolism in elderly subjects (Desborough, 2000).

This study therefore aimed (i) at finding whether EAAs could reduce the incidence rate of GIRC-acquired infection in a population of elderly patients and (ii) to identify possible risk factors for developing infection.

2. Subjects and methods

2.1. Clinical setting

Our structure is a 220 bed-geriatric institute located in Milan (Italy), dedicated to the rehabilitation of geriatric post acute illness. The patients are from both acute medical and surgical settings (85%) and directly from home (15%). Patients from acute hospitals are usually admitted to our GIRC 10–21 days after the index event.

2.2. Population and measures

The patients enrolled in this study were consecutively admitted to two wards of the GIRC (80 beds in total; at most 90 days of stay) from March 1st, to December 31st 2008. Patients, who were at admission on antibiotic therapy or with body temperature $>36.8\ ^{\circ}C$, with diabetes on insulin treatment, non-operated cancer, pressure ulcers, hematological tumors, on artificial nutrition (enteral/parenteral nutrition) cognitive dysfunction (mini-mental state examination = MMSE score < 24) were excluded. Written informed consent was obtained from all participants after the nature of the study was explained. The study was approved by the local technical ethics committee.

During the first 72 h after admission, patients underwent the following investigations: (1) anthropometrics: body weight (kg) was determined using a mechanical weight lifter and height (m) was calculated from knee height (Chumlea et al., 1985). Body mass index (BMI) was calculated as kg/m^2. (2) Bio-humoral variables after overnight fasting, at 7:00 a.m., blood samples from the peripheral vein were drawn to determine routine variables. In addition, variables relative to body protein status (albumin), nutrition adequacy (prealbumin), body inflammation CRP were also determined.

2.3. Nutritional intakes

For each patient, a 3-day alimentary diary was collected by GIRC nurses who had been previously trained ad hoc. GIRC nurses reported in dietetic sheet for 3 days, before and after patient meals, the type and weight of cooked or uncooked food selected by patients from hospital catering menu.

Thereafter, the first author of this article (R.A.) converted the amount of food really ingested to the RAW equivalent, when necessary, using appropriate tables (Istituto Superiore Nazionale della Nutrizione, 1989). Nutritional analysis allowed to calculate actual ingested calories and macro/micronutrients. Nutritional analysis was carried out by using a computer program set up by our group (Aquilani et al., 1999).

2.4. Co-morbidities

Associated disease(s) with the primary disease was analyzed using the Charlson index (Charlson et al., 1987).

Table 1
Nutritional composition of an individual packet containing 4 g of an amino acid mixture used in this study.

Kinectic parameters and amino acids	Quantity
K_{cal}	35.3
K_j	149.9
Total aminoacids of which (in mg)	4 g
L-Leucine	1250
L-Lysine	650
L-Isoleucine	625
L-Valine	625
L-Threonine	350
L-Cysteine	150
L-Histidine	150
L-Phenylalanine	100
L-Methionine	50
L-Tyrosine	30
L-Tryptophan	20

2.5. Patient randomization

After completing all these procedures, the patients were assigned to treatment according to a randomized allocation procedure. A randomization list was generated using SAS statistical software, A and B were the identifiers of the blinded treatment. The list was made available to both physicians (G.Z. and F.A.) and the hospital pharmacist. The physicians sequentially allocated patients to treatment A or B according to a randomization list. The first author (R.A.) who interpreted all results, was blinded to the patient allocation. The experimental group (EAA group) received an oral nutritional mixture supplement which provided 8 g of EAAs/day (Aminotrophic, Professional Dietetics, Milan, Italy; see Table 1; 4 g in the morning + 4 g in afternoon diluted in half a glass of water) for 30 days. The placebo group (control-group) was given a similar isocaloric product containing maltodextrine instead of EAAs. GIRC nurses assisted every patient during the intake of placebo or EAAs in order to be certain about patient compliance.

The duration of the study was30 days from the randomization procedure. After randomization, patients were surveyed daily by GIRC nurses for evidence of infection. Diagnosis of infection was set by physician on the basis of described minima criteria for initiating antibiotics in residents of long-term care facilities (Loeb et al., 2001).

