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Continuous 4 Percent Albumin *Versus* Intermittent 20 Percent Albumin in Adults with Septic Shock: A Prospective, Phase IV, Open-label Randomized Trial

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Abstract: Whether a specific way of infusing albumin affects outcome in patients with major oxidative stress remains uncertain. To determine whether outcome measurements (survival, organ failure and care-related infections) are different according to different regimens of albumin infusion, we conducted a phase IV, randomized, open-label trial to compare the effects of continuous infusion of 4 percent albumin *versus* intermittent 20 percent albumin on outcome measurements in three third level-hospital intensive care unit (ICU) patients with septic shock. We randomly assigned 125 consecutive patients with septic shock when serum albumin became ≤ 20 g/L. Patients received either 4 percent albumin (12.5mL/kg) continuously or 20 percent albumin (100mL over 1h/8h) intermittently (controls) until serum albumin ranged between 25 and 30g/L and norepinephrine could be weaned. The primary outcome measure was death from any cause during the 28-day period after randomization. The other outcome parameters were ICU- and hospital length of stay, serum albumin concentrations, SOFA score and lactate over the 4 days following inclusion, care-related infections and tolerance of albumin over the 28-day period after randomization. Data were analyzed with Bayesian methods. Of the 125 patients who underwent randomization, 63 received 4 percent albumin and 62 received 20 percent albumin; groups had balanced baseline characteristics. There were 19 deaths in the experimental group, as compared with 20 in the control group (Pr=0.40). The proportion of patients with new multiple-organ failure (assessed by daily SOFA) was similar in the groups (RR=0.71 [0.29-1.41], Pr=0.14). There were no differences in the medians [IQR] numbers of days spent in the ICU (12.0 [7.5; 22.0] *versus* 13 [8.0; 24.5] days, Pr=0.23), in

days spent within hospital (29.0 [10.5; 44.0] *versus* 24 [14.0; 46.8] days, $Pr=0.32$). In contrast, there were fewer patients with care-related infection in the study group, (14.3% *versus* 45.2%, $Pr<0.001$). Limitations concern lack of double blinding related to different regimens of infusion: this may impact results. To conclude, the continuous supply of 4 percent albumin in septic shock patients with serum albumin ≤ 20 g/L decreases care-related infection (by two third) but does not result in better survival.

Keywords: Albumin, Care-related Infection, Outcome, Oxidative Stress, Septic Shock

1. Introduction

Meta-analyses about resuscitation using albumin in critically ill patients at risk of complications have concluded that human albumin solutions were not robustly effective at reducing all-cause mortality [1-3]. Attention has mainly been paid to fluid resuscitation of shock. These reports have left unsolved issues related to non-oncotic properties of therapeutic albumin [4], among which are timing of albumin administration, amounts of albumin to deliver, and the concentrations of the drug required to impact positively on outcome. Well-designed studies suggest that: 1) an early timing of albumin improves microvascular perfusion [5]; 2) 4 percent albumin be better for early fluid resuscitation [3] and 20 percent albumin for maintaining normal serum albumin concentrations [6] and at improving oxygenation in ARDS (acute respiratory distress syndrome) patients as well [7]. Hyper-oncotic colloid solutions *per se* do not injure the kidney [8], whereas 4 percent albumin worsens prognosis in patients with traumatic brain injury [9]. Whether any form of therapeutic albumin protects *in vivo* against infection is suggested in some studies [10, 11], but the benefit of albumin in septic conditions seems of borderline importance [2].

Serum albumin is reported as having a powerful antioxidant activity [4] whose expression is related to the preservation of its structural and functional activity [12]. Oxidative stress present at the acute phase of critical illnesses deteriorates the functional activity of albumin and is associated with increased mortality [13, 14]. We took into account that, in patients requiring volume resuscitation and norepinephrine for acute circulatory failure, endogenous albumin may no longer display proper antioxidant and scavenging activities, binding and transport of substances, regulation of endothelial function and of the systemic inflammatory response [15]. In this setting, acute and short-term infusions of therapeutic albumin increase serum albumin concentrations, but whether - and how long - exogenous albumin remains functionally efficient for non-oncotic properties is unknown [16]. An antioxidant being “any substance that, when present at low concentration, compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate” [17], we set the hypothesis that a long lasting infusion of small amounts of albumin may permanently preserve some low amounts of non-oxidized antioxidant albumin available for physiological purposes rather than would do a large and acute supply. Our group has recently reported such a possibility for the restoration of activity of the CGA-derived peptide Vasostatin-I whose functional activity is lost by oxidation

[18]. Finally, a rapid supply of large amounts of albumin during full blown oxidative stress will also necessarily end up in large amounts of oxidized albumin potentially related to pathological conditions induction [4].

The objective of the present trial was to evaluate the effect of low and continuous 4 percent albumin supplementation as an anti-oxidant drug in critically ill patients with acute circulatory failure requiring the infusion of norepinephrine and developing acute albumin ≤ 20 g/L when compared to a standard strategy of hyper oncotic albumin administration intermittently. Our *a priori* hypothesis was that this supplementation would impact on outcome. We present herein the final analysis of a randomized trial which was stopped for futility on mortality and for an ethical issue linked to unexpected good impact of the experimental treatment on a secondary outcome (care-related infection occurrence).

