Plasma L-citrulline concentrations and its relationship with inflammation at the onset of septic shock: A pilot study.

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List of Abbreviations: COIITSS: corticosteroids and intensive insulin therapy for septic shock; CRP: C-reactive protein; I-FABP: intestinal fatty acid binding protein; ICU: intensive care unit; IL-10 interleukin-10; MOF: multiple organ failure; SD: standard deviation; SAPS: simplified acute physiology score; SOFA: sequential organ failure assessment; TNFα: tumor necrosis factor α.

Abstract

Purpose: Hypocitrullinemia has been suggested to be a prognostic factor for patients in intensive care. The aim of this ancillary study of the Corticosteroids and Intensive Insulin Therapy for Septic Shock prospective study was to investigate plasma L-citrulline concentrations and its relationship with inflammation and digestive bacterial translocation in septic shock multiorgan failure patients, without primary intestinal disease or chronic renal failure.

Methods: Sixteen adult patients were selected. They were studied on day (D) 0 at hours (H) 0, 6, 12, 18, 24 and on D4 (H96). Selected plasma amino acids and proteins, pro- (TNFα) and anti-inflammatory (IL-10) cytokine concentrations, and bacterial translocation were measured.

Results: Eight day-14 survivors and 8 day-14 non-survivors patients were studied. Citrulline was decreased during D0 (H0: 29±10 *vs.* nadir: 18±6 µmol/L, $P < 0.05$). The citrulline nadir was lower ($P < 0.01$) in patients with digestive bacterial translocation than in those without. H0-H96 mean citrulline concentrations were not significantly different between survivors and non-survivors. In both groups, citrulline was significantly inversely correlated with CRP ($r^2=0.10$, $P<0.01$) during D0. No significant correlations were found between citrulline and albumin, transthyretin, TNFα, IL-10 or TNFα/IL-10 ratio.

Conclusions: At the onset of septic shock, plasma citrulline decreases and varies inversely with CRP and is lower when digestive bacterial translocation occurs. This finding could reflect an early acute intestinal dysfunction but measurement of citrulline concentration does not appear to be able to predict the patients' mortality.

Key words: citrulline; septic shock; plasma cytokines; intensive care unit; enterocytes

Introduction

Acute intestinal dysfunction has been postulated as a cause of systemic inflammatory response syndrome and multiple organ failure (MOF) in patients with septic shock in intensive care unit (ICU). This "gut hypothesis" is difficult to evaluate, except when gastric tonometry identifies intramucosal acidosis, reflecting digestive mucosal ischemia [1, 2]. Reported prevalence of intestinal dysfunction in ICU ranges from 20% to 60%, depending on recruitment and the clinical criteria used, notably food intolerance and (or) intra-abdominal hypertension [3]. Circulating L-citrulline is a biomarker of enterocyte functional metabolic mass and is not difficult to be determined by ion-exchange chromatography [4].. Indeed, this amino acid is synthesized in the enterocytes of intestinal mucosa from glutamine and arginine, and is, moreover, not a component of proteins, or included in nutrition products. Plasma citrulline has a half-life of 3 hours in normal conditions and is converted to arginine by the proximal convoluted kidney tubules [5]. These metabolic features explain why plasma citrulline concentration (normal values: 30–50 μmol/L) is therefore, in the clinical situation, a reliable biomarker of gut metabolic mass with a high positive relationship with functional remnant length of the small bowel in various intestinal diseases [6], either post-surgical (short bowel syndrome) [7] or medical (villous atrophy) [8]. In addition, low plasma citrulline has been suggested as a risk factor for intestinal bacterial translocation in the context of allogenic bone marrow transplantation [9] and is associated with endotoxemia in post-cardiac arrest patients [10]. Moreover, a low plasma citrulline concentration appears to be an independent risk factor for catheter-related bloodstream infections in children with intestinal failure [11]. Patients with septic shock have been shown to have a marked reduction of citrulline synthesis [12-14]. However, another study shown conflicting results, with an increase in citrulline synthesis compared with control subjects [15]. Authors found that hypocitrullinemia below 10 μmol/L occurs 24 h (H24) after admission to ICU, due to a decreased intestinal production, possibly

