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ORIGINAL ARTICLE

Is stroke rehabilitation a metabolic problem?

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Abstract

Background: This study looks at the impact of inflammation during the rehabilitation stage of strokes and its effect on neuro-functional recovery.
Methods: This study investigated 94 patients suffering from strokes and admitted to rehabilitation. Anthropometric characteristics, serum proteins and inflammatory markers, plasma amino acids and neurofunction were all assessed.
Results: 55.3% patients had an inflammatory status (Interleukin-6 = $19.24 \pm 23.01\,\mathrm{pg\,ml^{-1}}$ vs. $4.1 \pm 1.6\,\mathrm{pg\,ml^{-1}}$ for non-inflamed subjects ($p < 0.001$). Inflammation was positively linked to positive proteins (alpha-1 globulin, $p < 0.02$) and negatively linked to negative proteins (albumin, $p < 0.02$; prealbumin, $p < 0.01$; transferrin, $p < 0.05$) of the acute-phase response. Inflammation was associated with low plasma concentrations of total amino acids. For the multiple logistic regression analysis, albumin ($p < 0.001$) and body weight maintenance ($p < 0.001$) were independent predictors of patient functional independence. Inflammation in dysphagic stroke (31.9%) patients was associated with more accentuated disability compared to non-inflamed dysphagics. The serum positive reactant alpha 1 globulin was the most powerful predictor of dysphagia severity ($p < 0.001$). At discharge, dysphagia improvement was associated with improved acute-phase negative proteins.
Conclusions: An inflammatory status may persist for most patients with strokes during the rehabiliation stage of the disease, its prevalence being higher in dysphagic compared to non-dysphagic subjects. The improvement in circulating albumin and body weight maintenance are predictors of neuro-function, even in dysphagic subjects.

Keywords

Inflammation, neurofunction, protein status

History

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Introduction

Strokes have metabolic consequences that are partially mediated by inflammation. Acute strokes (ischaemic or haemorrhagic) cause an inflammatory reaction that is mediated by cytokines [1–4]. Increases of pro-inflammatory tumour necrosis factor alpha (TNFα), interleukin-1 (IL-1) and interleukin-6 (IL-6) have been detected in the ischaemic cortex 1 hour after a mid-cerebral artery occlusion in an experimental model of brain ischaemia [1]. Results from both animal and human studies have found that pro-inflammatory cytokines increase brain injury. Indeed in rats, intra-ventricular injections of IL-1 and TNFα enlarged infarct volume and brain oedema after middle cerebral artery occlusions, whereas antibodies against IL-1 and TNFα injected into the animals reduced brain injury [5, 6]. In humans, a study reported that serum IL-6 [7] and TNFα levels were significantly higher in ischaemic stroke patients who had suffered clinical deterioration 48 hours after the acute event. Finally, a highly significant correlation was found between plasma and cerebrospinal fluid concentrations of IL-6 [7].

Importantly, increases of IL-6 predict a deterioration of the general clinical situation independent of the topography, initial size or mechanism of the infarction [7]. These findings were confirmed by another study showing that, in ischaemic stroke subjects [8], increased serum levels of C-reactive protein (CRP) were associated with unfavourable outcomes and initial lesion volume. It should be noted that this is an IL-6 induced-major marker of inflammation and was measured 24–48 hours after the onset of the symptoms.

Inflammation in subjects with sub-arachnoid and cerebral haemorrhage further impairs the anatomical lesion and deteriorates the clinical situation as in cerebral ischaemia. In individuals with aneurismal subarachnoid haemorrhages, the development of a systemic inflammatory response and extra-cerebral organ system failure were associated with a significant

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increase in the serum of the soluble TNFα receptor-I and IL-1_{RA} (although not in the cerebrospinal fluid) [2].

Plasma inflammatory markers (TNFα, IL-6) in subjects with intracerebral haemorrhage were associated with subsequent enlargement of the haematoma [9]. Similar to ischaemic strokes, inflammation predicted early neurological deterioration [10], as found by increased serum fibrinogen in patients with intra-cerebral haemorrhage.

All these studies confirm that central nervous system injury causes a cytokine shift across the disrupted blood–brain barrier (BBB) [11], priming a systemic inflammatory response. Systemic mediators released by peripheral immune endothelial or parenchimal cells can, in turn, reduce the integrity of BBB, creating a bi-directional communication of inflammatory mediators [12–14]. Thus, the brain acts both as an effector and a target organ [2].

Based on these studies, three hypotheses have been formulated. First, in stroke patients, an inflammatory state may persist in the rehabilitation stage of the disease either as a residual condition of the acute event and/or a consequence of post-acute complications. Second, inflammation may be prevalent in dysphagic subjects compared to non-dysphagic ones, given that dysphagia is a major risk factor for developing infection and in particular pneumonia [15]. Third, the inflammation-induced shift of hepatic protein synthesis [16] could help in the retrieval of a patient's functional ability and deglutition capacity. This last hypothesis relies on several studies which indicated that albumin, a negative protein of acute phase response, plays a role in neuro-protection [17–19]. Furthermore, both albumin and transferrin, another negative reactant, are associated with the functional status of brain injured patients [20]. On the contrary, the positive reactants of acute phase response such as CRP, haptoglobin and α-1 globulin system could negatively impact patient neuro-rehabilitation.

Methods

Population

One hundred and eight stroke patients consecutively admitted to the rehabilitation unit were enrolled within 90 days of their acute event (37.7 ± 27 days, median $= 28$ days). These patients came from the following origins: stroke units (12.8%), neurological settings (13.8%), general intensive care units (11.7%), homes (56.4%) and neurosurgery (5.3%). Fourteen patients were excluded for having nephrotic syndrome ($n = 1$), cancer ($n = 2$), oedema ($n = 4$) and steroid therapy ($n = 7$), as these events would impact the reactants of the acute-phase response. Thus, a total of 94 patients were studied.

Vascular brain insult documented by computerized tomography was ischaemic in 75/94 patients (79.8%) and there was haemorrhagic injury in 19/94 patients (20.2%). On the basis of computerized tomography or magnetic resonance imaging, the damaged stroke areas were classified in relation to the location of the ischaemic obstruction as PACI (partial anterior circulation infarction), TACI (total anterior circulation infarction), POCI (posterior anterior circulation infarction) or LACI (lacunar infarction). The study was approved by the ethical-technical scientific committee of the institute. Written informed consent was obtained from participants or whenever relevant from their caregivers, after the nature of the study had been fully explained.

Procedures

Within the first week of admission and before discharge from rehabilitation (45 ± 7 days after admission), the following variables were measured:

(a) Anthropometric characteristics: body weight (BW, kg), found using a mechanical weight lifter; height (m), calculated from knee height [21]. Body mass index (BMI) was calculated as $kg\,m^{-2}$. Patients (or their caregivers) were asked for their pre-acute BW. Loss of actual BW in relation to habitual (pre-acute) BW $> 5\%$ (i.e. actual/habitual BW $< 95\%$) was considered an index of significant under-nutrition [22].