2. Methods

2.1. Study Oversight and Design

After approval by legal administrative authorities (Comité de Protection des Personnes Est-IV, and Agence Nationale de Sécurité du Médicament, both in 2016), this study was performed in two university-affiliated departments of either medical critical care (30 beds) or surgical (18 beds) critical care, and in one medical critical care department of a third-level referral hospital (22 beds). Details of the ALBALSACE study are available at Clinicaltrials.gov: NCT02755155 [19].

2.2. Patient Selection (Inclusion and Exclusion Criteria)

As indicated in Figure 1, 128 consecutive adults who were admitted to participating ICUs with shock (acute circulatory failure and lactate >2 mmol/L) requiring the infusion of norepinephrine after correction of hypovolemia with saline, and developing subsequently acute serum albumin ≤ 20 g/L were screened for treatment allocation. Written informed consent was obtained for all included patients according to an emergency consent by a next of kin (and confirmed *a posteriori* by the patient himself in case of survival). The inclusion criteria were diagnosis of acute circulatory failure from suspected septic origin with mean arterial pressure <60 mm Hg, ongoing norepinephrine at any dosage to maintain blood pressure as required by guidelines [20], and occurrence of a serum albumin concentration ≤ 20 g/L. The exclusion criteria were allergy to human albumin, traumatic brain injury, pregnancy, age <18 years, chronic hypoalbuminemia (as met in undernutrition or nephrotic syndrome), patients

under guardianship or curators, and patients given therapeutic albumin in the month preceding inclusion.

2.3. Study Treatments

Before inclusion, patients were treated according to early goal-directed therapy and fluid resuscitation was performed as indicated by the attending physician with the exclusion of colloids and albumin. No other drug was forbidden. At inclusion, using a centralized randomization, stratified by center, with blocks of size 8, we randomly assigned patients to receive either 12.5mL/kg of 4 percent albumin infused continuously over 24h (treatment group) or 20 percent albumin intermittently (100mL over 1h, every 8h (control group), from inclusion to the day when norepinephrine was weaned. During the albumin treatment, daily serum albumin concentrations were measured so that serum albumin be maintained not higher than 30g/L. Therapeutic albumin was supplied by the pharmacy from each institution, and purchased at LFB Biomedicaments (Les Ulis, France) either as Vialebex^R 200mg/mL, bottle of 100mL, or Vialebex^R 40mg/mL, bottle of 500mL. The rate of norepinephrine infusion was set to maintain mean arterial pressure above 65mm Hg, and weaning of this drug was tested hourly. Of importance is the fact that every patient was given antibiotics for a presumed infection on inclusion in both groups. Finally, all patients were under sedation for ventilation support at first albumin.

2.4. Outcomes Measures

The primary outcome measure was all causes mortality rates at 28 days after inclusion. Secondary outcome measures were: 1) the length of both circulatory failure (measured as time from starting to stopping norepinephrine) and the length of albumin treatment (measured from first albumin to end of last albumin infusion); 2) the daily SOFA score change from inclusion to norepinephrine weaning; 3) the incidence of care-related colonization and infection within the hospital; 4) the amount of albumin and its tolerance in each group.

2.5. Baseline Assessment

We obtained baseline information on age, gender, causes of referral and severity of the disease. The SAPS II was calculated from the worst values in the 24h of admission [21] and the SOFA [22] both on day of admission, day of inclusion and throughout the 96h thereafter. We recorded the simultaneous lactate and creatinine. For the purpose of this study, we collected - on inclusion and then each 3 days from inclusion to day 28 - blood samples, lower respiratory tract, urines and skin (or abdominal) samples to check the presence of microbes (bacteria, fungi) and to detect care-related infection. Respiratory samples were only obtained as long as intubation was present and/or a bronco-alveolar lavage was required. When in-dwelling catheters were removed, they were sent for microbial culture. Care-related infections were defined as episodes with clinical signs of focal infection occurring at least after two days of inclusion in the protocol,

with fever ($>38^{\circ}\text{C}$) or hypothermia ($<35^{\circ}\text{C}$), increased C-reactive protein ($>8\text{mg/L}$, or an increase by 50% of basal value) and the presence of a microbe at the focus of infection; to be definitively considered as a new infection, these episodes required also that antibiotics be adapted, surgery or drainage be performed to obtain improvement of the patient. Colonization was defined as the presence of bacteria or fungi in an expectedly sterile site, in the absence of a simultaneous increase in C-reactive protein and fever. Immune suppression was defined as any condition or treatment capable of decreasing immunity either by alteration of the functioning of neutrophils or lymphocytes.

2.6. Statistics

On the basis of the averaged hospital mortality rates of our three ICUs, and of our current rates of colonization and care-related infections, the calculated sample size was 550 patients (alpha level=0.05, 80% power, 20% versus 30% of mortality at Day 28). The study protocol planned interim analysis every 50 patients. All analyses were conducted on an intention-to-treat basis. Data are presented as medians [IQR] or as percentages (%). The between-group mean or percentage difference or relative risk (RR) are given with their 95% credibility interval and the probability that the value is larger in the 4 percent Albumin group than in the 20 percent albumin group (Pr). A Pr value near 1 indicates a larger value in the 4 percent group and a Pr value near 0 indicates a smaller value. Repeated data are analyzed with mixed models and count data with Poisson models. Parameters estimates are given with the 95% credibility interval.