related to small bowel ischemia [16]. In addition, hypocitrullinemia was shown to be an independent factor of mortality adding to sequential organ failure assessment (SOFA) [16], an index that does not take digestive function into account [17]. Another study in ICU patients with varied pathologies suggests that low plasma citrulline is associated with intestinal dysfunction, similarly to a high residual gastric volume, ileus, diarrhea or gastrointestinal bleeding, but not with mortality [18]. However, the behavior of citrulline and its relationship with metabolic and inflammatory parameters in ICU patients is not known. In this pilot study, our aim was to further investigate the presence of an association between plasma L-citrulline concentration, inflammation and bacterial translocation Therefore we investigated, in ICU patients with severe septic shock and MOF treated with conventional insulin therapy and hydrocortisone hemisuccinate: (i) citrulline concentrations over a 4-day period, and (ii) its potential relationship with biological nutritional and inflammatory parameters and the occurrence of a digestive bacterial translocation.

Materials and Methods

Patient's inclusion and exclusion criteria

We performed an ancillary investigation within the multicentre Corticosteroids and Intensive Insulin Therapy for Septic Shock (COIITSS) study (trial registration: clinicaltrials.gov NCT00320099) [19] in patients recruited between January 2006 and January 2009. The COIITSS study protocol was approved by the "Comité de Protection des Personnes de Saint-Germain-en-Laye" ethical committee on 24th May, 2005. Written informed consent was obtained from the patients or their relatives in compliance with the French regulations for clinical research in emergency and critical care.

Patients were included from the conventional insulin therapy arm, in which blood glucose levels were maintained at approximately 8.3 mmol/L (150 mg/dL), in accordance with recommendations, [20] by the administration of either subcutaneous or intravenous insulin. In addition, all patients received hydrocortisone hemisuccinate in a 50 mg intravenous bolus every 6 h for 7 days. We selected patients with septic shock and a global baseline SOFA $\geq$ 8 (indicating MOF). However, because plasma citrulline increases in significant renal failure [5], renal SOFA had to be $\leq$ 2 (i.e. plasma creatinine < 299 μmol/L). Organ system failure was defined for each of the six major organ systems as a SOFA score of 3 or 4 points on a scale of 0–4 for each organ system, for an aggregate score of 0–24, with higher scores indicating more severe organ dysfunction [17]. We excluded patients with primary intestinal disease, diabetes mellitus or chronic renal failure. All the patients presented with septic shock and MOF at admission. They received intravenous fluids and vasopressor therapy (dopamine, adrenaline, noradrenalin, or any other vasoconstrictor agent) to maintain systolic blood pressure above 90 mm Hg or mean blood pressure above 60 mm Hg. Patients received enteral nutrition using a standard polymeric product by nasogastric tube after Day 1. When parenteral nutrition was

used, it was only for a short period with conventional non-glutamine supplemented solutions. Neither enteral nor parenteral products contained citrulline.

Sixteen patients (7 males, 9 females, mean age 61 yrs, Table 1) from the 138 patients (including 60 with fatal outcome during the study) of the COIITSS study who were randomized to receive conventional insulin therapy with hydrocortisone only [19], were selected on the above criteria and studied over a 4-day period. The patients were divided into two groups: survivors at day 14 following ICU admission (n = 8), and non-survivors, with time of death ranging from day 6 to day 14 (n = 8).

Variables recorded

Recorded parameters were creatinine, arterial lactates, total bilirubin, CRP, basal cortisol concentrations and cortisol concentrations at the peak 1 h after a 250 μg intravenous corticotropin injection. Severity of illness was determined using the SOFA and SAPS II (simplified acute physiology score) scales [21] (Table 1). In addition, specific biological data were measured.