(b) Bio-humoral measurements:
   (1) routine variables, including serum protein electrophoresis,
   (2) biomarkers of body inflammatory status:
      - serum levels of interleukine-6 (IL-6; normal value $<7\,pg\,ml^{-1}$), determined in duplicate using a high-sensitivity commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit from Mabtech (Agilent Technologies GmbH, Boblingen, Germany);
      - C-reactive protein (CRP; normal value $<0.3\,mg\,dl^{-1}$), determined by an immune-turbidimetric method;
      - acute-phase reactant proteins (haptoglobin, normal values $30\,200\,mg\,dl^{-1}$; α-1 globulin system, normal value $0.21–0.35\,g\,dl^{-1}$; non-reactant proteins (albumin, normal values $4.02–4.76\,g\,dl^{-1}$; prealbumin, normal values $18–30\,mg\,dl^{-1}$ and transferrin, normal values $202–364\,mg\,dl^{-1}$).
   (3) plasma amino acids. The concentrations of free amino acids in the plasma were measured using an AminoQuant II amino acid analyser, based on the HP 1090 HPLC system, with fully automated pre-column derivatization, using both ortho-phthalaldehyde and 9-fluorenyl-methyl-chloroformate reaction chemistries according to the manufacturer's protocol. Results were made by essentially injecting 1 µL of the derivatized mixture and measuring absorbance simultaneously at 338 and 262 nm. Plasma concentrations were expressed as $\mu mol\,L^{-1}$. Amino acids were measured as they are influenced by an inflammatory state [16] and in turn influence cerebral amino acid/protein metabolism [17].

(c) Functional status: evaluated using the functional independence measure (FIM) as elsewhere reported [20].

(d) Dysphagia. Identification of dysphagia was carried out clinically for the entire population. In case of positive or uncertain diagnosis, the patients underwent a video fluoroscopy examination. The severity of the dysphagia was evaluated using the Dysphagia Outcome and Severity Scale (DOSS), a 7-point scale developed to

systematically rate the functional severity of dysphagia [23]. The score range was 1–7, where level 1 denotes severe dysphagia, level 2 moderately severe dysphagia, level 3 moderate dysphagia, level 4 mild-to-moderate dysphagia, level 5 mild dysphagia, level 6 within functional limit/modified independence and level 7 normal in all situations.

(e) Nutritional intake: for self-feeding patients (84%) (with modified diet when necessary) a 3-day alimentary diary was kept by the rehabilitation nurses, who had been previously trained *ad hoc*. The nurses recorded the type and weight of cooked or uncooked food selected by patients from the hospital catering menu on a diet sheet for 3 days before and after the patients' meals. The amount of food actually ingested was converted (by R.A. of the article) to the raw equivalent when necessary, using appropriate tables [24]. Nutritional analysis, carried out using a computer program designed by this group [25], was used to calculate actual ingested calories and macro-/micro-nutrients.

Statistical analysis

This is an observational exploratory study; hence no formal sample size calculation was performed. Patients meeting inclusion/exclusion criteria were consecutively enrolled from 28 January 2009 to 28 January 2011.

Descriptive statistics were carried out for all recorded variables, reporting means and standard deviations for quantitative variables and distribution frequencies for qualitative variables. Median values are also reported for any not normally distributed variables. Chi-squared test was used for categorical variables.

Repeated measurement analysis of variance was used to assess any differences in trends over time between patients with or without inflammation and between patients with or without dysphagia. Baseline differences between groups (presence/absence of inflammation; presence/absence of dysphagia) were tested by means of an unpaired Student *t*-test.

FIM and DOSS measures were tested for their correlation with anthropometric and biohumoral variables. Pearson correlation coefficients were estimated at both admission to and discharge from the rehabilitation ward. Linear multiple regression analyses were performed in order to point out the variables with high association with FIM and DOSS. The level of statistical significance was set at $p<0.05$.

Results

Fourteen patients were excluded from the analysis for technical reasons and their IL-6 levels were not measured. The analysis of results was therefore carried out on 94 patients. The results showed that, except for age, which was lower in haemorrhagic (58.2 ± 11.7 year) compared to ischaemic subjects (75 ± 14 years) ($p<0.01$), there were no important differences between the two populations for anthropometric-, clinical-, biohumoral-characteristics or functional disability. The variables of the groups were therefore pooled together (Table I) for all subsequent analyses.

Inflammation

Table II shows the anthropometric-, biohumoral-, amino acid profiles and functional independence measures in subjects with inflammation (inflammation) or without inflammation (non-inflammation) both at admission to and discharge from rehabilitation.

At admission to rehabilitation

An inflammatory status was found in 55.3% (52) of 94 patients. IL-6 was $19.24\pm23.01\ \mathrm{pg\,ml^{-1}}$ in inflamed subjects and 4.1 ± 1.6 in non-inflamed ones ($p<0.001$). Significant differences were found in both functional disability and biohumoral variables between the two groups, Patients with inflammation had more severe functional disability compared to non-inflamed subjects, with ~19.2 lower FIM points (56.1 ± 26 vs. 75.3 ± 23.6 scores, $p<0.01$), notwithstanding the fact that the topography of ischaemic lesions was similar.

For the bio-humoral variables, inflammation was associated with significantly higher serum levels of alpha 1 globulin ($p<0.02$), haptoglobin ($p<0.05$), circulating neutrophil ($p<0.01$) percentage and Erythrocyte Sedimentation Rate (ESR) ($p<0.05$), but lower levels of albumin ($p<0.02$), prealbumin ($p<0.01$), transferrin ($p<0.05$), blood Hb content ($p<0.01$), plasma concentrations of histidine ($p<0.02$) and total amino acids ($p<0.05$). Anthropometric variables were similar for both groups of patients.

There was also no significant correlation between functional disability and the inflammation IL-6 marker, although a significant relation was found between functional disability and the liver shift of protein synthesis. Indeed, FIM correlated negatively with positive reactants and positively with the negative reactants of acute phase response (Table III, Figure 1). Moreover, FIM was positively associated with body weight maintenance (actual/pre-event BW > 95%), blood Hb, plasma levels of tryptophan and histidine. IL-6 levels were in inverse relation to serum albumin ($r=-0.52$, $p<0.001$). No significant relation was found between IL-6 and body weight conservation or between CRP and body weight conservation.

For predictors of patient function, only serum albumin ($r=+0.535$, $p<0.001$) and BW maintenance (actual/pre-event BW >95%, $r=+0.489$, $p<0.001$) were independent predictors of patient functional independence (Table III) at the multiple logistic regression analysis.

The rehabilitation stage of the disease

The time-courses of the variables considered were similar between non-inflamed and inflamed individuals, except for inflammation and circulating total leukocyte count indicators and neutrophil and lymphocyte percentages which improved for inflamed subjects (Table II). Inflamed subjects significantly increased their amino acid threonine levels ($p=0.03$).

During rehabilitation, serum IL-6 and albumin maintained their inverse relation. However, the slope of this relation was considerably lower than that found at admission ($r=-0.28$, $p<0.007$). The gain in FIM averaged 17.8 points for inflamed strokes and 15.6 points in non-inflamed ones (ns).

Table I. Demographic, clinical, anthropometric, biohumoral and nutritional variables and devices in the stroke population at admission to the rehabilitation ward.