For all analyses, the prior distribution were defined before the study and were based on mild assumptions derived from expert knowledge. In the interim analysis, we computed the predictive probability [23] of superiority of albumin 4 percent group over the 20 percent group. We remind that Bayesian methods do not use p-values and that Pr (see above) and the predictive probability must not be confused with p-values. All computations were done with R 3.5.3 and JAGS (Just Another Gibbs Sampler) 4.3.0.

2.7. Role of the Funding Source

The trial was funded by a competitive peer-reviewed grant from the Direction de la Recherche Clinique of our university hospital (Appel à Projets Internes-2016: Grant n°6371).

The funder had no role in study design, data collection, data analysis, data interpretation, or writing the report. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

3. Results

From January 2017 to December 2018, as indicated in the flow diagram of the study (Figure 1), 128 patients (out of 4777 patients screened on admission in the participating

ICUs) were randomly assigned to receive either continuous 4 percent albumin (12.5mL/kg over 24h, n=64) or intermittent 20 percent albumin (100mL over 1h/8h, n=64). Three patients, who were screened but did not receive any treatment, were excluded because of consent withdrawal. Therefore the intention-to-treat analysis included 63 patients in the 4 percent group and 62 patients in the control group.

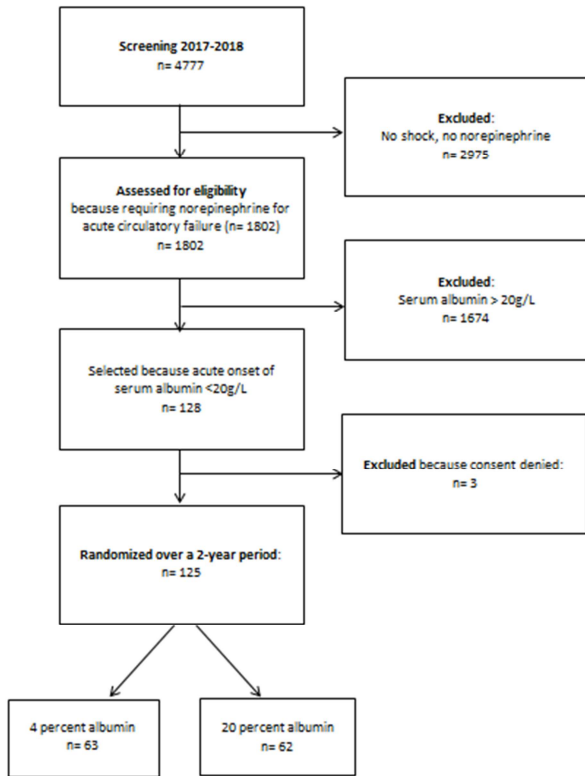


Figure 1. Flow diagram of the progress of the study.

Follow up: there were neither lost to follow-up nor discontinued intervention. Patients analyzed: n=125.

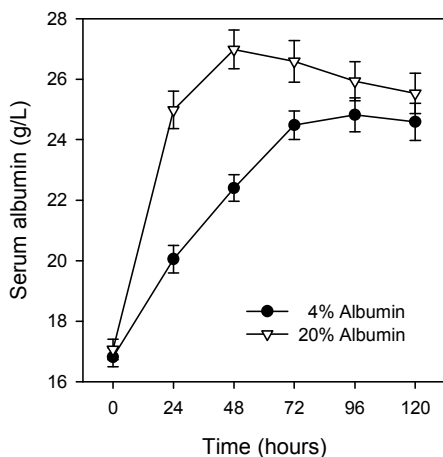


Figure 2. Time-dependent serum albumin concentrations during albumin infusion. To complete see p. 9.

At ICU admission, demographic, clinical, and laboratory features were balanced between the two groups (Table 1), although admission category was more frequently medical

than surgical. There were more chronic renal insufficiency patients in the 20 percent albumin group (Pr=0.02) (Table 1), and patients with solid cancer in remission >1 year were more represented in the 4 percent albumin group (Pr=0.99) (Table 1). At inclusion, the proportion of septic shock patients with positive microbial samples was significantly higher in the 20 percent group than in the 4 percent group (Pr=0.001) (Table 1).

The length of norepinephrine-infusion time was similar in both groups and so was the time length from norepinephrine treatment starting to the infusion of the first albumin (Table 2).

There was no group differences as far as duration from first albumin to peak of norepinephrine infusion is concerned (Table 2). Neither the duration from admission to first albumin infusion nor the duration from first albumin to last albumin was different between groups (Table 2). The median mass of albumin infused per patient was similar in both groups, but the volume of infusion was increased in the 4 percent albumin group (Table 2). Although the calculation of the treatment time by albumin - assessed from first albumin to last albumin infusion - is similar in both groups (Table 2), it must be kept in mind that the duration of exposition to non-oxidized albumin corresponding to the time interval from first albumin to last albumin in the 4 percent group, is only represented by 1h of infusion per bottle of 20 percent albumin in the control group: this leads to a shorter period of fresh albumin availability in this group (Table 2).