Specific assays

Plasma samples were stored at −80 °C. Before assay, all samples were thawed to room temperature and mixed by gentle swirling or inversion. Assays were carried out on D0 [admission (H0), H6, H12, H18 and H24], and at H96. Arterial blood lactates were measured at H0, H24, and H96 only. Sampling was performed in patients with no clinical evidence of acute dehydration. Plasma citrulline, glutamine and arginine, respectively the main precursor and the main metabolite of citrulline at the whole-body level [5], were measured by automated ion exchange chromatography as previously described [4]. Plasma was deproteinized (with sulfosalicylic acid, 30 mg/mL) and plasma-free amino acid concentrations were determined in

the supernatant by ion-exchange chromatography with ninhydrin detection [22] using an AminoTac JLC-500V analyzer (Jeol, Tokyo, Japan). The between-run precision for plasma citrulline determination had a coefficient of variation of less than 7.5%. The assay laboratory is a participant in the European Quality Control scheme, thus guaranteeing the accuracy of the amino acid determinations. Thirty healthy subjects, sampled after an overnight fast, served as controls. They presented no evidence of metabolic or digestive diseases and had normal body mass indexes, serum albumin levels and renal function. Albumin and transthyretin were determined by immunonephelemetry using a BNII® (Siemens, Paris, France), CRP (C reactive protein) by turbidimetry on a Modular P® (Roche Diagnostics, Meylan, France) and lactates by an enzymatic method (Dimension RXL®, Siemens). TNF-α and IL-10 levels were measured using a solid-phase chemiluminescent immunometric assay method (TNF-α and IL-10 Immulite®**,** Siemens Healthcare Diagnostics Products, UK) using an Immulite 1000® System. The TNFα/IL-10 ratio was used as an index of proinflammatory/anti-inflammatory balance [23].

Digestive bacterial translocation

Digestive bacterial translocation was defined as highly presumable in 12 out of 16 patients [24]. It included patients with blood cultures positive for enterobacteria with negative microbiological urine analysis (n = 7), spontaneous peritoneal infection (n = 3) or occurrence of a new episode of septic shock after remission of the initial episode but with negative blood or other sites cultures (n = 2).

Statistical analysis

Results are expressed as means ± SD. Plasma citrulline and other quantitative parameters were compared between the two groups using Student's *t*-test, ANOVA with repeated

measures, Mann-Whitney in cases of non normality distributions or chi-squared and Fisher exact test when appropriate. Pearson or Spearman correlations were performed to test associations between plasma citrulline and other biological variables. SPSS software version 11.5 (SPSS Inc, Chicago, IL) was used for the statistical analysis. Statistical significance was set at $p < 0.05$.

Results

Baseline data

Clinical, bacteriological and biological data at H0 are given in Table 1. There were no differences in baseline clinical, biological and bacteriological parameters (Table 2, 3 and 4) between the two groups, survivors and non survivors, with the exception of plasma glutamine concentration (Table 3).

Behavior of citrulline over the 4-day study period (Figures 1 A and 1 B)

Plasma citrulline decreased significantly ($P < 0.05$) during D0 in 11 of the 16 patients, 18±6 μmol/L at the nadir versus 29±10 μmol/L at H0. In 5 patients, citrulline concentration did not change significantly during D0. The early decrease in plasma citrulline during D0 occurred in both groups: in 6 and 5 patients of the survivor and non-survivor groups respectively. The plasma citrulline nadir was observed at H6 in 4 patients, H12 in 5, H18 in 1 and H24 in 1. When the behavior of mean plasma citrulline concentrations in survivor and non-survivor patients was considered, there were no significant differences between the two groups (Table 3) with the exception of a huge range at H96 in non-survivor patients (*Figure 1 B*). Analysis of individual data in the non-survivor group showed two patterns of behavior of plasma citrulline at H96: 3 patients developed high plasma citrulline concentrations (> 50 μmol/L, with a maximum of 201 μmol/L), whereas the other 5 patients had concentrations between 0 and 30 μmol/L. In the survivors, plasma citrulline concentration remained between 10 and 30 μmol/L in 6 patients and normalized in 2 patients.

