BMJ Open

Efficacy of coupled plasma filtration adsorption (CPFA) in patients with septic shock: A multicenter randomised controlled clinical trial

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To cite: Livigni S, Bertolini G, Rossi C, *et al*. Efficacy of coupled plasma filtration adsorption (CPFA) in patients with septic shock: A multicenter randomised controlled clinical trial. *BMJ Open* 2014;**4**:e003536. doi:10.1136/bmjopen-2013-003536

▸ Prepublication history and additional material for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2013-003536).

Received 17 July 2013
Revised 9 December 2013
Accepted 11 December 2013

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ABSTRACT

Objectives: Coupled plasma filtration adsorption (CPFA, Bellco, Italy), to remove inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomised, non-blinded trial compared CPFA with standard care in the treatment of critically ill patients with septic shock.

Design: Prospective, multicenter, randomised, open-label, two parallel group and superiority clinical trial.

Setting: 18 Italian adult, general, intensive care units (ICUs).

Participants: Of the planned 330 adult patients with septic shock, 192 were randomised to either have CPFA added to the standard care, or not. The external monitoring committee excluded eight ineligible patients who were erroneously included.

Interventions: CPFA was to be performed daily for 5 days, lasting at least 10 h/day.

Primary and secondary outcome measures: The primary endpoint was mortality at discharge from the hospital at which the patient last stayed. Secondary endpoints were: 90-day mortality, new organ failures and ICU-free days within 30 days.

Results: There was no statistical difference in hospital mortality (47.3% controls, 45.1% CPFA; p=0.76), nor in secondary endpoints, namely the occurrence of new organ failures (55.9% vs 56.0%; p=0.99) or free-ICU days during the first 30 days (6.8 vs 7.5; p=0.35). The study was terminated on the grounds of futility. Several patients randomised to CPFA were subsequently found to be undertreated. An a priori planned subgroup analysis showed those receiving a CPFA dose >0.18 L/kg/day had a lower mortality compared with controls (OR 0.36, 95% CI 0.13 to 0.99).

Conclusions: CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested that CPFA could reduce mortality, when a high volume of plasma is treated. Owing to the inherent potential biases of such a subgroup analysis, this result can only be viewed as a hypothesis generator and should be confirmed in future studies.

ClinicalTrials.gov: NCT00332371; ISRCTN24534559.

INTRODUCTION

The host response against pathogens is a complex one. It is modulated through the production of numerous mediators that, among other mechanisms, promote proinflammatory and anti-inflammatory responses.[1–4] The balance between these two pathways heavily influences the outcome.[4–9] The amount and timing of release of different mediators, their relatively short half-lives, their limited range of action, their considerable redundancy and pleiomorphisms and the underexpression or overexpression of their receptors[1 10–12] have negatively affected the numerous therapeutic attempts to neutralise specific molecules.[12] The repeated failure of this strategy suggested a potentially greater utility may be achieved through simultaneous removal of several mediators to rebalance the immune response. This can be accomplished by various blood purification techniques, of which coupled plasma filtration adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators.[13]

Early experience with CPFA showed an increased survival in a rabbit model of endotoxin-induced septic shock.[14] The first clinical study showed that a single treatment lasting 10 h significantly improved the haemodynamic status.[15] These preliminary observations were confirmed in a study of 10 septic shock patients in whom norepinephrine requirements were progressively reduced and eventually discontinued after an average of

five daily CPFA sessions,[16] without adverse events. Subsequently, several Italian ICUs adopted CPFA in septic shock patients with promising results, and were willing to formally evaluate its efficacy. GiViTI, the Italian ICU network, thus launched a randomised multicentre clinical trial to assess the efficacy of CPFA in reducing mortality of critically ill patients with septic shock.

METHODS

Written consent was obtained from the patient when possible; otherwise physicians enroled patients according to the article 4.8.15 of the Guidelines for Good Clinical Practice.[17].

Setting and participants

The study was performed in 18 adult ICUs who regularly used CPFA in the treatment of septic shock. Patients >18 years of age with septic shock either at or during their admission to the ICU were eligible for study entry, provided that CPFA could be started within 6 h from the occurrence of hypotension refractory to fluid resuscitation. This was made by the attending physician (present 24/7) using explicit criteria.[18] Reasons for exclusion prior to randomisation were: pregnancy, cardiopulmonary resuscitation, coma (GCS≤8) due to an organic cerebral disease, metastatic cancer, contraindication to a haemopurification technique, an estimated life expectancy less than 2 weeks, prior inclusion in the study, admission from another ICU where the patient remained for >24 h and lack of informed consent.

The Project Margherita electronic case report form (eCRF) was used for this study.[19 20] The core data included demographics, admission diagnoses, severity of infection on admission, comorbidities, location of the patient prior to ICU admission, surgical status, reasons for ICU admission, Simplified Acute Physiology Score II (SAPS II) variables[21] on admission, organ failures and diseases occurring during their ICU stay, the severity of infection reached, major procedures and interventions and ICU and hospital outcomes. For enroled patients, their clinical conditions, including the sequential organ failure assessment (SOFA) score,[22] the Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage kidney disease (RIFLE) criteria[22a] for acute renal dysfunction and CPFA parameters were collected at the time of randomisation and then daily until ICU discharge or for a maximum of 21 days. Interventions to assure study homogeneity and quality are described in the online supplementary material.

Randomisation and interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enrol the patient or to register reasons for not doing so. Once enroled, the patients were randomly allocated by the eCRF on a 1 : 1 basis to either have CPFA added to the standard care, or not. A blocked randomisation schedule (randomly permuting blocks of four and six)[23] was implemented in the eCRF, with stratification according to the centre and the presence of septic shock on admission. The allocation was securely saved in the database and revealed only once baseline additional data collection were completed. All these procedures were implemented to guarantee allocation concealment.[24]

Coupled plasma filtration adsorption

CPFA was developed to non-specifically remove larger mediators during systemic inflammation with an extracorporeal circuit consisting of a plasma filter, a resin cartridge and a high-flux dialyser.[25]

CPFA was performed with the use of a four-pump modular treatment (Lynda, Bellco, Mirandola, Italy) consisting of a plasma filter (0.45 m^2 polyethersulfone) and a following absorption on an unselective hydrophobic resin cartridge (140 mL for 70 g, with a surface of about 700 m^2/g) and a final passage of the reconstituted blood through a high-permeability 1.4 m^2 polyethersulfone haemofilter, in which convective exchanges may be applied in a postdilution mode (figure 1).[26]

The postdilution reinfusion rate could be set up to 4 L/h. The blood flow was maintained between 150 and 200 mL min, while the plasma flow was controlled by a filtration fraction ranging from 10% to 18% of blood flow.[27] More specifically, the filtration fraction should be set to 10% in the first hour and then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 L, corresponding to a blood flow of 150 mL min and a filtration fraction of 12%.