The intermittent infusion of 20 percent albumin was associated with a rapid increase in serum albumin concentrations in the range of 25-30g/L until day 4 when the serum albumin levels became similar in the two groups (Figure 2). At that time, according to the study protocol and data in Table 2, the infusion of norepinephrine had been stopped in both groups.

During the 4-day period following inclusion, there was no difference between groups in either SOFA decrease (time x treatment interaction 0.70 [-0.25; 1.64], Pr=0.93, Figure 3), in the plasma creatinine decrease (time x treatment interaction 8.83 [-10.2; 27.7], Pr=0.82, data not illustrated), or in the lactate decrease (time x treatment interaction 0.13 [-0.85; 1.11], Pr=0.60, Figure 4).

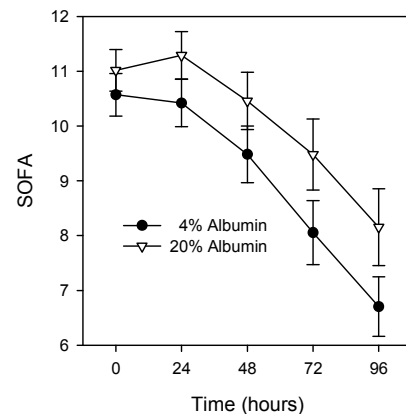


Figure 3. SOFA changes under treatment from inclusion to H96.

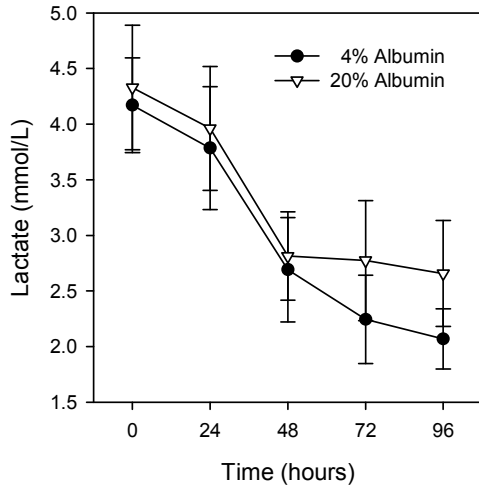
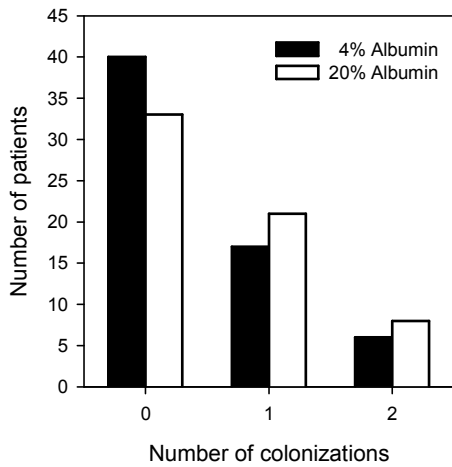
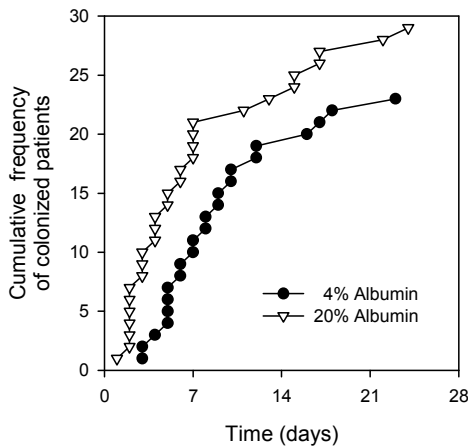


Figure 4. Lactate changes from inclusion to resolution of shock (weaning of norepinephrine, H96).

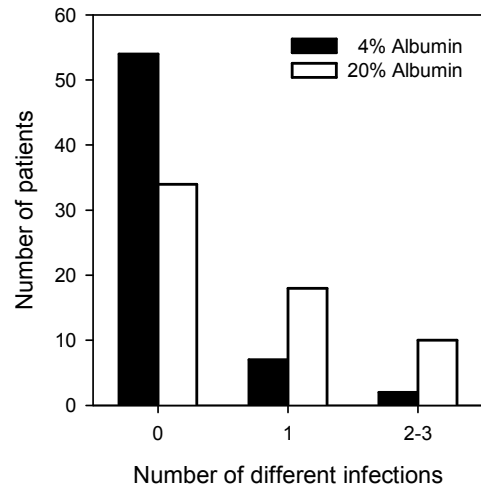
As far as colonization is concerned, there was no difference between groups (36.5% in the 4 percent group versus 46.8% in the 20 percent group (Pr=0.12, Figure 5 Panel a). However, there was a right shift of the occurrence of colonization in the 4 percent group during the ICU stay as shown in Figure 5 Panel b (RR=0.86 [0.47-1.43], Pr=0.252).



(a)



(b)



(c)

Figure 5. Occurrence of colonization and of care-related infections in the study population. To complete see p. 9.