Relationship between plasma citrulline and metabolic, nutritional and inflammatory parameters

The behavior of the biological parameters tested is given in Tables 3 and 4. Plasma citrulline concentration was positively correlated with plasma arginine ($r = 0.92$, $r^2 = 0.85$, $P < 0.0001$) and glutamine ($r = 0.95$, $r^2 = 0.90$, $P < 0.0001$) concentrations in both patient groups. No significant correlations were found either between plasma citrulline and albumin, or with transthyretin. Plasma citrulline levels measured at baseline correlated with baseline creatinine level ($r = 0.25$, $r^2 = 0.06$, $P = 0.04$), but the correlation was not significant at H96. A significant positive correlation was found between glutamine and lactates at H24 ($r = 0.81$, $r^2 = 0.66$, $P < 0.01$) and H96 ($r = 0.79$, $r^2 = 0.62$, $P < 0.01$). In addition, a similar correlation between citrulline and lactates was observed at H24 ($r = 0.57$, $r^2 = 0.32$, $P = 0.053$) and H96 ($r = 0.80$, $r^2 = 0.64$, $P < 0.01$). At most of the evaluations (H0, H12, H18, H24 and H96), plasma glutamine concentrations were significantly lower ($P < 0.05$) in survivors than in non-survivors (Table 3).

In both groups, plasma citrulline concentration was negatively related to CRP at all the D0 evaluations ($r = -0.31$, $r^2 = 0.10$, $P = 0.0017$) (*Figure 2* for H0), but not at H96. No significant correlations were found between plasma citrulline and TNFα, IL-10 or TNFα/IL-10 ratio. Similarly, no significant correlations were found for glutamine and arginine and circulating TNFα, IL-10 or TNFα/IL-10 ratio, whereas both glutamine and arginine were significantly negatively correlated with CRP ($r = -0.35$, $r^2 = 0.12$, $P < 0.001$ and $r = -0.39$, $r^2 = 0.15$, $P < 0.001$ respectively). Circulating IL-10 was higher ($P < 0.05$), especially at H6 and H12, in non-survivor than in survivor patients (Table 4). Inflammatory balance estimated by TNFα/IL-10 ratio was significantly higher (ANOVA, $P < 0.05$) in survivor than in non-survivor patients (Table 4), however the only time for significant differences between the groups was H12.

Citrulline and digestive bacterial translocation

The nadir of plasma citrulline, which occurred during D0 in all cases, was significantly lower ($P < 0.01$) in patients with presumable translocation than without (Table 5). All the patients with a plasma citrulline nadir below 10 μmol/L ($n = 4$) had presumable bacterial translocation. There were no significant differences between patients with or without digestive bacterial translocation for the other biological parameters tested at the time of the citrulline nadir, including TNFα and IL-10 and its ratio (Table 4).