The reinfusion solution, sterile and pyrogen-free, with bicarbonate buffer, contained the following composition (mmol L): Na 140, K 1.5, Ca 2, Mg 0.75, Cl 108, bicarbonate 35, acetate 4 and glucose 5.55.

All fluids were administered at room temperature. During treatment, the patient's temperature was to be maintained possibly within physiological limits, and anyway higher than 35°C. The anticoagulation protocol is described in the online supplementary material.

According to the available clinical evidence, CPFA was to be repeated daily for the first 5 days, lasting at least 10 h each time, so that an average of 0.15 L/kg/day of plasma should have been treated per day.

Outcomes, follow-up and plan of analysis

The primary endpoint was mortality at discharge from the hospital in which the patients were last treated. Thus, for patients transferred to another hospital, mortality was assessed at the discharge from the hospital in which the patients last stayed. To minimise the bias due to the decision to have the relative dying at home, patients discharged in a terminal condition (life expectancy <2 weeks as estimated by the attending physician) were considered to have died at the time of hospital discharge. The primary analysis was by intention-to-treat;

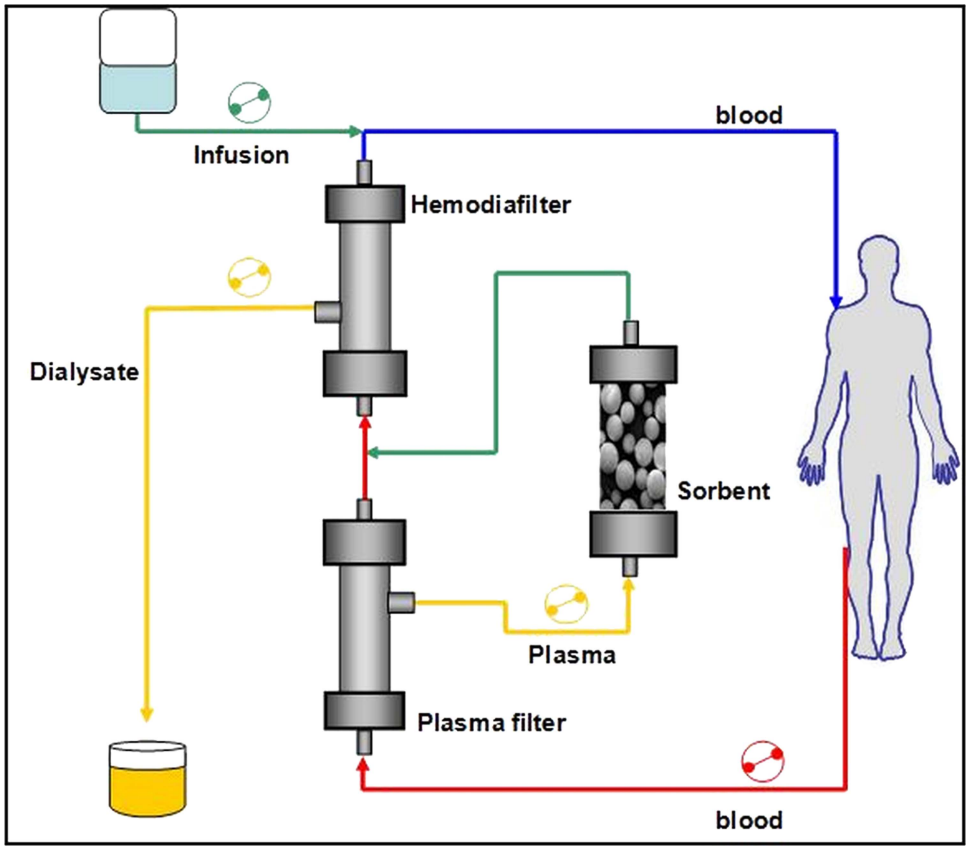


Figure 1 Coupled plasma filtration adsorption schema.

however, a per-protocol analysis was also planned to assess the impact of protocol violations, if any, on the primary endpoint. Secondary endpoints were: mortality within 90 days of randomisation; the proportion of patients who developed ≥1 new organ failures during their ICU stay (defined by an organ SOFA score of 3 or 4[22]) and ICU-free days during the first 30 days from randomisation.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely the assessment of outcomes in patients with septic shock on ICU admission or who developed it during their ICU stay and patients starting CPFA within or later than 4 h of randomisation.

The study was sized to have 80% power to detect an improvement in hospital mortality from an expected 63% in the controls to 47% with CPFA (25% relative improvement), with a two-tailed 5% type I error. A total of 330 patients were required. A Bayesian approach (see online supplementary material) was adopted for interim analyses.[23]

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted an early termination of the study on the grounds of futility. To reach the a priori determined goal of a 25% reduction in mortality, in the second part of the study, a 23% hospital mortality in the CPFA group would have been required, which was considered implausible. Further concerns were the low recruitment rate and the high number of protocol violations in the CPFA arm in terms of low volume of plasma treated per day.

Statistical analyses

Hospital mortality was analysed using the χ^2 test. The effect size was expressed in terms of absolute risk difference with its 95% CI.[28] With regard to secondary endpoints and subgroup analyses, categorical variables were compared with χ^2 or Fisher's exact tests, while a Student t test was used for continuous variables, after having assessed normality through the Kolmogorov-Smirnov, the Shapiro-Wilks tests and the normal probability plot, and homoscedasticity through the Levene's test. Mortality within 90 days of randomisation was assessed using Kaplan-Meier curves with any differences investigated through logrank testing.