2.6. Statistical analysis

2.6.1. Sample size estimate

The sample size estimate was based on the expected reduction in infection occurrence in supplemented patients compared with the control group. Based on previous experience we knew that infections occur in 85% of non-supplemented patients and a reduction of 25% was considered clinically significant. Starting from these hypotheses and assuming a type I error of 5% and a type II error of 20% (power = 80%), the sample needed was of 40 patients per group.

2.6.2. Data analysis

Descriptive statistics were performed for all recorded variables and mean ± S.D. are presented. χ^2-test was applied to test differences in infection occurrence, primary disease and co-morbidities between supplemented and non-supplemented patients. An unpaired *t*-test was used to compare the differences in continuous variables between groups (supplemented vs. non-supplemented patients; patients with vs. those without infections).

Logistic regression analysis was applied to assess the relation between infections and supposed risk factors (demographic-,

Table 2
Demographic, anthrophometric, clinical, nutritional variables and co-morbidity index, in the placebo and EAAs groups after randomization, *n* or mean ± S.D.

Variables	Normal value	Placebo group	%RDA	EAA group	%RDA	$p <$
Demographic						
Male/Female	–	13/27		14/26	–	0.2
Age (years)	–	82.13 ± 6.15	–	79.5 ± 7.71	–	0.10
Anthropometric						
BW (kg)	–	61.95 ± 15.39	–	61.12 ± 16.41	–	0.82
BMI (kg/m^2)	–	25.84 ± 7.93	–	24 ± 5.68	–	0.36
Co-morbidity index (scores)	–	1.65 ± 1.2	–	2.07 ± 1.6	–	0.8
Blood white cells ($n \times 10^9$/l)	4–9	6.4 ± 1.56	–	6.5 ± 2.4	–	0.3
Hb (g/dl) $F > 12$, $M > 13$		11.65 ± 1.61	–	11.45 ± 1.65	–	0.58
Glycated Hb (%)	≤6	5.92 ± 1.14	–	6.06 ± 1.44	–	0.67
CRP (mg/dl)	<0.8	2.4 ± 3.51	–	1.2 ± 2.6	–	0.55
Fibrinogen (mg/dl)	230–500	415.1 ± 128.59	–	400 ± 129.1	–	0.62
Albumin (g/dl)	3.5–4.8	3.46 ± 0.40	–	3.47 ± 0.52	–	0.94
Prealbumin (mg/dl)	18–38	16.31 ± 5.46	–	14.69 ± 5.41	–	0.20
Lymphocytes (n/mm^3)	≥1500	1645 ± 617.66	–	1630 ± 953.32		0.93
Creatinine (mg/dl)	0.5–0.9	1.06 ± 0.433	–	0.9198 ± 0.48		0.17
Urea nitrogen (mg/dl)	4.67–23.3	22.9 ± 8.9	–	21.8 ± 14.3	–	0.70
Daily nutritional intake						
Energy						
K_{cal}		1331.6 ± 304.3	–	1291.44 ± 305.96	–	–
K_{cal}/kg	≥25	21.49 ± 5.37	86	21.12 ± 5.28	84	0.59
Proteins						
g		56.3 ± 14.11	–	54.46 ± 14.18	–	–
g/kg	≥1.1	0.90 ± 0.22	82	0.89 ± 0.22	81	0.61
Lipids						
g	–	58.58 ± 15.83		53.80 ± 16.41		
g/kg	≤1	0.94 ± 0.23	94	0.88 ± 0.22	88	0.22
Carbohydrates						
g		157.8 ± 43.29	–	56.21 ± 44.83	–	–
g/kg	2.5–4	2.54 ± 0.63	100	2.55 ± 0.63	100	0.88
Fiber (g)	30	9.52 ± 4.20	32	9.50 ± 3.35	31.6	0.99
Calcium (mg)	1000	581.96 ± 336.82	58	545.16 ± 319.19	54	0.64
Phosphorus (mg)	1000	834.67 ± 234.39	83	821.33 ± 256.66	82	0.83
Potassium (mg)	3100	1511.6 ± 465.72	49	1404.74 ± 337.67	45	0.28
Sodium (mg)	nd	693.35 ± 495.65	–	668.55 ± 516.58	–	0.84
Iron (mg)	10	5.67 ± 1.75	57	5.58 ± 1.83	56	0.84
Zinc (mg)	10	5.77 ± 2.30	58	5.47 ± 2.70	55	0.64
Copper (mg)	1.2	0.70 ± 0.26	58	0.71 ± 0.18	60	0.93
Vit. B_1 (mg)	0.8	0.52 ± 0.21	65	0.51 ± 0.21	64	0.93
Riboflavin (mg)	1.6	1 ± 0.29	63	1.11 ± 1.05	69	0.57
Niacin (mg)	18	8.88 ± 3.36	49	8.22 ± 3.11	46	0.42
Vit. A (μg)	700	305.94 ± 174.83	44	287.83 ± 158.16	41	0.67
Vit. C (mg)	60	19.01 ± 7.9	32	19.18 ± 14.34	32	0.95
H_2O (ml) (water content in food)	–	593.12 ± 195.70	–	534.20 ± 142.18	–	0.18
Devices (urinary catheter)	–	12/40	–	10/40	–	0.7