Discussion

In a group of ICU patients with septic shock treated with conventional insulin therapy and hydrocortisone hemisuccinate, plasma citrulline concentrations decreased during D0 in the majority of patients. An important finding in this study is that patients with the lowest citrulline nadir had the highest risk of digestive translocation, confirming results observed in studies of allogenic bone marrow transplantation [9] and cardiac arrest for endotoxemia [10]. However, bacterial translocation was estimated by an indirect approach because we had no access to mesenteric lymph node cultures. In addition, for some patients, pneumonia following microaspiration of gastric fluid containing Gram-negative bacteria could cause an overestimation of bacterial translocation. Nevertheless the role of inflammation on citrulline level is difficult to appraise. The negative relationship with CRP suggests a role of inflammation in altered citrulline metabolism, but without a direct role of TNFα and IL-10. The results did not show any significant relationship between plasma citrulline and pro-inflammatory cytokines (TNFα), anti-inflammatory cytokines (IL-10) or inflammatory balance estimated by the TNFα/IL-10 ratio. However, a negative correlation was confirmed between citrulline and CRP, at least on D0, as has previously been found in critically ill children [25]. In another ICU study, no such relationship was found [18]. The difference in behavior between the cytokines under study and CRP may be explained by the fact that CRP production is mostly controlled by IL-6 but this latter cytokine was not measured. In an experimental rat model of sepsis, citrulline supplementation decrease IL-6 response but have no effect on IL-10 secretion [26]. Although mean plasma citrulline was not significantly different between survivors and non-survivors in this small group of patients, our results show that citrulline levels may normalize at H96 in patients with the best prognosis. It would be interesting to have citrulline data after a more prolonged period. Our results did not show any

relationship between citrulline level and prognosis, but this may be due to the limited power of this study.

Two limitations of this study are the small number of patients and the absence of direct intestinal evaluation. Although the results may thus not be representative of the overall ICU patient population, they may nevertheless be representative of patients with severe septic shock. These patients are characteristically unstable, which may explain the heterogeneity of some of the results. In a previous study of 65 patients, the decrease in citrulline at H24 was more marked in patients with septic shock than in those who did not have septic shock [16]. In our study, citrulline behavior was not clearly related to renal impairment. The results showed that there was no correlation between H96 plasma creatinine and citrulline in patients with a baseline renal SOFA $\leq$ 2, confirming previous data in ICU patients with acute renal failure [16, 18]. However, this key point deserves further specific study.

Possible explanations for the variations and changes in citrulline levels may be related to tissue and (or) metabolic parameters. It is known that hypocitrullinemia is always due to a decreased intestinal production [4]. In the present study, an impairment of intestinal function for example secondary to mucosal intestinal ischemia, could be a possible explanation [27, 28]. Hence, the results found during D0 could reflect an acute intestinal dysfunction in some ICU patients with septic shock; however citrulline, as a surrogate marker, only gives an indirect intestinal evaluation. To our knowledge, there is currently only one ICU study that focuses on the relationship between citrulline level and digestive symptoms [18] but it does not report on the relationship between citrulline and intestinal lesions. On the other hand, it is known that the decrease in citrulline cannot be contemporaneously related to clinical symptoms, due to the fast decrease in plasma citrulline and the possible adaptation of the digestive tract as shown in acute radiation enteritis [29]. In addition, enteral nutrition or other modes of nutrition seems do not influence plasma citrulline in ICU patients[18].. As expected, we found a strong

correlation between plasma citrulline and its major precursor (glutamine) and major metabolite (arginine). A metabolic cause of hypocitrullinemia, notably by a decrease in its main precursor, glutamine, cannot be excluded[14], but a purely metabolic explanation is unlikely. In our study, plasma glutamine was significantly lower in survivor patients as has previously been shown in some stressed patients during the first day of admission to ICU [30]. On the other hand, a study with stable isotope tracers including [5,5-2H2]citrulline in patients with sepsis (n = 8) or septic shock (n = 5) showed a reduction in citrulline flux and citrulline and arginine concentrations, with, in consequence, an insufficient arginine synthesis [12]. This was also shown in another isotopic study of patients with septic shock, in whom a decrease in nitric oxide (NO) production was also suggested [13]. In contrast, an increase in citrulline flux and NO production, calculated as the conversion rate of arginine to citrulline has been observed in critically ill children with sepsis [15]... This could question the relevance of citrulline as a surrogate intestinal biomarker as compared to other test like I-FABP (intestinal fatty acid binding protein) assay [31]. Circulating I-FABP increases during the acute phase of sepsis, is associated with a worse 28-day prognosis [31] and has been considered as a marker of enterocyte damage in ICU [32].