As a number of protocol violations in the CPFA arm were registered due to a lower than planned volume of plasma treated, we also performed a per-protocol analysis of the primary endpoint, as determined a priori. The analysis by the 'adhesion to the protocol' was indeed planned to involve patients who did not have relevant protocol violations, to assess the possible influence of such violations on the outcome.

Hospital mortality was evaluated according to tertiles of the mean volume of plasma treated per kg per day.

Any association between tertiles and hospital mortality was tested with the χ^2 test and the Cochran-Armitage test for trend. As any benefit of randomisation was lost, comparison with the control group was performed through a logistic regression model that allowed to adjust for possible confounders (see online supplementary material).

RESULTS

Between January 2007 and November 2010, a total of 192 patients had been randomised. Recruitment in each ICU lasted a median of 22 months (IQR 13–26). During this period, 386 patients with septic shock were excluded as being non-eligible (see online supplementary material). Central monitoring subsequently identified 14 enroled patients whose eligibility criteria were doubtful. Further clinical information was retrieved and provided to the EDSMC who determined that eight of these patients (5 CPFA, 3 control) were erroneously enroled (see online supplementary material). Analysis was performed by intention-to-treat on the 184 remaining patients.[29] Figure 2 denotes the flow of participants.

Table 1 shows the patients' characteristics; further details are provided in the online supplementary material. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving drotrecogin alfa (activated).

Overall, 44 patients (48.4%) had less than the minimum amount, as recommended by the protocol, of plasma treated over the first 5 days. They were evenly distributed across centres. To better express and investigate the phenomenon of undertreatment, and following the emerging concept of dose of renal replacement therapy,[30] we computed the volume of plasma treated in L/kg/day. In the 91 patients randomised in the CPFA arm, a mean of 0.15 L/kg/day were treated for the first 5 days (tertiles 0.12–0.18), and 0.18 for the first 3 days. Table 2 lists the reasons for undertreatment. Four patients died during CPFA, one before initiating the treatment, two at the very first moment and one after the first 0.09 L kg of plasma treated. The mean time to start CPFA after septic shock identification was 5.7 h (SD 3.8); 38 patients started within 4 h. In the control group, in violation of the protocol, two patients were treated with CPFA; one died at 7 days post-randomisation, the other was discharged alive from the hospital 37 days after randomisation.

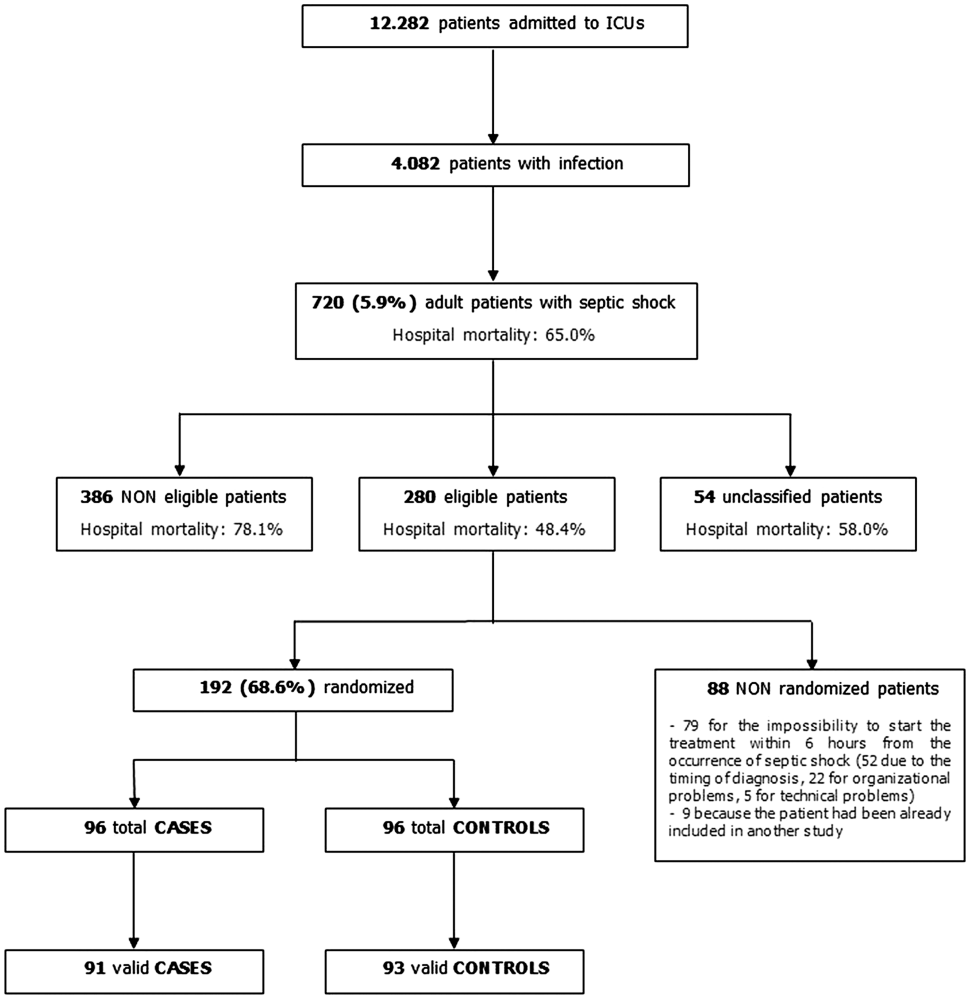


Figure 2 Flow chart of participants.