Notes: Statistical analysis: unpaired *t*-test and χ^2-test when the case; RDA = recommended daily allowance; nd = not defined.

Table 3
Distribution of disease in the patients after randomization.

	Placebo group		EAA supplemented group		$p =$
	n/*n*	%	*n*/*n*	%	
Major trauma-orthopedic surgery	24/40	60	27/40	67.5	ns
Neurocognitive degeneration	12/40	30	10/40	25	ns
Stroke	12/40	30	9/40	22.5	ns
Atrial fibrillation	11/40	27.5	8/40	20	ns
Arterial hypertension	5/40	25	7/40	17.5	ns
Diabetes	7/40	17.5	10/40	25	ns
CAD	7/40	17.5	6/40	15	ns
COPD	6/40	15	7/40	17.5	ns
Invalidating polyarthropaties	6/40	15	7/40	17.5	ns
CHF	3/40	7.5	3/40	7.5	ns
Cachexia	3/40	7.5	4/40	10	ns
CABG	2/40	5	1/40	2.5	ns
Acute/chronic renal failure	2/40	5	2/40	5	ns
Rheumatoid arthritis	2/40	5	1/40	2.5	ns
Postanoxic coma	1/40	2.5	–	–	–
Depression	1/40	2.5	3/40	7.5	ns
Peripheral neuropathy	1/40	2.5	1/40	2.5	ns
Abdominal surgery	–	–	2/40	5	–

Notes: Statistical comparisons of groups: χ^2-test: CAD = coronary artery disease; COPD = chronic obstructive pulmonary disease; CHF = chronic heart failure; CABG = coronary artery bypass graft.

Table 4
Proportion and types of GIRC-acquired infections in study population.

	Placebo group	%	EAA group	%	$p=$
Wound	2/33	6	–	–	–
Lower respiratory tract	12/33	36.4	6/21	28.5	ns
Urinary tract	19/33	57.6	14/21	66.7	ns
Gastrointestinal tract	–	–	1/21	4.8	–

Notes: Statistical comparisons by the χ^2-test.

anthropometric-, nutritional-, and bio-humoral variables). A model including independent dichotomous variables was tested and odd ratios for high risk categories and 95% confidence intervals were estimated. A stepwise procedure was applied to identify variables with the highest association with infection occurrence. All analyses were performed using SPSS statistical software.

3. Results

The study found that supplemented and placebo patients were similar in their demographic-, anthropometric-, bio-humoral-, nutritional-, clinical-characteristics as well as in their co-morbidity index (Tables 2 and 3). Table 2 shows that the two patient subgroups had normal body weight but marginal intakes in protein, energy, and profound reductions in nearly all micronutrients (Istituto Superiore Nazionale della Nutrizione, 1989). Moreover, increased body inflammation process (CRP), reduced protein synthesis of non-reactant proteins (albumin and prealbumin) were found in the two subgroups. In addition, the two subgroups were anemic.

No EAA patient complained for amino acid solution. No patient on placebo group nor in EAA one dropped out of the study.

Considering all patients as an entire group, the study showed that the infection rate was 67.5% (54/80 patients) over the first month of hospital stay. When the two subgroups were analyzed separately, the infection rate was 30% lower in the EAA group (52.5%; 21/40 patients) compared to the placebo one (82.5%; 33/40 patients) ($p < 0.02$). The distribution of infection types was similar between placebo and EAA groups (Table 4).