Variables	Stroke population ($n = 94$)	Normal values	%RDA
Demographic			
Male/Female	57/37	–	–
Age (years)	67 ± 15	–	–
Clinical			
Aetiology			
Ischaemic	61 (64.9%)	–	–
Haemorrhagic	33 (35.1%)	–	–
Ischemic stroke location		–	–
LACI	30/61 (49.2%)		
PACI	3/61 (4.92%)		
POCI	3/61 (4.9%)		
TACI	25/61 (41%)		
Anthropometric			
Actual body weight (kg)	71.5 ± 15	–	–
Body mass index (BMI) (kg/m^2)	25.6 ± 4.4		
Pre-event body weight (kg)	74.4 ± 15.6		
Actual/pre-event BW (%)	97.6 ± 7.2	–	–
Blood			
ESR 1st hour (mm)	31.42 ± 26.26	<20	–
Haemoglobin (g dl^{-1})	13.2 ± 1.9	12–15	–
Blood urea (mg dl^{-1})	40.8 ± 21	20–40	–
Serum creatinine (mg dl^{-1})	0.99 ± 0.28	0.7–1.2	–
Plasma glucose (mg dl^{-1})	110 ± 37.7	80–110	–
α_1 globulin (g dl^{-1})	0.21 ± 0.04	0.21–0.35	
Serum albumin (g dl^{-1})	3.39 ± 0.52	4.02–4.76	–
Serum prealbumin (mg dl^{-1})	21.6 ± 5.6	18–30	–
Serum haptoglobin (mg dl^{-1})	179 ± 100	30–200	
Interleukin-6 (pg ml^{-1})	12.5 ± 18.68 (median 7.78)	<7	–
Serum transferrin (mg dl^{-1})	209.51 ± 49	202–364	
Serum C-reactive protein (CRP) (mg dl^{-1})	1.04 ± 1.74 (median 0.75)	<0.3	–
Daily nutritional intake			
Energy			
Kcal	1781 ± 294	–	–
Kcal kg^{-1}	25 ± 4.5	≥25	100
Proteins			
g	69 ± 5.9	–	–
g kg^{-1}	0.97 ± 0.21	≥1	97
Lipids			
g	65 ± 6.7	–	–
g kg^{-1}	0.91 ± 0.2	≤1	92
Carbohydrates			
g	230 ± 72	140–180 g	127.7
g kg^{-1}	3.22 ± 0.81	2.5–4	–
Calcium (mg)	1125 ± 135	1000	112
Phosphorus (mg)	1190 ± 158	1000	112
Potassium (mg)	2951 ± 605	3100	95
Sodium (mg)	1595 ± 124	nd	–
Iron (mg)	10.8 ± 2.1	10	108
Zinc (mg)	8.8 ± 1.1	10	82
Copper (mg)	1.01 ± 0.4	1.2	84
Iodide (mcg)	110 ± 29	nd	–
Devices			
Tube feeding	12 (12.7%)		

Data are expressed as mean ± standard deviation (SD) or median, whenever appropriate.
LACI, lacunar infarction; PACI, partial anterior circulation infarction; POCI, posterior circulation infarction; TACI, total anterior circulation infarction; ESR, erythrocyte sedimentation rate; RDA, recommended daily allowance; nd, not defined.

At discharge from rehabilitation

The inflammation rate, although reduced, was still present in 59.6% (31/52) of the inflamed subjects. When compared to non-inflamed subjects, the inflamed ones were discharged with lower levels of haemoglobin ($p < 0.01$), prealbumin ($p < 0.01$), histidine ($p < 0.05$) and poorer functional status ($p < 0.03$) (Table II).

Compared to the relations between inflammation and functional independence (Table IV), FIM positively correlated with body weight and its conservation over time, blood Hb levels, serum concentrations of albumin, prealbumin, transferrin, plasma histidine-, tryptophan- and tyrosine concentrations. The correlation between FIM and albumin remained constant, even when the two variables

Table II. Anthropometric, biohumoral and amino acid profiles, functional independence measured in subjects without inflammation (non-inflammation) or with inflammation (inflammation) at admission and at discharge.

Variables	Inflammation ($n=52$)		No inflammation ($n=42$)		Trend over time (p level) interaction
	Admission	Discharge	Admission	Discharge	
Anthropometric					
Actual body weight (kg)	73.2 ± 17.2	69.8 ± 14.7	75.9 ± 12.5	74.6 ± 12.6	ns
Actual/pre-event BW (%)	96.3 ± 6.2	96 ± 6.4	99.2 ± 8.2	98.5 ± 7.7	ns
Blood					
ESR 1st hour (mm)	37.1 ± 28.7*	34.7 ± 24.5	21.2 ± 13.8	25.8 ± 18.1	ns
Haemoglobin (g dl^{-1})	12.6 ± 2.0***	12.5 ± 1.6^^^	13.8 ± 1.6	13.4 ± 1.32	ns
Blood urea (mg dl^{-1})	42.5 ± 25.4	37.8 ± 13.3	39.2 ± 14.2	38.3 ± 15.2	ns
Serum creatinine (mg dl^{-1})	1.03 ± 0.31	1.02 ± 0.3	0.94 ± 0.24	0.99 ± 0.27	ns
Plasma glucose (mg dl^{-1})	109 ± 33.4	97.5 ± 23	108.2 ± 38.3	95.9 ± 27	ns
Blood white cell count (n° mm^{-3})	6783 ± 2070	6026.6 ± 1827.5	6144 ± 1827	6462.7 ± 2685.2	0.008
of which:					
Neutrophils (%)	61.3 ± 10***	57.8 ± 9.1	57.5 ± 10.5	58 ± 10.5	0.012
Lymphocytes (%)	25.9 ± 9.2	29.4 ± 9.7	30.7 ± 10	30.2 ± 10.3	0.003
Monocytes (%)	8.8 ± 2.6	9.1 ± 2.5	8.7 ± 2.1	8.4 ± 2.5	ns
α_1 globulin (g dl^{-1})	0.22 ± 0.04**	0.21 ± 0.04	0.19 ± 0.029	0.20 ± 0.03	ns
Serum albumin (g dl^{-1})	3.2 ± 0.55**	3.39 ± 0.46	3.6 ± 0.4	3.6 ± 0.38	0.051
Serum prealbumin (mg dl^{-1})	19.3 ± 5.5***	20 ± 5.4^^^	24.7 ± 4.2	23.9 ± 4.5	ns
Serum haptoglobin (mg dl^{-1})	194.7 ± 115.3*	161 ± 81.7	158.6 ± 70.6	171 ± 66.5	0.001
Interleukin-6 (pg ml^{-1})	19.24 ± 23.01****	10.39 ± 7.8	4.1 ± 1.6	5.58 ± 3.41	ns
Serum transferrin (mg dl^{-1})	202.4 ± 46.1*	208.9 ± 46.3	218.7 ± 50.9	219 ± 39.1	ns
Serum C-reactive protein (CRP) (mg dl^{-1})	1.48 ± 2.2	0.85 ± 1.68	0.48 ± 0.53	0.68 ± 1.26	0.037
Amino acid profiles (μmol L^{-1})					
Aspartate	11.2 ± 2.4	12.1 ± 2.6	11.8 ± 1.9	12.3 ± 2.5	ns
Glutamate	70.7 ± 20.3	67 ± 21	73.8 ± 22.5	78.7 ± 29.6	ns
Histidine	69.4 ± 12.4**	72.6 ± 13.1^	80.3 ± 15	81.4 ± 25.3	ns
Asparagine	48 ± 10.5	50.7 ± 9.8	51.5 ± 14.5	49.6 ± 8.6	ns
Serine	115 ± 28.2	125.9 ± 81.3	115.2 ± 33.1	113.7 ± 30.8	ns
Glutamine	567.6 ± 116.4	597.7 ± 87.1	609 ± 125.4	588.6 ± 121.5	0.050
3methylhistidine	4.2 ± 1.2	4.1 ± 0.82	3.98 ± 0.84	4.06 ± 0.81	ns
Arginine	59.2 ± 19.5	61.1 ± 20.5	60.1 ± 19.8	66.5 ± 16.1	ns
Citrulline	33.2 ± 10.7	35 ± 11	34.2 ± 7.8	33.3 ± 9.6	ns
Glycine	267.2 ± 76.6	285.6 ± 82.5	273.9 ± 65.3	276.9 ± 87.7	ns
Threonine	137.2 ± 39	156.3 ± 61.8	146.6 ± 46.4	142.6 ± 34.2	0.030
Alanine	335.9 ± 86.5	369.3 ± 102.6	382.8 ± 92.7	382.9 ± 91.6	ns
Taurine	94.2 ± 28.4	88.5 ± 25.5	91.6 ± 30.1	92.5 ± 31	ns
Tyrosine	63.4 ± 14.5	64.5 ± 18.7	68.7 ± 22.3	64.3 ± 20.4	ns
Valine	242.7 ± 53.4	234.1 ± 63.3	272.7 ± 84.4	249.5 ± 50.7	ns
Methionine	27.2 ± 6.9	28.8 ± 7.4	31.1 ± 9.5	28.5 ± 7.3	0.019
Tryptophan	40.4 ± 8.5	42.5 ± 9.2	45.9 ± 15.3	45.4 ± 11.8	ns
Phenylalanine	61.7 ± 14.6	59.4 ± 12	61.2 ± 20.9	58.6 ± 12.5	ns
Isoleucine	71.7 ± 17	70.8 ± 27.7	72.8 ± 19.2	73.5 ± 20.2	ns
Leucine	136 ± 36.5	133 ± 58.2	143.2 ± 42.7	135.6 ± 34.6	ns
Ornithine	88.3 ± 29.4	87 ± 31	91 ± 33.4	83.8 ± 34.1	ns
Lysine	214.4 ± 51.1	210 ± 57.8	225.9 ± 62.8	219.4 ± 45.6	ns
Total-amino acids	2778.8 ± 345.8*	2870.9 ± 457.3	2969.7 ± 523	2894.1 ± 374.5	ns
FIM score	56.1 ± 26.0***	73.9 ± 28.5§	75.3 ± 23.6	90.9 ± 24.1	ns