Table 1 Characteristics of the patients before randomisation

	Controls (n=93)	CPFA (n=91)
Sex (male), n (%)	65 (69.9)	56 (61.5)
Age (years), n (%)		
Overall, mean [SD]	64.9 [13.3]	63.6 [14.4]
17–45	10 (10.8)	9 (9.9)
46–65	34 (36.6)	35 (38.5)
66–75	23 (24.7)	27 (29.7)
>75	26 (28.0)	20 (22.0)
BMI (%)		
Underweight	5 (5.4)	2 (2.2)
Normal weight	34 (36.6)	27 (29.7)
Overweight	24 (25.8)	31 (34.1)
Obese	30 (32.3)	31 (34.1)
Length of stay before ICU admission (days), mean [SD]	6.5 [13.8]	6.2 [11.8]
Source of admission, n (%)		
Emergency room	16 (17.2)	31 (34.1)
Surgical ward	43 (46.2)	31 (34.1)
Medical ward	29 (31.2)	27 (29.7)
Other ICU	5 (5.4)	2 (2.2)
Surgical status, n (%)		
Not surgical	43 (46.2)	54 (59.3)
Elective surgical	8 (8.6)	6 (6.6)
Emergency surgical	42 (45.2)	31 (34.1)
Trauma, n (%)	6 (6.5)	5 (5.5)
Comorbidities, n (%)		
None	12 (12.9)	18 (19.8)
Mary Charlson Index, median [Q1–Q3]	2 [0–3]	1 [0–2]
Reason for admission, n (%)		
Monitoring/weaning	7 (7.5)	7 (7.7)
Respiratory failures	80 (86.0)	69 (75.8)
Cardiovascular failures	50 (53.8)	58 (63.7)
Neurological failures	12 (12.9)	9 (9.9)
Renal failure	24 (25.8)	33 (36.3)
Multiple organ failures	59 (63.4)	65 (71.4)
Top 3 non-infectious diseases on admission, n (%)		
Metabolic disorder	23 (24.7)	25 (27.5)
Gastrointestinal perforation	16 (17.2)	15 (16.5)
ALI	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1–Q3]	53 [43–67]	51 [42–65]
SOFA at randomisation, median [Q1–Q3]	9 [8–11]	9 [8–11]
RIFLE at randomisation, n (%)		
No risk	51 (54.8)	29 (31.9)
Risk	16 (17.2)	22 (24.2)
Injury	10 (10.8)	21 (23.1)
Failure	16 (17.2)	19 (20.9)
Septic shock on admission, n (%)		
Missing	39 (42.4)	43 (47.8)
	1	1
Site of infection, n (%)		
Pneumonia	25 (26.9)	30 (33.0)
Peritonitis	28 (30.1)	25 (27.5)
Primary bacteraemia	1 (1.1)	8 (8.8)
Colecistitis/colangitis	5 (4.3)	3 (3.3)
Urinary tract infection	1 (1.1)	2 (2.2)
Other	23 (24.7)	19 (20.9)
Multisite	10 (10.8)	4 (4.4)
Top five microorganisms isolated, n (%)		
Non-ESBL producing *Escherichia coli*	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)

Continued

Table 1 Continued

	Controls (n=93)	CPFA (n=91)
Methicillin-resistant *Staphylococcus aureus*	10 (10.5)	4 (4.5)
Penicillin sensitive *Pneumococcus*	2 (2.1)	4 (4.5)
Ampicillin-resistant vancomycin-sensitive *Enterococcus faecalis*	3 (3.2)	3 (3.4)
Gram-positive bacteria	25 (26.3)	27 (30.7)
Gram-negative bacteria	29 (30.5)	27 (30.7)

Q1–Q3=first and third quartiles; underweight=for male, BMI<20, for woman, BMI<19; normal weight=for man, BMI 20–25, for woman, BMI 19–24; overweight=for male, BMI 25–30, for female, BMI 24–29; obese=for male, BMI>30, for female, BMI>29; respiratory failure=need of ventilatory support to maintain gas exchange; cardiovascular failure=need of vasoactive drugs to provide sufficient pump action; neurological failures (GCS≤8); Renal failure=RIFLE score: injury or higher.
ALI, acute lung injury; BMI, body mass index; CPFA, coupled plasma filtration adsorption; ESBL, extended-spectrum β-lactamase; GCS, Glasgow Coma Scale; ICU, intensive care unit; RIFLE, Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage kidney disease; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment.

No statistical difference was found in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% dying in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95% CI −12.2% to 16.6%). The 90-day survival curves of the two groups substantially overlapped (logrank test, p=0.48; figure 3). Secondary endpoints did not statistically differ: the occurrence of new organ failure was 55.9% in the control versus 56% for CPFA patients (p=0.99); the free ICU days during the first 30 days postrandomisation were 6.8 in the control group versus 7.5 in the CPFA group (p=0.35). There were also no statistical differences in the a priori determined subgroups. Hospital mortality in patients with septic shock on ICU admission was comparable (16/39 (41%) for control vs 19/43 (44.2%) for CPFA; p=0.77). The same was observed for the subgroup of patients who developed septic shock during their ICU stay (27/53 (50.9%) control vs 21/47 (44.7%) CPFA; p=0.53). Likewise, no statistical difference in mortality was observed between controls 44/93 (47.3%), and patients starting CPFA within 4 h from randomisation (17/38 (44.7%); p=0.88), nor in those who started CPFA after 4 h (20/46 (43.5%); p=0.76). In seven patients, the timing of CPFA initiation was missing. Eventually, no effect on the number of patients per ICU was observed.

The per-protocol analysis revealed a non-significant trend in hospital mortality according to the tertiles of volume of plasma treated per kg per day over the first 5 days (figure 4). The characteristics of the groups defined by the tertiles are shown in the online supplementary material. The logistic regression model, aimed at adjusting for possible confounders, verified that hospital mortality in patients falling within the third tertile (≥0.18 L/kg/day of plasma treated over the first 5 days) was statistically lower than in the control group (OR 0.36, 95% CI 0.13 to 0.99; see table 3). We then performed two sensitivity analyses, namely limiting the evaluation of the volume of plasma treated to the first 3 days and excluding from the control and treated groups patients who died in the first 24 h postrandomisation. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days; the second was intended to minimise any possible selection bias as patients who died early could not have entered the highest tertile of treated plasma due to insufficient time. Both sensitivity analyses (presented in the online supplementary material) confirmed the same estimates, even though statistical significance was lost for lack of power.