Conclusions

A major decrease in plasma L-citrulline can occur in ICU patients with septic shock, additionally associated with digestive bacterial translocation and increased CRP. However, in this pilot study, we were unable either to assert or to deny the prognostic value of plasma citrulline, based on its concentrations in patients with poor prognoses. Meanwhile, the influence of intestinal perfusion, hemodynamic instability and metabolic and hormonal management on citrulline level in this context remains to be more precisely determined. In addition, a more powerful and larger study is needed, for example using the citrulline

generation test (stimulation of citrulline production with enteral glutamine) [33], in order to examine more closely the behavior of plasma citrulline found in this study.

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Table 1. Baseline (H0) clinical, bacteriological and biological (plasma concentration) characteristics of the 16 septic shock patients

Characteristics	
Age (years)	61±18
Sex: male/female (*n*)	7/9
Creatinine (μmol/L)	136.2±82
Bilirubin (μmol/L)	69±72
SOFA	11.9±3.0
SAPS II	58±16.2
Albumin (g/L)	17.8±6.4
Sites of infection (*n*)	26 sites
Chest	17
Peritoneal	5
Urogenital	1
Septicemia	3
Wound	1
Central nervous system	1
Pathogens, Gram-negative (*n*)	9 (56%)
Presumable digestive bacterial translocation (*n*)	12
Citrulline (N: 30–50 μmol/L)	23±13
Glutamine (N: 480–670 μmol/L)	489±328
Arginine (N: 60–80 μmol/L)	44±42
CRP (N < 5 ng/mL)	167±14
TNF alpha □ (N< 8.1 pg/mL□)	37 (4-895)
IL-10 (N < 9.1 pg/mL)	19 (4-1000)
TNF □alpha/IL-10 ratio	1.8 (0.21-223)
Lactates (N < 2.4 mmol/L)	11±23

Values are mean±SD, median (range) for TNF alpha, IL-10 and TNF alpha/IL-10 ratio or quantitative number (*n*)

Table 2. Baseline (H0) and bacteriological characteristics of the two groups of septic shock patients

Characteristics	**Survivors (*n* = 8)**	**Non-survivors (*n* = 8)**
Age (years)	60 (±16.5)	62.9 (±18.5)
Sex: male/female (*n*)	3/5	4/4
Creatinine (µmol/L)	145.4 (±105.1)	132.1 (±77.3)
Arterial lactates (mmol/L)	16.7 (±34.9)	7.8 (±6.0)
Bilirubin (µmol/L)	71 (±83)	66 (±63)
Prothrombin ratio (%)[1]	43 (±19)	47 (±24)
SOFA	11.75 (±2.5)	12.4 (±4.5)
SAPS II	56 (±20.8)	62.1 (±14.5)
CRP (mg/L)	169 (±83)	164 (±144)
Albumin (g/L)	18.4 (±4.9)	17 (±7.9)
Cortisol levels (µg/dL)		
Basal	25.7 (±14.9)	41.1 (±33.8)
Peak	25.8 (±17.5)	44.5 (±30.9)
Sites of infection (*n*)	12 sites	16 sites
Chest	6	11
Peritoneal	4	1
Urogenital	0	1
Septicemia	1	2
Wound	1	0
Central nervous system	0	1
Pathogens, Gram-negative (*n*)	4 (50%)	5 (62.5%)
Presumable digestive bacterial translocation (*n*)	6	6

[1] Value at H24

Values are mean (±SD) or quantitative number (*n*)

None of the characteristics were significantly different between the 2 groups (Chi 2 for qualitative data and *t*-test for quantitative data).