Data are expressed as mean ± standard deviation (SD).
Statistical analysis: repeated measures analysis of variance. Trend over time: interaction differences in trends between groups.
Comparisons between the two groups: at admission *$p<0.05$, **$p<0.02$, ***$p<0.01$, ****$p<0.001$; at discharge ^$p<0.05$, ^^$p<0.02$, ^^^$p<0.01$; §$p<0.03$.
ns, not significant; ESR, erythrocyte sedimentation rate; FIM, functional independence measure.

were expressed in terms of gains over time ($r=+0.3$, $p=0.0020$) (Figure 2). FIM was negatively linked to the positive reactants of acute-phase response and ESR. Final FIM was positively correlated with admission FIM ($r=+0.88$; $p<0.0001$). For the multiple regression analysis model, FIM at admission ($r=+0.88$, $p<0.0001$), albumin ($r=+0.511$, $p<0.001$), transferrin ($r=+0.425$, $p<0.002$) and actual/pre-event BW ($r=+0.394$, $p<0.005$) were significant predictors of patient functional independence.

Inflammation in dysphagic patients

At admission to rehabilitation

Dysphagia was diagnosed in 31.9% of patients (30/94). Altered deglutition was detected by video fluoroscopic examination in 10/30 patients (33.3%) because of dubious clinical assessment. Severe-to-moderately severe dysphagia (DOSS levels 1–2) was found in 50% of patients (15/30) and mild-to-moderate dysphagia (DOSS level 4) was found

Table III. Relation between function (FIM) and anthropometric and biohumoral variables at patient admission to the rehabilitation ward.

		r^a	p	Multiple regression
FIM admission vs	Actual body weight (BW)	0.314	0.001	
	Actual/pre-event BW	0.489	<0.001	Actual/pre-event BW $p<0.001$
	Albumin[b]	0.535	<0.001	Albumin $p<0.001$
	α_1 globulin[c]	−0.48	<0.001	
	ESR	−0.44	<0.001	
	CRP	−0.40	<0.001	
	Prealbumin[b]	0.393	<0.001	
	Transferrin[b]	0.383	<0.001	
	Haemoglobin	0.33	0.001	
	Haptoglobin[c]	−0.39	<0.001	
	Histidine	0.345	<0.001	
	Tryptophan	0.27	0.005	

FIM, Functional Independence Measure; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein.
[a]Pearson's correlation co-efficient.
[b]Negative reactant proteins of acute-phase response.
[c]Positive reactant proteins of acute-phase response.

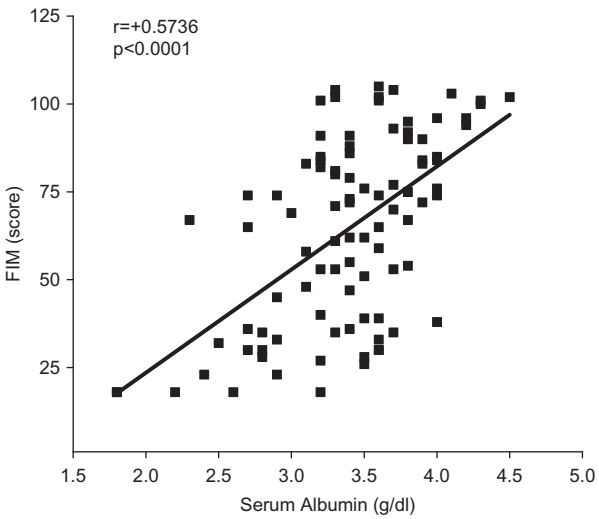

Figure 1. Correlation between circulating serum albumin and functional independence measure (FIM) at patient admission to rehabilitation. The relation clearly shows the link between serum albumin level and patient functional independence.

in 46.7% (14/30). One patient (3.3%) had deglutition capacity within functional limits (DOSS level 6).

As ancillary data, dysphagia was similarly distributed between the former groups of ischaemic (34.9%) and haemorrhagic (36.8%) individuals. In Table V demographic-, anthropometric-, biohumoral-, amino acid profiles and functional independence in inflamed subject without dysphagia (no-dysph) or with dyspagia (dysph) are shown. The prevalence of inflammation was higher in dysphagics (73.3%, 22/30) than in non-dysphagics (43.7%, 28/64) ($p<0.01$), but the rate of inflammation was similar between the two groups.

The loss of functional independence was more accentuated in inflamed dysphagics with 35.3-FIM points lower than in non-dysphagics ($p<0.001$). Notwithstanding a similar degrees of inflammation, the shift of hepatic protein synthesis was more pronounced in the dysphagic stroke patients. Inflammation-associated alpha 1 globulin system, ESR and circulating neutrophils were within the normal range of values but significantly higher in dysphagic than in non-dysphagic subjects. Lymphocytes were higher in non-dysphagic than in dysphagic patients. Only dysphagic subjects lost body weight (−6.7% pre-event BW, $p<0.001$). The loss of body weight occurred in 43.3% (13/30) of dysphagics and in 10.9% (7/64) of non-dysphagics ($p<0.001$). Even though it was within the normal range of values, histidine was lower and arginine and glycine higher in the dysphagic group. Blood urea, glucose and Hb concentrations were normal and similar between the two groups of patients.