Table 2 Reasons for undertreatment in the coupled plasma filtration adsorption arm (n=44)

	n	Per cent
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organisational problems	4	9.1
Patient's death	4	9.1
Lack of specialised personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

DISCUSSION

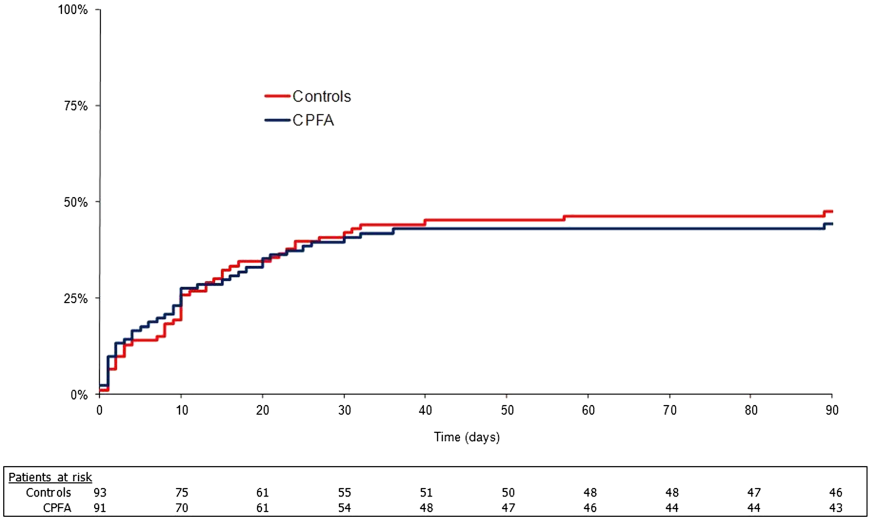
The prognosis of critically ill patients with septic shock remains poor, with mortality rates still around 50–60%.[20 31] All attempts to find a 'magic bullet' to restore immune derangements during sepsis and improve the outcomes have failed, highlighting the complexity of the immune response, including a marked intrapatient variability in terms of magnitude of response, timing and trajectory and our continued lack of full understanding.

Rather than targeting a specific molecule, CPFA offered a more general means of reducing the circulating inflammatory mediator load. Following promising results in early phase studies,[15 16 25] GiViTI performed this randomised clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock.

The main findings

After randomising more than half the planned number of patients, we found no statistical difference with the use of CPFA in hospital mortality, the occurrence of new organ failures or the overall clinical evolution. To

Figure 3 Survival curves.



reverse these results, with the sample still to be randomised, implausible data should have been observed from then on. Furthermore, this study was powered from an anticipated 63% hospital mortality in the control group. Although such an estimation, coming from previous GiViTI data, was confirmed in the whole sample (figure 2), the eligibility criteria selected a subgroup where mortality was sensibly lower (47.3%), thereby reducing the power of the study. Thus, the EDSMC considered that continuing to spend money in a clinical trial that had a little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, non-binding Bayesian futility criteria for stopping the trial were not fulfilled.

The dilemma of primary endpoint

The correct primary endpoint of clinical trials in septic shock is still debated.[32] Most of the studies have adopted 28-day mortality due to Food and Drug Administration stipulations. However, the mortality rate attributable to sepsis continues long after the initiation of the acute event[33]; indeed, 16.8% of our study patients were still in the ICU beyond 28 days after randomisation. On the other hand, overextending the follow-up period has the disadvantage of diluting the phenomenon, with the inclusion of competing causes of death. We thus considered mortality at the time of discharge from the hospital into which they were last admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialised, interdisciplinary care, which means he or she had survived the septic shock episode. A 90-day mortality was anyway recorded and considered as secondary endpoint.

The problem of undertreatment

Nearly half of the patients randomised to CPFA were undertreated as per protocol stipulation. This poses two

Figure 4 Hospital mortality according to the quantity of volume of plasma treated (whiskers represent 95% CI).

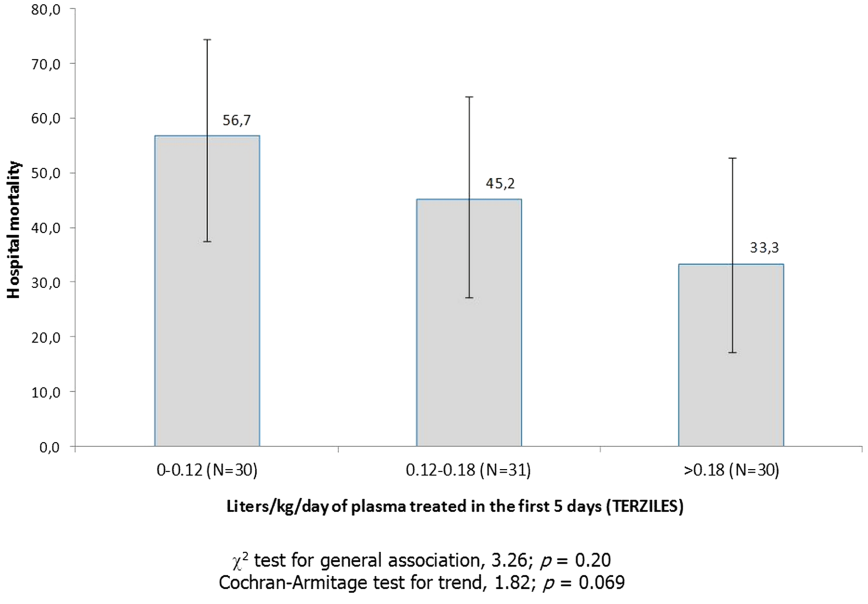


Table 3 Results of the logistic regression model on hospital mortality

Variable	OR	95% CI	p Value
Volume of plasma treated (L/kg/day)			
CPFA, ≤0.18 (1° and 2° tertiles) vs controls	1.52	0.73 to 3.17	0.033
CPFA, >0.18 (3° tertile) vs controls	0.36	0.13 to 0.99	
Age (decades)	1.57	1.19 to 2.07	0.001
Source of admission			
Other ICU vs medical ward	0.28	0.04 to 1.89	
Emergency room vs medical ward	0.27	0.11 to 0.67	0.021
Surgical ward vs medical ward	0.34	0.15 to 0.77	
Renal failure at admission	4.08	1.47 to 11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04 to 0.75	0.018

Dependent variable: hospital mortality. Number of patients = 184. Prediction: likelihood ratio test: 39.93, degrees of freedom: 8, p<0.0001; % pairs: concordant 77.4%; discordant 22.2%; Somers' D: 0.55; receiver operating characteristic (ROC) curve area: 0.78. Goodness of fit Hosmer-Lemeshow goodness-of-fit C test: 8.22; eight degrees of freedom; p value = 0.41.
ICU, intensive care unit.

crucial questions: the true feasibility of the technique in the ICU and the possible relationship between the overall negative result and such undertreatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centres.