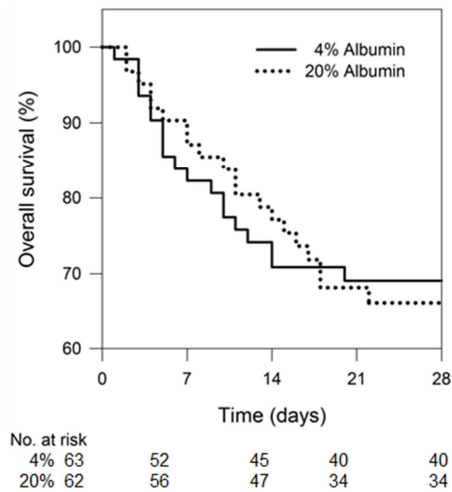


Figure 6. Overall survival to Day 28. To complete see p. 9, 10.

In addition, there were fewer subjects with nosocomial infections in the 4 percent group than in the 20 percent group (14.3% versus 45.2%, Pr<0.001, Figure 5 Panel c) with a smaller number of nosocomial infections between groups (RR=0.36 [0.18-0.61], Pr<0.001). The characteristics of nosocomial infections are summarized in Table 3. Bayesian predictive probability at interim analysis showed that the probability of a higher infection rate in the 20 percent albumin group by the end of the study is >0.999, with a risk difference estimated to be 29.9% [14.8-44.6], Pr>0.999.

The 28-day all-cause mortality rate was similar in both group (Table 4, Pr=0.40) and so was further mortality at Day-28 using the Kaplan-Meier estimates for the probability of overall survival in the intention-to-treat population (Figure 6). The Bayesian predictive probability analysis ruled out the possibility that the treatment by 4 percent albumin may reach the main objective at the final 550 inclusion-target, suggesting that the current study be stopped for futility (the risk difference is 2.0% [-17.9, 14.0] and the predictive probability to conclude to a larger death rate in the 20 percent

group on final sample target is only 0.402.

Table 1. Characteristics of the patients.

At ICU admission	4% Albumin (n=63)	20% Albumin (n=62)	Pr
Age – yr	68.0 [58.5-75.5]	68.0 [58.2-74.0]	0.58
Male gender - no. (%)	41 (65.1)	37 (59.7)	0.73
Body Mass Index	26.0 [23.1-29.6]	25.2 [23.2-30.6]	0.71
Admission category – no. (%)			
Medical	39 (62)	53 (85.5)	0.001
Surgical (emergency surgery)	24 (38)	9 (14.5)	
SAPS II score	58.0 [50.0 -70.8]	60.0 [50.2-75.0]	0.30
SOFA	9.0 [6.0 -12.5]	9.0 [7.0 - 12.0]	0.16
Arterial lactate	3.1 [1.9 -5.1]	2.7 [1.9 - 4.8]	0.53
Albumin (g/L)	18.0 [15.5 - 18.5]	17.5 [16.0 - 19.0]	0.23
Presence of shock no. (%)			
Yes	61 (96.8)	56 (90.3)	0.92
No	2 (3.2)	6 (9.7)	
Comorbidities - no. (%)			
Pulmonary diseases (COPD, asthma, OAS, miscellaneous)	18 (28.6)	25 (40.3)	0.08
Chronic heart diseases (coronary heart disease, left ventricular failure, arrhythmia...)	31 (49.2)	12 (19.4)	>0.99
Hypertension	35 (55.5)	40 (64.5)	0.16
Metabolic diseases (diabetes, hypercholesterolemia, thyroid dysfunctioning...)	38 (60.3)	36 (58.1)	0.60
Chronic renal failure (<30mL/min)	5 (7.9)	13 (21.0)	0.02
Malignant hemopathy:			
no aplasia	7 (11.1)	4 (6.5)	0.81
with aplasia	7 (11.1)	10 (16.1)	0.21
Solid cancer (remission > 1yr)	6 (9.5)	0 (0)	0.99
Recent cancer (< 1 yr; no aplasia)	17 (27.0)	9 (14.5)	0.96
Immunosuppressive therapy	6 (9.5)	13 (21.0)	0.04
Cirrhosis	11 (17.5)	14 (22.6)	0.24
At study inclusion			
SOFA	10 [8-13]	11 [9-13]	0.21
Albumin (g/L)	18.0 [15.5 - 18.5]	17.5 [16-19]	0.29
Serum creatinine (µmol/L)	127.0 [80.0-183.5]	114 [73.5-182.0]	0.88
Arterial lactate (mmol/L)	2.7 [1.8 - 5.5]	2.5 [1.7 - 5.4]	0.41
Cause of shock no. (%)			
Septic shock with positive microbial samples	39 (61.9)	51 (82.3)	0.001
Septic shock without positive microbial samples	14 (38.1)	11 (17.2)	

Data are medians [IQR] or frequency (%). SOFA=Sequential Organ Failure Assessment; SAPS II: Simplified Acute Physiologic Score two. To complete see p. 9.

Table 2. Characteristics of study treatments (albumin and norepinephrine).