Table VI shows the relation between the dysphagia outcome and severity scale (DOSS) and anthropometric, biohumoral and FIM variables. The severity of dysphagia (i.e. lower DOSS value) correlated with positive proteins of the acute phase response (mainly alpha 1 globulin system), ESR, plasma glucose. On the other hand, dysphagia was less severe with increasing concentrations of negative proteins of the acute phase response (albumin, transferrin, prealbumin), Hb, plasma histidine and glycine, FIM, actual/pre-event BW. In the logistic regression analysis, serum alpha 1 globulin system levels were the most powerful predictors of dysphagia severity ($p<0.001$).

During the rehabilitation stage

Fifty per cent of patients with dysphagia had their deglutition alteration improved. Dysphagia decreased in 53.3% of patients with DOSS 1–2: five patients increased DOSS to 3–5 scores, four to DOSS 6–7.

Both dysphagic and non-dysphagic patients improved their functional disability for all study variables. They had similar time-courses of changes including the gain in FIM (+14.6 scores in dysphagics and +17.3 scores in non-dysphagics). Functional status improved even given the infection (mainly of the urinary tract), which developed in 66.6% (20/30) of dysphagics and 37.5% (24/64) of the non-dysphagic population ($p=0.004$).

The study showed that the improvement in dysphagia (Table VII) was associated with improved time courses of serum albumin (interaction $p=0.01$), with decreased concentrations of haptoglobin (interaction $p=0.05$) and CRP (interaction $p=0.05$), with improved FIM (interaction $p=0.02$) and with reduced plasma levels of threonine (interaction $p=0.03$).

Table IV. Relation between function (FIM) and anthropometric and biohumoral variables at patient discharge from the rehabilitation ward.

		r^a	p	Multiple regression
FIM discharge vs	Actual body weight (BW)	0.361	<0.001	
	Actual/pre-event BW	0.394	<0.001	Actual/pre-event BW $p<0.005$
	Albumin[b]	0.511	<0.001	Albumin $p<0.001$
	ESR	−0.2	0.029	
	CRP	−0.21	0.033	
	Prealbumin[b]	0.327	0.001	
	Transferrin[b]	0.425	<0.001	Transferrin $p<0.002$
	Haemoglobin	0.29	0.003	
	Histidine	0.279	0.004	
	Tyrosine	0.21	0.031	
	Tryptophan	0.425	<0.001	
	FIM admission	0.88	<0.0001	FIM admission $p<0.0001$

FIM, Functional Independence Measure; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein.
[a]Pearson's correlation coefficient.
[b]Negative reactant proteins of acute-phase response.

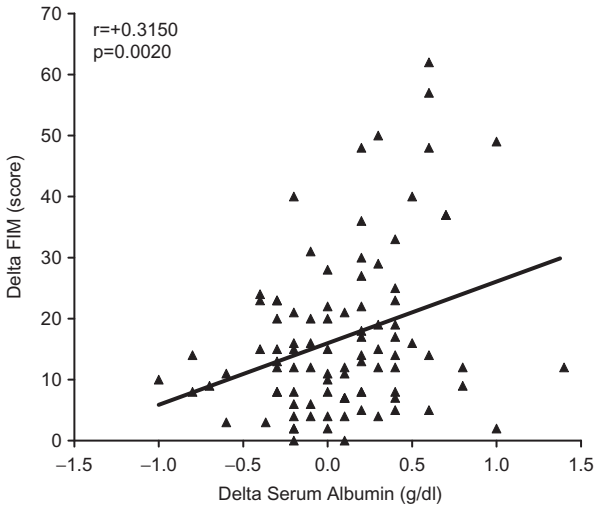

Figure 2. Correlation between the over-time gains in circulating serum albumin and functional independence measure (FIM). Delta = discharge value – admission value. The relation clearly shows the importance of the level of circulating albumin for the improvement of patient functional independence.

At discharge from rehabilitation

Compared to patients who improved dysphagia (Table VII), the patients who did not improve deglutition were admitted with lower body weight ($p<0.02$) and higher transferrin and threonine ($p<0.05$) and were discharged with lower albumin ($p<0.01$), prealbumin ($p<0.02$), haemoglobin ($p<0.02$), functional independence ($p<0.01$) and plasma histidine ($p<0.05$) but higher ESR ($p<0.05$), plasma glutamine ($p<0.05$) and glycine ($p<0.05$) levels.

Discussion

This study confirms the hypothesis that an inflammatory status may persist during the rehabilitation stage of strokes, is more prevalent in dysphagic than in non-dysphagic subject and that the retrieval of functional independence and of dysphagia is linked to the level of negative proteins of the acute-phase response and to the maintenance of body weight and not to the reduced rate of inflammation.

Moreover, the study showed that inflammation is associated with more severe patient functional disability, mainly in individuals with dysphagia.

Prevalence of inflammation at admission to rehabilitation

The fact that inflammation was present in most but not all admitted stroke patients may be explained by several factors. One is that, in some individuals, brain ischaemia may cause a low grade of inflammation that subsequently progressively decreases until completely disappearing. Alternatively/additively ischaemia induced-inflammation may be perpetuated/enhanced over time by infectious complications, occurring frequently (25–65%) [26] during the post-acute period of the stroke. Furthermore, pre-existing co-morbidities such as diabetes mellitus, hypertension and malnutrition can exacerbate an individual's response to stress [27].

Inflammation is not to be attributed to the different topography of brain lesions, as lesion sites were similarly distributed between inflamed and non-inflamed subjects. These are in line with a previous study reporting that, in acute strokes, clinical deterioration, associated with IL-6 increments, were independent of topography, initial size or mechanism of infarction [7].

The association of inflammation with more severe disability confirms data already documented in acute stroke patients, i.e. increased serum levels of inflammatory markers predict deteriorating neurological symptoms both in brain ischaemia [7] and intracerebral haemorrhage [10].

Inflammation accounts for the observed inter-group differences in serum levels of positive and negative reactants of acute phase response and in circulating neutrophil percentage. The differences in albumin and transferrin serum levels between the two groups were not due to different nutritional intakes as energy-protein intakes/supplies were adequate and similar between inflamed and not-inflamed subjects.

Inflammation may also explain the reduced blood Hb, plasma levels of histidine and total amino acids. Indeed, an inflammatory state increases both Hb degradation [28] and muscle amino acids release into the blood stream. However, the concomitant increased use of circulating amino acids by

Table V. Demoghraphic, anthropometric, biohumoral and amino acid profiles and functional independence measurements in inflamed subjects without dysphagia (non-dysph) or with dysphagia (dysph) at admission to the rehabilitation ward.