Why did the training of the centres not have an effect? Many factors could have contributed. First, CPFA involves a complex circuit that includes a haemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions and anticoagulation. We used heparin for anticoagulation (see online supplementary material), the most frequently used drug in this regard, because the machine used in the study did not support regional anticoagulation with citrate. Nevertheless, heparin is difficult to manage, particularly in the critically ill. Many centres may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be an insufficient antithrombin substrate for the heparin to be effective.[34] Second, because of the high cost of the procedure (about €1.200 per treatment), in most of the cases, the physicians did not start a new course of CPFA on the same day, in case of clotting of the circuit. Third, the training may have been (partly) ineffective. On the one hand, it only reached a few people per ICU. It was often difficult to involve the nephrologists, who, in many centres, are in charge of the procedure. On the other hand, despite excellent feedbacks from the participants, we cannot *a posteriori* exclude it was qualitatively suboptimal.

At any rate, the feasibility problems we have encountered in the present clinical trial suggest that the procedure, as implemented in this study, is not practicable in everyday clinical practice. Interestingly, regional anticoagulation with citrate represents a valid alternative to heparin as its anticoagulatory effect is limited to the extracorporeal circuit, without any systemic effect, and can be safely applied in the ICU.[35 36] In a feasibility study carried out in 13 patients at high risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with the heparin.[37] The newer generation CPFA machine is able to apply citrate regional anticoagulation, and initial experiences in patients with septic shock demonstrate that a much higher volume of plasma can be safely treated.[38] Should these preliminary results be confirmed, the question whether the reason of our negative result was a problem of feasibility or efficacy would become essential, to avoid the risk of dismissing a potentially effective treatment for such a high mortality condition as septic shock.

The per-protocol analysis and its limits

Of note, patients who had a larger volume of plasma treated seemed to have reduced hospital mortality. This cannot be taken as conclusive evidence of the efficacy of CPFA. Even though the per-protocol analysis was planned a priori with the expected direction of the effect being stated in advance, and a dose–response relationship was found, a number of potential problems threaten the validity of this result. First, a subgroup definition for the per-protocol analysis (ie, tertiles of plasma treated) was based on characteristics measured after randomisation. Under such circumstances, the allocation to a subgroup may have been influenced by the intervention in relation to the severity of the patient, causing an important bias. This would be the case, for example, if the probability of circuit clotting was higher in the more severely ill patients. Actually, the characteristics of the three subgroups were somewhat unbalanced (see online supplementary material). We adjusted for possible confounders in the multivariate model to minimise this risk, but we were limited to prognostic factors collected in the database. Particularly, we have no data on the immunoinflammatory status of the patients to account for. Second, the subgroup allocation may have been influenced by the outcome. For example, early deaths could have prevented the treatment of high volume of plasma. Even if we standardised the treated volume to the duration in hours of CPFA, since the treatment started with a low filtration fraction to be gradually

increased to the target value (see online supplementary material), the first hours were characterised by a certain degree of undertreatment by design. In this case, an early death could have prevented the patient from being included in the third tertile, but not in the others, nor in the controls, spuriously influencing the result. We performed a sensitivity analysis by excluding early deaths from all groups, knowing that such an analysis could have greatly disadvantaged CPFA, if the lower number of early deaths were due to the efficacy of the technique. Interestingly, we verified that the strength of association was unchanged, albeit losing statistical significance for a lack of power, thereby excluding the presence of a differential outcome-related selection bias. Finally, the statistical significance of our results is quite thin; indeed, just one more death in the highest tertile subgroup would have rendered the difference in hospital mortality non-significant.

Study limitations

Almost 60% of patients with septic shock did not meet the inclusion criteria. The main reason was life expectancy less than 2 weeks. The mortality of these patients was in fact 98%. Nonetheless, we cannot exclude that the higher severity could have brought about a potentially greater possibility of response to intervention, at least for some patients. Future studies should consider this aspect.

One-third of the eligible patients were not randomised due to the very narrow window (6 h) for the patient's recruitment and initiation of the treatment. This would have particularly hampered the generalisability of results had the findings been positive.

Finally, the study was terminated early for reasons of futility, after almost 60% of the originally planned patients had been recruited. This reduced the possibility of studying phenomena emerging from the analyses with a significant power, as in the case of the volume of plasma treated. In any event, any subgroup analysis, regardless of the involved sample size, could only have generated hypotheses. Our interpretation of the findings is in itself a hypothesis, which would have been only more robust with a larger sample.

CONCLUSION

CPFA was not able to reduce mortality in patients with septic shock. This result strongly discourages the use of CPFA in the everyday clinical practice, as it was implemented in this study. Unfortunately, we were not able to discern whether the culprit of such a negative result was the lack of effectiveness (mainly due to widespread feasibility problems) rather than the lack of true efficacy. The subgroup analysis was suggestive of efficacy, if a high volume of plasma was treated. Although we have taken counter measures to minimise potential biases, these cannot be completely excluded. Hence, this result can only be viewed as hypothesis generating. Given the new availability of citrate regional anticoagulation, we have designed a confirmatory, adaptive trial whose first step will be to prove this new technique easily allows high volume of plasma treated with CPFA.

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Contributors All the authors substantially contributed to the conception and design interpretation of data, critically revising it and approved the final version of the manuscript , GB and CR were involved in the analysis of the data and GB involved in the drafting the article.

Funding This work was supported by the Bellco SpA. The study was funded by GiViTI and through an unconditional research grant from Bellco (Mirandola, Italy), the CPFA patent holder, who had no role in the study. GiViTI-Istituto di Ricerche Farmacologiche Mario Negri has full ownership of the data and of the dissemination policy of the results. GiViTI is the recipient of unconditional grants from Brahms and Astellas, who also had no role in this study.