	4 percent albumin (n=63)	20 percent Albumin (n=62)	Pr
Duration of norepinephrine support (h)	68 [50-141]	78 [37. 5-138.5]	0.87
Time from NE starting to first albumin (h)	16.8 [8.2 – 28.8]	18.8 [6.0 – 29.0]	0.57
1 st Albumin to peak of NE (length of time, h)	-3.8 [-11.8-4.9]	-2 [-13-4.0]	0.27
Admission to 1 st albumin (length of time, h)	23.5 [11.2 – 37.8]	24 [13 – 51.1]	0.21
Duration of albumin infusion (h)	68.5 [46.5 – 97.2]	56 [24.0 – 112.0]	0.92
Volume of albumin infused (mL/patient)	4000 [2000-6000]	1000 [600 - 1600]	1
Total albumin mass infused (g/patient)	160 [80-240]	200 [120-320]	0.61
Albumin exposure per hour of treatment (g/h)	2.3 [1.8 – 3.1]	3.8 [2.7 – 6.0]	0.001
Duration of fresh albumin exposure (h)	68.5 [46.5 – 97.2]	10.0 [6.0 – 16.0]	>0.999

Data are medians [IQR] or frequency (%).

SOFA: Sequential Organ Failure Assessment; SAPS II: Simplified Acute Physiologic Score two. NE: norepinephrine. To complete see p. 9.

Table 3. Care-related infections according to the arm of treatment (4 percent versus 20 percent albumin).

4 percent or 20 percent Albumin	Site of infection	Species involved	Length of time from first albumin to infection (days)
4	Blood	<i>S. epidermidis</i>	12
4	Blood	<i>S. epidermidis</i> & <i>C. albicans</i>	7
4	Blood	<i>S. epidermidis</i>	12
4	Surgical site (abdomen)	<i>E. faecium</i>	18
4	Blood & lower respiratory tract	<i>E. coli</i> & <i>E. faecium</i>	12
4	VAP	<i>P. aeruginosa</i> & <i>Imipenem R-A. baumannii</i>	3
4	Lower respiratory tract	<i>C. glabrata</i> & <i>C. albicans</i>	5
4	Surgical site (abdomen)	<i>C. glabrata</i> , <i>C. jeikeium</i> & <i>L. mucosa</i>	8

4 percent or 20 percent Albumin	Site of infection	Species involved	Length of time from first albumin to infection (days)
4	Blood	<i>S. aureus</i>	12
20	Blood	<i>C. glabrata</i>	4
20	VAP and Blood	<i>K. oxytoca</i> , <i>E. faecium</i> & <i>C. albicans</i>	17
20	Urine	<i>E. faecalis</i> & <i>H. alvei</i>	24
20	Blood	<i>C. parapsilosis</i>	14
20	Blood stream and lower respiratory tract	<i>P. aeruginosa</i> & <i>S. maltophilia</i>	18
20	Surgical site (biliary tract)	<i>E. faecium</i> & <i>S. epidermidis</i>	7
20	Surgical site (abdomen)	<i>E. faecium</i> & <i>E. aerogenes</i>	8
20	Blood	<i>S. aureus</i>	28
20	Lower respiratory tract	<i>S. epidermidis</i>	3
20	VAP	<i>P. aeruginosa</i>	7
20	Surgical site (biliary tract)	<i>E. cloacae</i> & <i>S. epidermidis</i>	3
20	VAP	<i>E. coli</i>	15
20	VAP	<i>P. aeruginosa</i>	3
20	Lower respiratory tract	<i>S. haemolyticus</i>	11
20	Urine	<i>S. maltophilia</i>	7
20	VAP	<i>E. coli</i> , <i>E. faecalis</i> & <i>Methicilin-R S. aureus</i>	8
20	Surgical site (abdomen)	<i>E. faecalis</i>	15
20	VAP & urine	<i>E. cloacae</i> , <i>P. maltophilia</i> & <i>P. aeruginosa</i>	7
20	Blood & catheter	<i>C. albicans</i>	6
20	VAP	<i>Pseudomonas aeruginosa</i> & <i>C. freundii</i>	4
20	VAP	<i>P. aeruginosa</i>	7
20	VAP	<i>S. marcescens</i>	25
20	VAP	<i>E. faecalis</i> & <i>C. freundii</i>	4
20	VAP	<i>E. faecalis</i>	24
20	Surgical site (abdomen)	<i>K. oxytoca</i> & <i>E. cloacae</i>	2
20	Surgical site (abdomen)	<i>S. aureus</i>	4
20	VAP	<i>E. faecalis</i> & <i>E. coli</i>	4
20	Lower respiratory tract	<i>C. argentoratense</i> & <i>H. simplex virus</i>	4
20	Blood & catheter	<i>Methicilin Resistant S. aureus</i>	20

VAP=ventilator-associated pneumonia were diagnosed using bronco-alveolar lavage fluid analysis and X-Ray changes. To complete see p. 9.

Table 4. Outcomes.

	4 percent Albumin (n=63)	20 percent Albumin (n=62)	Pr
Status at 28 days (n, %)			
Dead	19 (30.2)	20 (32.3)	0.40
Alive but still in ICU (n, %)	9 (14.3)	13 (21.0)	0.17
Alive in hospital	44 (69.8)	42 (67.7)	0.60
Duration of ICU stay (days)	12 [7.5-22.0]	13 [8.0-24.5]	0.23
Duration of hospital stay (days)	29 [10.5-44.0]	24 [14.0 -46.8]	0.32
Care-related infections			
Colonization (number of patients)	23 (36.5)	29 (46.8)	0.12
Care-related infections (number of patients)	9 (14.3)	28 (45.2)	<0.001

Data are medians [IQR] or frequency (%).