Variables	Normal values	No-dysph ($n = 64$)	Dysph ($n = 30$)	p Value
Demographic				
Age (years)	–	67.17 ± 14.7	66.74 ± 13.6	ns
Anthropometric				
Actual body weight (kg)	–	72.40 ± 15.5	69.37 ± 14.1	ns
Pre-event Body weight (kg)		74.03 ± 16	73.88 ± 15.7	
Actual/pre-event BW (%)		99.6 ± 6.5	93.38 ± 7.24	<0.001
Blood				
ESR 1st hour (mm)	<20	28.28 ± 20.5	45.92 ± 35.63	0.009
Haemoglobin ($g\,dl^{-1}$)	12–15	13.33 ± 1.84	12.6 ± 1.9	ns
Blood urea ($mg\,dl^{-1}$)	20–40	41.36 ± 18.35	40.84 ± 27.23	ns
Serum creatinine ($mg\,dl^{-1}$)	0.7–1.2	1 ± 0.26	0.93 ± 0.31	ns
Plasma glucose ($mg\,dl^{-1}$)	80–110	106.71 ± 36.37	116.4 ± 39.19	ns
α_1 globulin ($g\,dl^{-1}$)	0.21–0.35	0.20 ± 0.035	0.23 ± 0.05	0.002
Blood white cell count ($n^{\circ}\,mm^{-3}$)		6384.57 ± 2044.21	6799.73 ± 1854.85	ns
of which:				ns
Neutrophils (%)	50–70	58.32 ± 9.64	63.35 ± 10.45	0.014
Lymphocytes (%)	20–40	29.18 ± 9.46	24.7 ± 9.5	0.021
Monocytes (%)		8.9 ± 2.36	8.7 ± 2.35	ns
Serum albumin ($g\,dl^{-1}$)	4.02–4.76	3.51 ± 0.41	3.11 ± 0.62	0.001
Serum prealbumin ($mg\,dl^{-1}$)	18–30	22.38 ± 5.26	19.03 ± 5.9	0.004
Serum haptoglobin ($mg\,dl^{-1}$)	30–200	165.8 ± 81.5	232.7 ± 123.56	0.005
Interleukin-6 ($pg\,ml^{-1}$)	<7	13.13 ± 36.55	20.74 ± 26.4	ns
Serum transferrin ($mg\,dl^{-1}$)	202–364	217 ± 45.99	191.66 ± 48.73	0.011
Serum C-reactive protein (CRP) ($mg\,dl^{-1}$)	<0.3	0.84 ± 1.12	1.75 ± 2.41	0.032
Amino acid profiles ($\mu mol\,L^{-1}$)				ns
Aspartate	20–37	11.44 ± 2.44	11.52 ± 1.87	ns
Glutamate	43–82	72.7 ± 22.39	66.26 ± 20.63	ns
Histidine	61–116	75.6 ± 13.50	69.1 ± 16.37	0.030
Asparagine	44–78	48.4 ± 9.8	52.44 ± 15.66	ns
Serine	106–150	112.62 ± 27	118.7 ± 97	ns
Glutamine	466–805	575.21 ± 108.6	601.76 ± 138.4	ns
3methylhistidine	0.53–5	4.13 ± 1	4.28 ± 1.20	ns
Arginine	52–89	52.83 ± 17	67.47 ± 21.68	0.003
Citrulline	25–49	34.51 ± 8.92	31.97 ± 10.55	ns
Glycine	186–577	260.5 ± 63.03	289.29 ± 81.74	0.044
Threonine	168–288	136.21 ± 42.99	148.9 ± 40.29	ns
Alanine	265–573	351.24 ± 89.13	347.9 ± 94.5	ns
Taurine	62–206	95.3 ± 29.77	85.44 ± 23.95	ns
Tyrosine	66–90	64.26 ± 17.35	66.31 ± 18.9	ns
Valine	123–307	256 ± 66.1	243.73 ± 75.8	ns
Methionine	6–40	28.99 ± 8.93	28.4 ± 6	ns
Tryptophan	37–57	43.31 ± 8.99	41.26 ± 15.83	ns
Phenylalanine	39–64	59.7 ± 14.42	64.6 ± 21.33	ns
Isoleucine	52–80	70.47 ± 17.17	74.23 ± 19.6	ns
Leucine	94–160	137.4 ± 29.44	137.82 ± 52.71	ns
Ornithine	29–125	90.13 ± 30.93	82.44 ± 28.18	ns
Lysine	151–226	217.3 ± 53.17	218.1 ± 63.94	ns
Total-amino acids	2200–3332	2798 ± 559.6	2851.92 ± 538	ns
FIM score	125	75 ± 22	39.68 ± 21	<0.001

Data are expressed as mean ± standard deviation (SD).
Statistical analysis: unpaired *t*-test; No-dysph vs. dysph group.
ns, not significant; ESR, erythrocyte sedimentation rate; FIM, functional independence measure.

the liver lowers plasma amino acids concentrations [16]. This may partially be reduced by increased erythropoietic activity of the bone marrow, which compensates for excess peripheral Hb degradation [29].

The study shows that the co-presence of inflammation and dysphagia appears to be even more deleterious for brain repair and function than inflammation alone. In addition to inflammation, nutritional alterations associated with dysphagia [30, 31] may negatively impact brain function. Indeed, dysphagic patients have a loss of pre-event body weight to indicate inadequate nutrition to body requirements following the acute event [32]. This may explain the plasma content in arginine and glycine being higher than in non-dysphagics as a consequence of increased skeletal muscle release of amino acids.

Inflammation during rehabilitation

During rehabilitation, the patients improved their functional independence and biohumoral variables. Moreover, 50% of dysphagic subjects improved their deglutition capacity. Unexpectedly, the gains in FIM scale occurred in all patient groups, i.e. without inflammation, with inflammation, with combined inflammation and dysphagia.

Table VI. Relation between dysphagia outcome and severity scale (DOSS) and anthropometric and biohumoral variables at patient admission to the rehabilitation ward.

		r^a	p	Multiple regression
DOSS	Actual/pre-event BW	0.468	0.007	
admission vs	Albumin[b]	0.480	0.002	
	α_1 globulin[c]	−0.717	<0.001	α_1 globulin
	Glucose	−0.517	0.001	$p<0.001$
	ESR	−0.452	0.006	
	CRP[a]	−0.343	0.035	
	Transferrin[a,b]	0.559	<0.001	
	Haemoglobin	0.351	0.031	
	Haptoglobin[c]	−0.367	0.028	
	Histidine	0.358	0.027	
	Glicine	0.357	0.028	
	FIM	0.548	<0.001	

BW, body weight; FIM, Functional Independence Measure; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein.
[a]Non-parametric correlation (Spearman).
[b]Negative reactant proteins of acute-phase response.
[c]Positive reactant proteins of acute-phase response.

This provides strong evidence that, at admission to rehabilitation, the patients may have similar retrieval rates of brain repair and function. This study cannot explain the mechanisms leading to similar gains in neuro-function in each group. It is suspected that IL-6, in a context of body anabolic improvement, could play a role by limiting the rate of inflammation and by inducing neuro-protection. Indeed, inflammatory cytokines also have concomitant anti-inflammatory properties by down-regulating inflammatory tumour necrosis factor-α and IL-1 [33] and by acting on the hypothalamus-pituitary axis regulating the production of anti-inflammatory glucocorticoids [34].

IL-6 directly influences brain function by various mechanisms including nerve cell regeneration after injury [34], neuronal differentiation [35] and increased secretion of neurotrofic factor by astrocytes [35]. Furthermore, the cytokine regulates learning and memory process as it is highly expressed in brain structures essential for learning and memory [36], such as the hippocampus [37], cerebral cortex

Table VII. Anthropometric-, biohumoral variables, amino acid profile, function amino acid profile in dysphagic subjects who improved or did not improve deglutition capacity after rehabilitation.