Competing interests None.

Ethics approval Ethics Committee of all centers.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis. Further analyses will be provided on request to the corresponding author. Raw data are available on justified request.

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Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in Septic Shock patients: multicenter randomized clinical trial

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva

(Italian Group for the Evaluation of Interventions in Intensive Care Medicine)

Online supplement

Homogeneity and quality of the study

In each ICU a senior intensivist (see Appendix of the paper) was responsible for protocol and data integrity. A detailed on-line operating manual, which was easily accessible during data input, explained all the definitions employed. As many as 140 different validity checks were performed concurrently with data entry. The system allowed inconsistent or implausible data to be saved, but marked the record as problematic. Data were further reviewed by the coordinating center, and any queries solved with the individual ICUs. A call center was fully operative during the study. Each ICU ran its own pilot phase during which the experimental protocol (5 days of early CPFA) had to be correctly performed and fully documented. All units were visited by the clinical PI of the project (SL) during the pilot phase to ensure CPFA was performed according to the standard procedures. During the recruitment we provided each ICU with general and personalized progress reports focusing on problems experienced by investigators; 6 investigators' meetings were organized, centered on patient recruitment and problems encountered, during which a machine was available for in depth tutorial; a total of 52 ad hoc site visits to ICUs with specific problems were performed during the study.

Central monitoring of the study identified 14 randomized patients whose eligibility criteria were in doubt. Further clinical information were retrieved for each patient and provided to the EDSMC, without revealing the randomization arm. According to internationally accepted criteria[1], the EDSMC determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled as they did not meet inclusion criteria. Due to human error the patients were inappropriately randomized, even though the exclusion criteria were already known at the time of randomization. This is a reason to exclude patients from the analysis[1]. More specifically, in four cases the patient was terminally ill (metastatic cancer in one case, where the advice of oncologist was not to proceed with further investigations or oncologic therapy during ICU stay; AIDS in terminal condition in one case; a severe autoimmune disease, for which the patient was assuming cyclosporine, accompanied by severe renal failure, ARDS, and metabolic imbalance in one other case, and diabetes complicated by end-stage renal failure and severe cerebral vasculopathy in the last case). In all these patients, life expectancy was less than two weeks (exclusion criterion). In one case the patient was in coma following an operated spontaneous intra-cerebral hemorrhage (exclusion criterion) and had a life expectancy less than two weeks (further exclusion criterion). In the remaining three cases, the diagnosis of infection was not confirmed (clinical sepsis) and the shock had an other than infective origin (inclusion criteria): obstructive in one case of pulmonary embolism, hypovolemic in the other two cases.

Reasons for excluding patients

As many as 386 patients were considered not eligible for the study. Table S1 lists the related reasons.

Table S1. Main reason for excluding adult patients from randomization

Exclusion criteria	**Patients** *n* (%)
Terminal conditions	192 (49.7)
Low dose of vasopressors	53 (13.7)
Contraindication to a haemopurification technique	48 (12.4)
Denied consent	21 (5.4)
Clinical decision of the attending physician	19 (4.9)
> 24 hours in another ICU	17 (4.4)
Coma for organic cerebral disease	8 (2.1)
Cardiopulmonary resuscitation	4 (1.0)
Metastatic cancer	3 (0.8)
Not reported	21 (5.4)

Anticoagulation protocol

Patient with no increased risk of bleeding:

Use non-fractionated heparin (UFH), PTT between 1 and 1.4 times the normal values, or low-molecular-weight heparin (LMWH), anti-Xa activity between 0.25 and 0.35

Heparin-induced thrombocytopenia:

Discontinue all types of heparin, UFH or LMWH. (Grade C)

Patient with increased risk of bleeding:

Prostaglandins can be considered (grade E).

Flolan (prostacyclin), dissolve contents of one 0.5-mg vial with 50 ml of sterile diluent for flolan, dilute everything in 500 ml of saline. The solution will contain 1000 ng ml^{-1}.

Priming the circuit with heparinized saline: 10,000 U of heparin in 2 liters of saline.

Connecting the patient to the circuit: initially infuse Flolan in the venous line at a dose of 3 ng kg^{-1} min^{-1} for 15 minutes. Closely monitor the hemodynamic parameters. After 15 minutes move the infusion line to the circuit input, before the pump, at double speed (6 ng kg^{-1} min^{-1}).

Initial setting of flows: set dialysis and reinfusion to 1,000 ml h^{-1}. Set the blood flow between 150 and 200 ml min^{-1}.

Patient with increased tendency to clot:

Add prostaglandins to UFH or LMWH (grade C):

The application of the predilution (grade C) or the combination of systemic and regional anticoagulation can be considered.

Regional anticoagulation

A protocol for regional anticoagulation for CVVH in critically ill patients has been developed by the group coordinated by dr. Lea Fabbri (University Hospital Careggi, Florence) [2] and can be adopted.

Treatment schedule

Prefilter:

- heparin 1000 U h^{-1}
- Prostacyclin (Flolan) 4 ng kg^{-1} min^{-1}

Postfilter:

- Protamine sulphate 1 mg $(100\ IU)^{-1}$ of heparin.

Important advices:

- Dilute prostacyclin as follows: 250,000 ng in 250 ml of saline
- Dilute protamine sulphate as follows: 250 mg in 250 ml of saline
- Connect protamine sulphate right at the entrance of the coaxial catheter, to avoid clots in the return line.

Interim Analyses

Bayesian approach was adopted for interim analyses, due to its remarkable practical and theoretical strengths [3]. As known, Bayesian approach combines a prior distribution and the gathered experimental evidence into a posterior distribution. The posterior distribution is the basis for the stopping decision. Hence, this analysis required a probabilistic formalization of two conflicting prior hypotheses: the skeptical and the enthusiastic ones. The trial was planned to be stopped early for benefit when the skeptic was convinced of the treatment efficacy or, in other words, when the posterior distribution starting from the skeptical prior was shifted enough toward benefit. Conversely, the trial was planned to be stopped early for futility when the enthusiastic was convinced of the treatment uselessness or, in other words, when the posterior distribution starting from the enthusiastic prior was shifted enough toward equivalence.