Infection timing was 7–10 days after randomization in 57.4% of patients, 12–18 days in 33.3%, from the third week in 9.3% patients.

Amoxicillin + clavulanic acid was used in 44.4% patients, laevofloxacin in 50%, i.v. cephalosporins in 3.7%, i.v. vancomycin in 1.8%. No significant differences were found between the two groups for the type of antibiotic being used. The number of days on antibiotics was higher in placebo that in EAA infected patients (298 ± 15 vs.150 ± 10 days; $p < 0.001$).

Table 5
Changes of the variables, and CMI in the patients who developed or not infections independently of their allocation to placebo or EAAs intervention, n or mean $\pm$ S.D.

Variables value	Normal	IG	%RDA	WIG	%RDA	$p<$
Demographic						
Male/Female	–	19/35	–	7/19	–	0.30
Age (years)	–	82.06 ± 5.63	–	77.92 ± 9.06	–	0.047
Anthropometric						
BW (kg)	–	61.78 ± 14.71	–	61.03 ± 18.20	–	0.84
BMI (kg/m^2)	–	24.01 ± 5.9	–	26.05 ± 7.88	–	0.32
Co-morbidity index median(range)	–	2 (0.6)	–	1 (0.7)	–	0.35
Blood						
Blood white cells ($n \times 10^9$/l)	4–9	8.8 ± 3.1	–	7.2 ± 2.3	–	<0.02
Hb (g/dl)	$F > 12$; $M > 13$	11.23 ± 1.6	–	12.24 ± 1.52	–	0.01
Glycated Hb (%)	≤6	5.91 ± 1.3	–	6.14 ± 1.28		0.49
CRP (mg/dl)	<0.8	28.66 ± 34.64	–	8.14 ± 12.33	–	<0.001
Fibrinogen (mg/dl)	230–500	417.02 ± 128.76	–	390.54 ± 127.86	–	0.41
Albumin (g/dl)	200–400	3.34 ± 0.47	–	3.69 ± 0.33	–	<0.001
Prealbumin (mg/dl)	18–38	14.45 ± 5.71	–	17.38 ± 4.43	–	<0.026
Lymphocytes (n/mm^3)	≥1500	1600 ± 657.03	–	1715.4 ± 1043.3	–	0.55
Creatinine (mg/dl)	0.5–0.9	1.07 ± 0.50	–	0.82 ± 0.31	–	= 0.009
Urea nitrogen (mg/dl)	4.67–23.3	23.8 ± 13.5	–	19.2 ± 6.9	–	0.048
Daily nutritional intake						
Energy						
K_{cal}	–	1265.8 ± 315.7	–	1421.3 ± 245.5		0.05
K_{cal}/kg	≥ 25	21 ± 5.5	84	23.3 ± 6	93	
Proteins						
g	–	53.01 ± 15.32		60.94 ± 8.39	–	0.008
g/kg	≥1.1	0.86 ± 0.25	78	0.99 ± 0.29	90	
Lipids						
g	–	54.85 ± 16.36	–	59.33 ± 153.70	–	0.30
g/kg	≤ 1	0.88 ± 0.21	88	0.97 ± 0.25	97	
Carbohydrates						
g	–	150.92 ± 44.12	–	171.60 ± 40.17	–	0.07
	2.5–4	2.5 ± 0.6	100	2.80 ± 0.7	100	
Fiber (g)	20–30	8.94 ± 3.84	45	10.89 ± 3.28	54	0.05
Calcium (mg)	1000	545.65 ± 330.54	55	606.55 ± 319.65	61	0.49
Phosphorus (mg)	1000	793.83 ± 240	79	912.53 ± 239	91	0.07
Potassium (mg)	3100	1418.21 ± 423.82	46	1554.05 ± 356.25	50	0.21
Sodium (mg)	nd	655.69 ± 502.35	–	741.58 ± 510.85	–	0.52
Iron (mg)	10	5.65 ± 1.88	57	5.57 ± 1.6	56	0.87
Zinc (mg)	10	5.47 ± 2.23	55	5.97 ± 2.99	60	0.46
Copper (mg)	1.2	0.68 ± 0.22	57	0.76 ± 0.22	63	0.24
Vit. B_1 (mg)	0.8	0.50 ± 0.23	62	0.54 ± 0.16	67	0.52
Riboflavin (mg)	1.6	1.06 ± 0.89	66	1.036 ± 0.28	65	0.9
Niacin (mg)	18	8.15 ± 3.46	45	9.47 ± 2.52	53	0.13
Vit. A (μg)	700	275.95 ± 170.84	39	343.25 ± 148.85	49	0.13
Vit. C (mg)	60	19.24 ± 12.8	32	18.76 ± 7.55	31	0.88
H_2O (ml) (water content in food)	–	539.21 ± 173.89	–	620.65 ± 163.15	–	0.08