ICU: intensive care unit. To complete see p. 9.

4. Discussion

In this randomized trial involving real-life practice critically ill patients with septic shock (including patients with aplasia), and developing thereafter acute hypoalbuminemia, the continuous infusion of 4 percent albumin was associated with less care-related infections when compared to intermittent 20 percent albumin. No impact on in-hospital survival or tolerance to albumin was detected.

The most seriously critically ill patients have increased mediators of oxidant stress and a higher incidence of both multiple organ failure and nosocomial infections [24]. The occurrence of the latter is associated with increased morbidity, costs of treatments and with the emergence of

threatening bacteria and fungi that are resistant to available antimicrobial drugs [25]. To improve outcome in such patients in addition to conventional precautions, we hypothesized that the restoration of the endogenous functional status of proteins belonging to innate defense would benefit most importantly the ICU-patients with the lowest serum albumin which are prone to care-related infections [26]. Reversing the oxidative stress-related loss of activity of such proteins by anti-oxidant albumin is possible *in vitro* as far as antimicrobial peptides are concerned with very low amounts of albumin [18]. *In vivo*, to restore total plasma antioxidant capacity [27] an adequate concentration ratio between the oxidized substrate and the anti-oxidant agent has to be achieved [17, 28]. Accordingly, we performed this study comparing the continuous supply of non-oxidized

4 percent albumin [28] with that of intermittent albumin during the acute phase of septic shock which is a model of severe oxidative stress. In our study, the serum albumin concentrations achieved in the experimental arm were constantly lower than in the control arm before the end of acute circulatory failure (which can be dated at the moment when norepinephrine was weaned). Before this trial, we had performed an *in vitro* assessment of the best concentration of albumin capable of displaying an efficient anti-oxidative power *in vivo* on oxidized Vasostatin-I, an endogenous antimicrobial peptide: this pilot dose-finding study suggested a possible effect in the range of 10-15mL/kg of 4 percent albumin per day, which additionally excluded harmful side-effects with reference to recommendation for albumin prescription in humans [18].

In our patients, ICU-mortality and medium term mortality are in the range of those of other septic shock populations in the present era [23, 29-30], and even lower than expected from both the SAPS II score, the admission SOFA and the comorbidities (which included many pathophysiological conditions with at least one immune defect). For long times, the question of why these patients who additionally displayed low serum albumin concentrations have been at high risk of developing care-related infection has not been answered satisfactorily [31]. Our results prove that the continuous infusion of 4 percent albumin was not able to modify mortality when compared to 20 percent albumin. This had also no impact on daily SOFA changes, which suggests the absence of effect on acute circulatory failure, and on each and every organ failure included in this score as well. Finally, lactate decreased to normal range without study group-differences suggesting that both albumin treatments impacted similarly on global organ perfusion. Thus, our data are in agreement with meta-analyses that indicate that the therapeutic supply by albumin for the maintenance of a properly working circulation can poorly be recommended to improve mortality [1-3]. Similarly, 4 percent albumin did impact neither on ICU nor on hospital length of stay as previously suggested [1, 3].

In contrast, our experimental treatment impacted tangibly on nosocomial infection-related morbidity. Although there was no impact of 4 percent albumin on the number of patients getting colonized during the hospital stay, we noted a difference between 4 percent and 20 percent albumin-treated patients as far as time of colonization is concerned. Microbes-related events occurred as if either 4 percent albumin delays the occurrence of colonization or as 20 percent albumin shortens the time of colonization onset; furthermore, this is occurring within the ICU, not later, most possibly because systematic samplings of microbes was performed rigorously in the ICU - in each and every site of potential infection - whereas it was no longer performed in the airways after ICU-discharge. A decreased rate of early colonization cannot be explained in one arm of treatment by broad spectrum antibiotics given at the acute phase of septic shock because this treatment was present in all patients at inclusion. Thus, our observations are in accordance with our

working hypothesis and suggest that a pharmacological manipulation of innate defense altered by endogenous stress-related oxidation is possible.

A handful of studies suggest the existence of a positive effect of albumin on infection rate lowering in selected cohorts of patients [10, 32]. The mechanism (s) by which the 4 percent albumin is associated with less infection than 20 percent is, remains unknown. Rarely, albumin has been reported as an antimicrobial agent with antifungal property related to its structure [33]; also, less colonization by fungi is reported to lower the risk of care-related infections [34]. However, the very rare disorder "congenital analbuminemia" has remarkably mild signs and symptoms at all ages without any association with recurrent infections [35]. Therefore, it seems reasonable to propose that a functional and/or indirect mechanism rather than an intrinsic property of albumin may explain our data on the lessening of care-related infection incidence. Posttranscriptional changes of oxidized serum albumin are independently associated with bacterial infections in patients with cirrhosis [36]; in this study, ischemia-modified albumin is reported as elevated in patients with cirrhosis and hyperdynamic circulation without shock [36] and that such an alteration is also associated with bacterial infections. More recently, Caraceni *et al.* confirmed *in vivo* that a long-term, low dose albumin administration in decompensated cirrhosis is associated with a significant decrease in spontaneous bacterial infection of ascites but also in other infections, thus confirming the possibility that less albumin than required to normalize the serum concentration range is good for the prevention of infection (32).