Variables	No improvement of dysphagia		Improvement of dysphagia		Trend over time (p level)
	Admission	Discharge	Admission	Discharge	Interaction
Anthropometric					
Actual body weight (kg)	63.38 ± 11.22**	63.67 ± 11.21	76.76 ± 14	75.6 ± 12	$p=0.072$
Actual/pre-event BW (%)	92.96 ± 0.56	93.38 ± 7	94 ± 7	93 ± 8.45	$p=0.20$
Blood					
ESR1st hr (mm)	41.9 ± 32.3	42.38 ± 29.94^	44.27 ± 32.31	30.45 ± 24	$p=0.15$
Haemoglobin (g/dl)	12.46 ± 1.88	12.21 ± 1.40^^	12.75 ± 1.95	12.62 ± 1.16	$p=0.82$
Glucose (mg/dl)	117.2 ± 40	98.8 ± 25.57	106.61 ± 20	94.53 ± 19	$p=0.40$
α1 globulin (mg/dl)	0.23 ± 0.050	0.22 ± 0.054	0.23 ± 0.50	0.20 ± 0.036	$p=0.3$
Serum albumin (g/dl)	3.162 ± 0.57	3.195 ± 0.54^^^	3.047 ± 0.68	3.46 ± 0.49	$p=0.01$
Serum prealbumin (mg/dl)	18.7 ± 4.34	17.58 ± 4.64^^	18.50 ± 7.12	21.56 ± 6.3	$p=0.010$
Serum haptoglobin (mg/dl)	209.33 ± 86.27	191.77 ± 84.15	254.33 ± 160.9	182.93 ± 98.15	$p=0.05$
Interleukin-6 (pg/ml)	22.14 ± 28.61	12.43 ± 9.29	22.4 ± 27.66	6.52 ± 5	$p=0.59$
Serum transferrin (mg/dl)	201.68 ± 50.88*	197.31 ± 43.40	181.13 ± 45.91	192.13 ± 22.66	$p=0.1$
Serum C-reactive protein (mg/dl)	1.32 ± 1.82	1.23 ± 2.36	2.28 ± 2.97	0.60 ± 0.70	$p=0.05$
Amino acid profiles (nmol/L)					
Aspartate	11.62 ± 1.58	11.7 ± 1.84	11.38 ± 1.93	12 ± 3.29	$p=0.2$
Glutamate	66 ± 17.1	63.7 ± 14.69	72.3 ± 16.93	68.5 ± 21.8	$p=0.3$
Histidine	72 ± 11.86	71.85 ± 12^	65.41 ± 20.44	67.94 ± 14.88	$p=0.62$
Asparagine	51.25 ± 13.1	55.25 ± 8.47	53.38 ± 22.4	51.23 ± 13.3	$p=0.09$
Serine	117.19 ± 33.81	117.85 ± 39.72	120.52 ± 37.31	117.41 ± 27.68	$p=0.70$
Glutamine	626.14 ± 119.8	630.4 ± 100.75^	571.64 ± 156.87	592.82 ± 90.19	$p=0.76$
3 methylhistidine	4.17 ± 1.20	3.96 ± 0.99	4.41 ± 1.21	3.9 ± 0.88	$p=0.38$
Arginine	69.81 ± 20.41	66.19 ± 19.72	64.58 ± 23.45	60.3 ± 17.9	$p=0.91$
Citrulline	34.5 ± 8.9	36.6 ± 10.1	31.9 ± 10.5	33.6 ± 11.7	$p=0.81$
Glycine	289.3 ± 81.7	295.7 ± 95.8^	260.5 ± 63	275.7 ± 80.32	$p=0.75$
Threonine	155.14 ± 36.11*	176.76 ± 59.9	141.17 ± 44.84	126 ± 36.48	$p=0.03$
Alanine	357.3 ± 78.9	370 ± 110.8	364.76 ± 125.5	378.4 ± 113.6	$p=0.4$
Taurine	88.61 ± 25.07	89.28 ± 27.72	81.52 ± 22.60	88.82 ± 24.79	$p=0.45$
Tyrosine	66.19 ± 16	67.76 ± 20.88	66.47 ± 22.44	61.52 ± 25.83	$p=0.42$
Valine	229.57 ± 47.9	228 ± 74.32	261.23 ± 99.14	227 ± 62.59	$p=0.20$
Methionine	29.14 ± 6.82	28.47 ± 7.86	27.47 ± 5	29.35 ± 9.74	$p=0.32$
Tryptophan	40.19 ± 9.71	39.90 ± 9.24	42.58 ± 21.41	42.35 ± 16.22	$p=0.99$
Phenylalanine	64.61 ± 20	60 ± 12.34	64.56 ± 23.54	57.68 ± 14.8	$p=0.75$
Isoleucine	70 ± 17.61	73.19 ± 36.4	79.75 ± 21.81	69.12 ± 16.55	$p=0.12$
Leucine	124.23 ± 27.16	112.9 ± 68.84	154.58 ± 70.44	130 ± 36.37	$p=0.16$
Ornithine	82.5 ± 28.2	83.6 ± 26.5	90.1 ± 30.9	95.4 ± 33.6	$p=0.75$
Lysine	209.4 ± 51.37	219.7 ± 53	222.47 ± 74.71	210.76 ± 41.62	$p=0.36$
Total-amino acids	2881.9 ± 293.75	2953.80 ± 437.45	2813.26 ± 731.17	2794.46 ± 515.10	$p=0.67$
FIM score	37.85 ± 19.45	47.61 ± 26.54^^^	41.94 ± 23.16	62.47 ± 22.84	$p=0.02$

Data are expressed as mean ± standard deviation (SD).
Statistical analysis: repeated measures analysis of variance. Trend over time: interaction differences in trends between groups.
Comparisons between the two groups: at admission *$p<0.05$, ** $p<0.02$; at discharge ^$p<0.05$, ^^$p<0.02$, ^^^$p<0.01$.
ESR, erythrocyte sedimentation rate; FIM, functional independence measure.

[38] and amygdale [39]. All these activities rely on increased protein synthesis.

The retrieval of neurofunction could be directly affected by improved patient anabolic/catabolic ratio. Body anabolic activity is of paramount importance for brain protein synthesis, repairing and functioning [17–19, 40]. Body anabolism may act in synergy with comprehensive standardized rehabilitation programmes in improving brain function [17]. That patients undergo improved body anabolic activity is suggested by the following: (1) over time body weight stability (notwithstanding infection complications) liver function oriented towards a more normal protein synthesis activity, (2) functional status dependent on the gains in both albumin and body weight and (3) improved deglutition capacity that occurred only in those subjects who significantly increased serum albumin and transferrin concentrations and, at the same time, reduced inflammatory proteins. The retrieval of deglutition capacity is part of a general improvement in brain function, as suggested by the association of dysphagia with functional status (FIM).

At present, one cannot explain the inverse relation between the time courses of plasma threonine concentrations and improved dysphagia. The correlation might be more than a casual finding given that, physiologically, threonine contributes to regulate neuron activity [41]. This point needs a well-planned investigation given that essential amino acids can be exogenously manipulated by diet.