Notes: Statistical analysis: unpaired t-test and χ^2-test when the case.

A stratification of all patients in the two subgroups, those who developed infection and those who did not, independently of randomization, allowed us to see (Table 5) that patients who developed infection compared to free-infection subgroup, were significantly older and more anemic and had a higher inflammation process, reduced serum concentration of non-reactant proteins, increased blood urea nitrogen and serum creatine levels, and lower energy and protein intakes.

The stepwise procedure identified, the following potential risk factors for infection; CRP and Hb levels are statistically associated with an increased risk for future infection. Indeed, patients with CRP $>$ 0.8 mg/dl had a risk for infection 4.1 times higher than patients with CRP $\leq$ 0.8 mg/dl (95%CI = 1.42–12.1, $p = 0.009$). Blood Hb concentration $<$13 g/dl in males and $<$12 g/dl in females was a risk 3.6 times higher for future infection (95%CI = 1.22–10.66, $p = 0.02$).

4. Discussion

Our study showed that oral supplementation of EAAs could reduce the incidence of GIRC-acquired infection by 30% and indicates that serum CRP level $>$0.8 mg/dl and blood Hb concentration $<$12 g/dl in females, $<$13 g/dl in males may be risk factors of infection.

4.1. EAAs and infection

The high incidence rate of infection found in the placebo group notwithstanding the adoption of preventive measures suggested the co-presence of post acute metabolic, nutritional sequelae and low immunological defense favoring the development of infection.

Given the activity of amino acids on both lymphocyte proliferation and monocyte/macrophage phagocytosis (Roth, 2007), decreased infection occurrence in supplemented patients could suggest an EAA-mediated improvement of this immunological alteration. Indeed, amino acids modulate immuno-function by boosting protein synthesis (Fafournoux et al., 2000), an essential biochemical process for proliferation and phagocyte activity of immune cells (Lesourd, 1995; Chandra, 2002).

Interestingly, the formula used here did not contain glutamine, the most important amino acid for immune function (Roth, 2008). The discrepancy between the positive effect of the formula used in the study and the absence of glutamine in it can easily be reconciled by considering that the principal amino acid in the formula, leucine, is a potent stimulator of glutamine formation (Ruderman, 1975). In our laboratory we observed (unpublished data) that 8 g/day of EAAs by elderly healthy subjects increased baseline plasma glutamine levels by 10% which lasted 3 h without impairing arginine concentration, another amino acid essential for immuno-function (Ochoa et al., 2001; Roth, 2007).

Furthermore, beyond to its glutamine-mediator effect, leucine can exert per se direct action on immune cell functions, as it is the most important amino acid for protein synthesis (Lynch et al., 2002; Rennie et al., 2006). In addition to leucine, other amino acids in the formula including cysteine and methionine, probably contributed to reduce the development of infection. Cysteine, modulates glutathione metabolism, and is indispensable for maintaining the redox potential of the cell (Dröge, 2002). Methionine (as well as cysteine) is a methylating amino acid essential for nucleic bases synthesis (Bradshaw et al., 1998).

4.2. Risk factors of infection development

Our study showed that in patients admitted to our GIRC, serum CRP and to a lesser extent, blood Hb were risk factors for future infection. High CRP, an acute-phase protein, reflects inflammation and indicates increased interleukine-6 (Krabbe et al., 2004) production and/or tumor necrosis factors α, two pro-inflammatory cytokines produced by pathogen-activated immuno-cells. We therefore showed that a large portion of patients had post acute persistent inflammatory status and we cannot totally exclude that in some of these, high CRP could be due to current incubating pathogens.