Table 3. Plasma citrulline, glutamine, and arginine over the study period

Plasma level (mean, SD)	**H 0**		**H 6**		**H 12**	
	Survivors	*Non-survivors*	*Survivors*	*Non-survivors*	*Survivors*	*Non-survivors*
Citrulline (N: 30–50 μmol/L)	23 (12)	24 (15)	25 (15)	20 (16)	19 (12)	20 (13)
Glutamine (N: 480–670 μmol/L)*	381 (69)**	598 (418)**	424 (256)	528 (280)	316 (166)**	562 (366)**
Arginine (N: 60–80 μmol/L)	37 (44)	53 (41)	49 (55)	39 (33)	44 (44)	49 (47)
	H 18		**H 24**		**H 96**	
	Survivors	*Non-survivors*	*Survivors*	*Non-survivors*	*Survivors*	*Non-survivors*
Citrulline	21 (12)	29 (32)	22 (12)	29 (18)	24 (8)	76 (76)
Glutamine*	335 (129)**	656 (493)**	335 (129)**	621 (312)**	427 (157)**	1224 (1037)**
Arginine	46 (40)	62 (48)	53 (38)	93 (48)	89 (45)	174 (152)

Values are mean (SD). * $P < 0.05$ (repeated measures ANOVA) between survivors and non-survivors, and $P < 0.05$ ** at different times.

Table 4: Plasma CRP, cytokines (TNFα and IL-10) and lactates over the study period

Plasma level	**H 0**		**H 6**		**H 12**	
	Survivors	*Non-survivors*	*Survivors*	*Non-survivors*	*Survivors*	*Non-survivors*
CRP (N < 5 ng/mL)	169 (83)	164 (144)	206 (110)	158 (110)	200 (132)	152 (108)
TNF alpha (N < 8.1 pg/mL)	32 (4–895)	42 (14–306)	27 (3–1100)	68 (14–212)	27 (6–1100)	56 (14-138)
IL-10 (N < 9.1 pg/mL)*	12 (4–400)	20 (4–1000)	6 (4–253)**	89 (12–718)**	5 (4–73)**	102 (9-311)**
TNF alpha/IL-10 ratio*	3.13 (0.21–223)	1.7 (0.3–11.8)	3.47 (0.18–275)	0.65 (0.21–3)	2.6 (0.38–275)**	0.82 (0.2-4.1)**
Lactates (N < 2.4 mmol/L)	16 (35)	7.8 (6)				
	H 18		**H 24**		**H 96**	
	Survivors	*Non-survivors*	*Survivors*	*Non-survivors*	*Survivors*	*Non-survivors*
CRP	203 (135)	133 (110)	190 (145)	75 (21)	84 (103)	84 (98)
TNF alpha	24 (6–810)	46 (14–102)	24 (4–627)	49 (14–72)	22 (4–569)	37 (32-47)
IL-10 *	10 (4–70)	39 (9–171)	8 (4–67)	47 (14–104)	4 (4–188)	17 (7.5-24)
TNF alpha/IL-10 ratio*	2.37 (0.27–72)	1.35 (0.32–3.77)	2.49 (0.2–156)	1.01 (0.47–.54)	1.95 (0.09–42)	2.78 (1.4-4.3)
Lactates			2.6 (1.7)	7.4 (7.5)	2.3 (1.8)	3.8 (3)

Values are mean (SD) for CRP and lactates and median (range) for TNF alpha, IL-10 and TNF alpha/IL-10 ratio.

* $P < 0.05$ (repeated measures ANOVA; non parametric tests for cytokines) between survivors and non-survivors, and $P < 0.05$ ** at different times.