The question of why hyperoxidized albumin does not further protect patients as native albumin does has also been addressed in severe alcoholic hepatitis patients who often undergo systemic inflammation: in fact, such albumin modulates neutrophils to further induce oxidative stress and inflammation with a subsequent increased risk of overwhelming bacterial infections (37). Conversely, the effect of albumin on infection prevention whatever the type or origin of infection has never been assessed as a primary objective in a clinical trial involving critically ill patients: more specifically, no study has ever compared 4 percent albumin *versus* 20 percent albumin probably because a consistent rationale for such a strategy was lacking. Proven in further studies, this concept of treatment may be interesting for large populations of patients at risk of systemic inflammation whether acute or chronic [38].

Our trial has limitations. The most common is that health care professionals were aware of the treatment assignments in agreement with the protocol of infusion. The lack of blinding may have improved the attendance to hygiene rules and thereby lowered the incidence of nosocomial infections, although we did not observe such a trend in other patients treated simultaneously in our ICUs. To compensate for this risk, we chose deliberately to include patients with severe underlying comorbidities (*ie*: post-chemotherapy aplasia or immunosuppressive drugs for transplantation) which are spontaneously at high risk of nosocomial infection. The real

impact of the lack of blinding on estimated treatment effects has however recently been questioned and may be less important than previously thought [39]. Finally, our study was stopped on the a planned interim analysis because 1) Bayesian statistics on the primary endpoint suggested futility and 2) for ethical issues based on a secondary endpoint improvement which was not expected to be of such an importance when starting the trial.

5. Conclusions

In critically ill patients undergoing an acute oxidative stress who also display recent-onset serum albumin concentrations of 20g/L or lower, a strategy of early and continuous infusion of 4 percent albumin at anti-oxidant dosage is associated with less care-related infection, but it does not result in a mortality rate decrease at 28 days. Large randomized clinical trials are required to validate our findings in other oxidative stress-related disorders before changing clinical practice in other settings.

Caption Figure 2: Serum albumin changes over 120 hours of treatment either by 4 percent albumin (black circles) or by 20 percent albumin (white triangles). Data are means +/- SEM.

Caption Figure 5: The proportion of colonization was similar in the two arms of treatment by albumin (Panel a, $\text{Pr}(4\%>20\%)=0.12$). As indicated in Panel b, the cumulative frequency of colonized patients was shifted rightwards in the 4 percent albumin treated patients when compared to controls. Care-related infections were significantly decreased in the 4 percent albumin group (Panel c) when compared to the controls ($\text{Pr}(4\%>20\%)<0.001$).

Caption Table 1: Bayesian methods do not use p-values; Pr must not be confused with p-values. Two values of Pr are of interest: value near 1 for the 4 percent group and the value near 0 for the 20 percent group.

Caption Table 2: The third column corresponds to the posterior probability that group 4 is higher than group 20, for the mean or percentage difference. Bayesian methods do not use p-values; Pr must not be confused with p-values. Two values of Pr are of interest: value near 1 for the 4 percent group and the value near 0 for the 20 percent group.

Caption Table 4: Bayesian methods do not use p-values; Pr must not be confused with p-values. Two values of Pr are of interest: value near 1 for the 4 percent group and the value near 0 for the 20 percent group.

Caption Table 3: Lower respiratory tract infections were diagnosed using tracheal fluid aspiration and ventilator-associated pneumonia using bronco-alveolar lavage. Blood stream infection was diagnosed using commercially available culture vials (BD Bactec™, USA). Positive catheter culture was defined by a catheter tip culture performed with the Brun-Buisson technique yielding $\geq 10^3$ colony-forming units/mL. Sampling on operative sites was performed by surgeons within the operating theater. In a few instances, infection has been related to fungi because only these microbes were found *in situ*, and also because antifungal

drugs were necessary to full recovery of the patient: this cannot rule out that the focus of infection could have been triggered by a viral species (see for instance patient 7).

Caption Figure 6: Kaplan-Meier estimates for the probability of overall survival in the intention-to-treat population. Dotted line=20 percent albumin treated patients; black line=4 percent albumin-treated patients. There was no difference in survival time within 28 days after inclusion.

Supplementary Informations

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Clinical Trial

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Conflict of Interest

None declared.

Declaration of Interests

None.

Author Contributions

FS designed the protocol, was study chief investigator, reviewed the data, including interpretation of the results and co-wrote the manuscript. VC and GM ran the study at the Hautepierre I hospital, and made intellectual input in the study protocol and manuscript writing. A-F D and A P ran the study at the Mulhouse hospital (GHMSA), whereas Pierre-Olivier Ludes ran the study at the Hautepierre II hospital. BS and TF checked the data and performed statistical analyses, NM co-designed the study protocol, designed the statistical plan, supervised the analyses, and co-wrote the manuscript. M-H M-B co-wrote the study protocol and the manuscript, reviewed the data, including interpretation of the results.

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