The reduced Hb can also be ascribed to a number of factors including the primary disease and co-morbidities, surgery blood loss, pre-surgery Hb levels, peri-post operative inadequate nutrition. Low Hb leads to impaired immunological capacity given that adequate oxygen availability for antigen-activated lymphocyte and monocyte metabolism is essential, both for protein synthesis linked to cell proliferation (lymphocytes) and to produce peptide messengers, interleukins, complement components and other proteins involved in inflammation/infection (monocyte/macrophages) (Roth, 2008).

Although not significant as risk factors in our statistical model, age and low protein-, calorie-, micronutrient intake should not be underestimated as factors favoring infection as they have been shown to affect immunological function (Prasad et al., 1993; Ahluwalia et al., 2004; Bunout et al., 2004). Inflammation may explain higher blood urea and serum creatinine concentrations in patients with infections as well as lower visceral protein status (serum albumin, prealbumin), whose reduction is magnified by reduced protein energy intake (Doweiko and Nompleggi, 1991; Aquilani et al., 2009).

5. Conclusions

This study shows that EAAs have the potential to substantially reduce GIRC-acquired infection in compromised post acute elderly patients, acting synergistically with other prevention measures.

5.1. Clinical considerations

This study suggests certain factors which could be useful for clinical practice to prevent GIRC-acquired infection as much as possible. Firstly, by determining serum CRP levels or, in its absence, considering blood Hb concentration, we can identify patients at high risk for future infection. Secondly, at least in these patients, nutritional intake should be surveyed and in particularly proteins, calories and micronutrients. The ingestion of adequate proteins with high biological value is particularly important in elderly patients (mainly, beef meat, poultry, eggs, fish) as they provide EAAs and micronutrients including iron and zinc relevant for immunocompetent (Prasad et al., 1993; Ahluwalia et al., 2004). In case of inadequate intake of proteins with high biological value for whatever clinical reason(s), EAAs should be supplemented independently of any infection prevention. Indeed, with 8 g/day of EAAs, as used in this study, patients are provided with the same amount of leucine (2.5 g) ingested with 160 g of lean beef meat.

5.2. Financial considerations

The study indicates that EAAs could reduce the consumption of antibiotics; appropriate pharmaco economics study will be necessary to deal with this important aspect relevant also to reduce antibiotic resistance. These considerations highlight the importance to perform larger trials with EAAs to confirm the study findings.

5.3. Limitations

We are aware that this study has several limitations. The population sample was relatively small, so there is a need to

investigate a larger number of patients in order to draw definite clinical conclusions. However, it should be kept in mind that the efficacy of EAAs observed here, relies on the scientific evidence of amino acid activity on immune cell function. Moreover, for clinical purposes, EAAs supplementation, independent of their action on the immune system, is necessary to reduce catabolism or ensure protein synthesis. We have chosen as placebo the maltodextrin instead of a mixture of non-EAAs for two reasons. First, since amino acids, are not used in clinical practice for lowering infection rate, we wanted to document whether amino acids, simple natural nutrients, be them EAAs or non-EAAs, could represent an added value to standard prevention protocols for controlling infection incidence rate. Second, choosing EAAs instead of non-EAAs was a more correct pathophysiological mechanism for complicated patients (see Section 1) in order to boost protein synthesis. Indeed for this aim, physiologically, EAAs can be anticipated to be promoter of protein synthesis more than non-EAAs. It is interesting to note, as ancillary finding, that in order to reduce infection, EAAs were superior to a polysaccharide (maltodextrine), a nutrient having potential immunomodulating capacity.

Adequate investigations are necessary to document the effect of EAAs on the prevention of specific infections such as urinary or respiratory or gastrointestinal or skin tracts. This implies also a need for documenting direct effects of EAAs on several immune cell functions. This is a particularly important point considering that more than 57% infection occurred in the first 10 days after randomization.

The highest infection incidence in GIRC calls for an interaction between GIRC and acute wards in order to reduce as much as possible patient metabolic, nutritional, immunological alterations since the first hours of acute event. The study was scheduled to document the incidence rate of infection over the first 30 days of admission to GIRC. Since the long of stay, in GIRC, can be extended to 90 days after admission, a future study address the incidence of infection and its control beyond the first month after admission.

Conflict of interest statement

None.

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