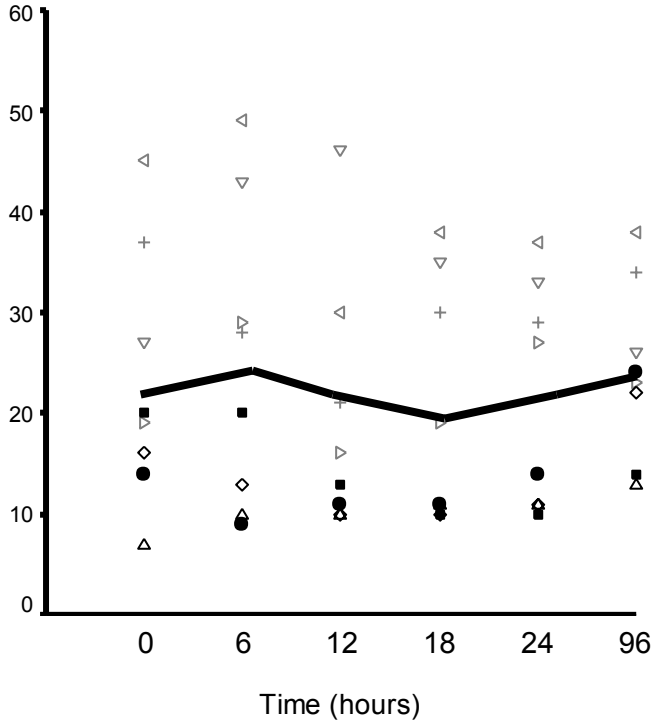
Table 5. Citrulline at nadir (D0) with other biological parameters collected at the same time, and bacterial digestive translocation

	Translocation (n = 12)	No translocation (n = 4)
Plasma citrulline nadir (μmol/L)	13±6*	29±7
Glutamine (μmol/L)	330±208	496±184
Arginine (μmol/L)	31±27	57±58
CRP (ng/ml)	189±129	110±103
TNFα (pg/mL)	42 (3–1100)	28 (21–69)
IL-10 (pg/mL)	16 (4–718)	56 (4–311)
TNFα/IL-10	1.7 (0.29–275)	0.38 (0.22–15)
Albumin (g/L)	19±8	16±2
Transthyretin (g/L)	0.11±10	0.05±0.01

Values are mean ± SD or median (range)
*P < 0.01 between translocation and no translocation

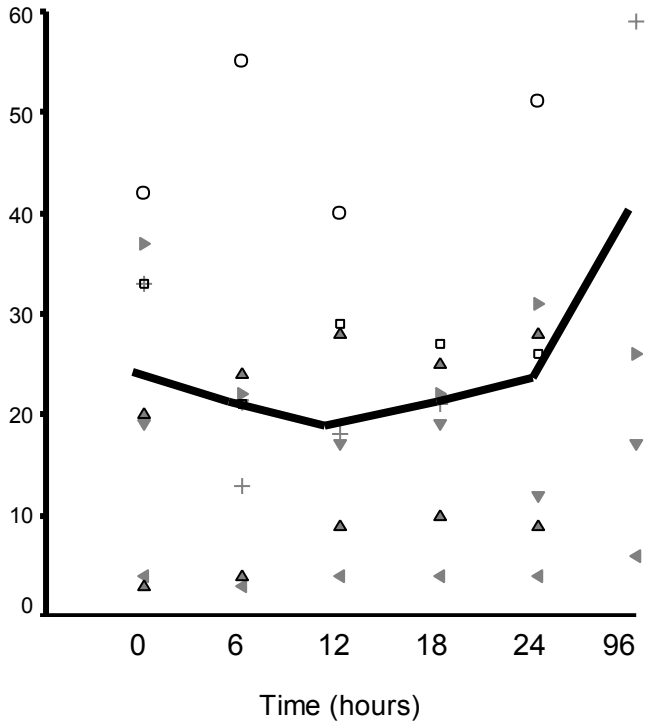
Figure:

Figure 1 A. Behavior of plasma citrulline concentration in the 8 septic shock survivor patients



Individual time course and median value at each time.

Figure 1 B. Behavior of plasma citrulline concentration in the 8 septic shock non survivor patients



Individual time course and median value at each time. Outliers are not on the Figure.

Figure 2. Relationship between plasma citrulline and CRP concentrations at H0 in survivor (n=8) and non-survivor (n=8) septic shock patients