This study provides clear evidence of the additional damage caused by dysphagia in inflamed subjects. Compared to patients with normal deglutition, dysphagics are discharged weighing less, with reduced circulating albumin, prealbumin, transferrin, histidine levels, blood haemoglobin and poor function. All these features are probably the consequences of both inadequate nutrition and more intense inflammation from complications suffered during the pre-rehabilitation stage of stroke. Supporting this argument, IL-6 was higher in dysphagic subjects both at admission and discharge from rehabilitation, although not significantly. In addition, plasma arginine and glycine levels at admission and additionally glutamine concentrations at discharge were higher in dysphagics, suggesting the effects of a more intense inflammation on skeletal muscle breakdown. Finally, glycine and glutamine may be useful to dysphagics, as glycin has cytoprotective effects [42] and glutamine improves immunological defense [43].

The study shows that the poorer function and biohumoral outcomes of the dysphagic patients at their discharge from rehabilitation are consequences of patient clinical time-course during the pre-rehabilitation stage of the disease as in the rehabilitation period patients with or without dysphagia have similar potential for neuro-rehabilitation outcome.

The relation between variables and neurofunction

The level of patient functional status at discharge from rehabilitation is conditioned by the level of function at admission and by protein status. Both in pre- and rehabilitation periods, the positive association of serum albumin and body weight with functional independence suggest that circulating albumin [44–47] and body protein status, due to inflammatory state/nutrition, play a major role for brain repair and function [17–19, 40]. Moderate-to-high dose human albumin therapy provides neuroprotection in rodent models of transient focal ischaemia [48–50], global ischaemia [51] and traumatic brain injury [52]. Here, the importance of albumin on brain functioning is even more given the correlation between the gain in protein levels and the gain in functional independence [20].

Both experimental and human studies have documented the importance of protein turnover in cerebral ischaemia. Protein synthesis in the brain is suppressed by cerebral ischaemia. This is an important factor limiting post-ischaemic recovery of neurons [53]. In subjects with ischaemic brain after cardiac arrest, brain protein synthesis is essential for neuronal survival [54] and supplemented protein may help the neurocognitive recovery in subjects with sub-acute ischaemic stroke [17, 18]. During rehabilitation, in addition to albumin, circulating transferrin emerged as an independent predictor of functional independence, confirming a recent study by this group [20]. Normal levels of serum transferrin are essential for binding within the brain an excess of free iron following ischaemia-induced neuronal damage, as an excess of free iron contributes greatly to the progression of stroke and a deteriorating outcome [55].

As regards body weight, this investigation agrees with previous studies reporting the importance of normal nutrition for functional states in stroke patients. Malnutrition decreases clinical outcomes in acute stroke [56, 57] and functional improvement during rehabilitation [30, 31].

Inflammation and dysphagia seem to be two mutually influencing conditions, in that dysphagia pre-disposes patients to more frequent infections complications [26], the inflammatory response of which, in turn, could determine the degree of dysphagia. Here, this is suggested by the correlation observed in the pre-rehabilitation period between antiprotease alpha-1 globulin and dysphagia. This suggests that inflammation-induced phagocitic activity of circulating neutrophils and macrophages may negatively impact the mechanisms underlying normal deglutition such as interneuronal activity and/or deglutition centre and/or peripheral neuromuscular function of deglutition [58]. Here, the fact that improved deglutition capacity was associated with improved acute-phase negative proteins and a more normal liver protein synthesis also suggests the influence of inflammation on dysphagia.

Clinical interpretation and implications

Inflammation, present in a large number of patients with stroke sequelae, can persist for a long time, sometimes at least 3 months after the acute event. The impact of inflammation on neuro-repair seems to be albumin-dependent at all stages of the post-acute stroke, as suggested by correlations found between functional improvement and serum albumin (positive association), albumin and IL-6 (negative association) and by the absence of any direct significant correlation between IL-6 and functional status.

The impact of inflammation on neuro-function seems to be dependent on stroke stages: negative in the pre-rehabilitation stage, but not negative during the rehabilitation stage. Although the correlation does not necessarily imply a cause–effect relationship, the existing scientific and clinical

evidence seems to suggest that the correlation between albumin and FIM may rely on a mechanistic relation [17–20].

The correlation between albumin and neurofunction retrieval may pose the question of whether and how it is possible to increase albumin levels in sub-acute strokes, i.e. in a phase of progressive decline of inflammation. This study suggests that, at least in the rehabilitation phase, the liver albumin synthesis could be in part susceptible to nutritional interventions. This is indirectly suggested by the relation between albumin and inflammation which is far weaker in the rehabilitation than in the pre-rehabilitation phase of the disease.

In expectation of a well-planned intervention aimed at improving circulating albumin in post-acute stroke, it is reasonable to expect that, in clinical practice, every effort should be paid to prevent any inflammation-inducing complication, mainly infection [20, 59] and to potentiate protein intake/supply above $1\,g\,kg^{-1}\,day^{-1}$ [60]. When a complication has occurred, any improvement in patient protein access may be particularly important to avoid inadequate nutrition. Increased protein intake/supply can also improve the plasma concentrations of histidine and tryptophan. This may be relevant for neuro-rehabilitation as these two amino acids are precursors of, respectively, the neuro-transmitter histamine and serotonin. Histamine is a regulator of 'whole brain activity' [61]. The fact that histidine concentrations were low both in the pre-rehabilitation and rehabilitation periods, even given adequate patient protein intake/supply, would suggest that, in strokes, amino acid may behave as essential amino acids. Tryptophan-derived serotonin is involved in cognition, motor functions and mood [62]. Moreover, tryptophan is the limiting amino acid of albumin synthesis [63].

This study shows that the use of albumin, prealbumin and transferrin as markers of nutrition is precluded in a large proportion of post-stroke rehabilitation patients until inflammation has disappeared [31].

In synthesis, these results confirm the huge importance of proteins on brain function and repair [17, 18, 20, 64, 65] in stroke subjects and shows the usefulness of monitoring both inflammatory markers and proteins of the acute phase response to inflammation over time. It is believed that nutritional intervention in strokes should be addressed not only for nutritional aims *per se*, but also might be targeted to specifically potentiate neurorehabilitation outcomes. This is more striking for a disease for which at present there is no drug for brain repair.

Limitations

The study has several limitations that require appropriate investigation to be resolved. Cytokines, hormones and peptides play key roles as mediators of inflammation and immunological response caused by brain ischaemia. More knowledge of these mediators and their interplay would allow one to understand and control the severity of both biochemical and metabolic alterations better during the post-acute stage of strokes. For instance, the determination of the anti-inflammatory cytokine IL-10 could provide some useful information about the balance between inflammatory and anti-inflammatory activities during patient rehabilitation. This is a relevant issue because inflammation is not only a tissue deteriorating process primed by tissue insult but at the same time limits the severity of the damage acting then as a neuro-protective process [36]. Determination of hormones, particularly insulin, insulin-like growth factor-1 and cortisol may provide information on body anabolic/catabolic ratio orientation.

Conclusions

An inflammatory status may persist in subjects with post-acute strokes and is more prevalent in dysphagic than in non-dysphagic individuals. Inflammation aggravates metabolic and neurofunction alterations, particularly in patients with altered deglutition. The patient retrieval of functional independence and dysphagia seem to be dependent on serum albumin levels and maintenance of body weight.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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