The skeptical prior postulated no difference (the null hypothesis) between the two treatments (the prior distribution has zero mean), with only a 2.5% credibility to observe an advantage of the experimental treatment greater than the protocol expected difference (the prior distribution had a standard deviation such as only 2.5% of values exceeded the 25% improvement). The enthusiastic prior postulated the expected difference (the protocol hypothesis) between the two treatments (the mean of the prior distribution was equal to a 25% improvement in favor of the experimental group), with a 2.5% credibility to observe no or negative effect (the prior distribution had a standard deviation such as only 2.5% of values lied below zero) [4]. Computing posterior probability distributions from both hypotheses during the data collection allowed to monitor the criteria to prematurely interrupt the study, that happened if it yielded: a) an at least 25% superiority of the experimental treatment, with only a 2.5% probability of being less effective, starting from a skeptic prior; b) an inferiority or a less than 25% superiority of the experimental treatment, with only a 2.5% probability of being more than 25% superior, from an enthusiastic prior.

Methods to develop the multivariate logistic regression model

In the per-protocol analysis we evaluated the association between hospital mortality and the tertiles of the average volume of plasma treated per kg per day. Since the volume of plasma treated was not the object of randomization but, rather, the result of the application of the technique to the randomized patients, we cannot guarantee that this was not related to the patient's severity. Thus, we adjusted the relationship between hospital mortality and the volume of plasma treated for possible confounders through a logistic regression model.

The dependent variable was the primary endpoint of the study, i.e. mortality at the discharge from the latest hospital where the patient stayed. We screened in a bivariate analysis, as possible confounders, all the variables identified as prognostically relevant in the 2009 GiViTI mortality-prediction model and all the sites of infection. Bivariate analyses were performed by means of the one-way ANOVA or Mann-Whitney *U*-test for quantitative variables and the chi-squared or Fisher exact test for qualitative variables. Each variable was tested in the model either if it was thought to be clinically relevant, or if it was associated to the dependent variable at a permissive significance level ($p<0.3$). We tested the assumption that the logit was linear in the quantitative variables by analyzing the estimated coefficients of designed variables representing the quartiles of the original variable distribution [5]. Whenever suggested by this analysis, we tested a second order model or log-transformation of the variable. If these approaches failed to fit the data, the variable was divided into classes, and dummy variables were used [5].

We forced in the model a four-level design variable identifying patients randomized to control (as reference category) and those belonging to the tertiles of the average volume of plasma treated per kg per day. After having introduced this variable in the model, we step-by-step added the covariate that maximized the increment in likelihood, in a forward approach. Model selection was based on the information criterion with a penalizing parameter equal to 1 and on the likelihood ratio test, using $p \leq 0.05$ as the level of significance.

All tests were two-tailed, with 0.05 as level of significance. Data were analyzed using SAS software, version 9.1.3 (Cary, NC, USA).

Patients characteristics

Table S2. Characteristics of the patients before randomization

	Controls (*n* = 93)	**CPFA (*n* = 91)**	**1st tertile of volume of plasma treated** (<0.12 L kg^{-1} day^{-1}) *n* = 30	**2nd tertile of volume of plasma treated** (0.12-0.18 L kg^{-1} day^{-1}) *n* = 31	**3rd tertile of volume of plasma treated** (>0.18 L kg^{-1} day^{-1}) *n* = 30
Physiological parameters, mean [*SD*]					
	167 [69]	197 [95]	189 [96]	186 [80]	215 [108]
PaO_2/FiO_2	1.6 [0.5]	1.5 [0.4]	1.6 [0.4]	1.4 [0.3]	1.6 [0.4]
INR	40.9 [12.0]	42.5 [15.4]	45.2 [19.4]	39.3 [14.0]	43.3 [12.0]
PTT	196 [137]	156 [122]	119 [99]	159 [113]	190 [143]
Platelet count (x 10^3)	575 [241]	534 [249]	502 [275]	633 [223]	463 [227]
Fibrinogen	2.2 [2.5]	2.0 [3.7]	1.5 [1.7]	2.8 [5.9]	1.6 [1.2]
Bilirubin	2.0 [1.4]	2.3 [1.5]	2.5 [1.7]	2.3 [1.5]	2.2 [1.3]
Creatinine					
Treatments, *n* (%)					
Steroids	21 (23.9)	29 (34.1)	7 (29.2)	12 (38.7)	10 (33.3)
Drotrecogin alfa (activated)	5 (5.5)	1 (1.1)	0 (0.0)	1 (3.2)	0 (0.0)
Vasoactive drugs*	65 (69.9)	62 (68.1)	18 (60.0)	19 (61.3)	25 (83.3)
CVVH**	45 (48.4)	54 (59.3)	12 (40.0)	27 (87.1)	15 (50.0)
Stress ulcer prophylaxis	84 (95.5)	84 (98.8)	24 (100.0)	31 (100.0)	29 (96.7)

* = Dopamine > 5 μg kg^{-1} min^{-1} or epinephrine or norepinephrine > 0.1 μg kg^{-1} min^{-1}
** = CVVH couldn't overcome the dose of 25 ml kg^{-1} hr^{-1}
SD=Standard deviation; *Q1-Q3*=first and third quartiles

Table S3. Characteristics of the subgroups defined by tertiles of volume of plasma treated, in the CPFA arm

	1st tertile of volume of plasma treated (<0.12 L kg^{-1} day^{-1}) *n* = 30	**2nd tertile of volume of plasma treated** (0.12-0.18 L kg^{-1} day^{-1}) *n* = 31	**3rd tertile of volume of plasma treated** (>0.18 L kg^{-1} day^{-1}) *n* = 30
Sex (Male) *n* (%)	18 (60)	23 (74.2)	15 (50.0)
Age (years) *n* (%)			
Overall mean [SD]	66.0 [12.4]	60.0 [15.8]	64.9 [14.4]
Body Mass Index *n* (%)			
Underweight	0 (0.0)	1 (3.2)	1 (3.3)
Normal weight	8 (26.7)	5 (16.1)	14 (46.7)
Overweight	12 (40.0)	10 (32.3)	9 (30.0)
Obese	10 (33.3)	15 (48.4)	6 (20.0)
Length of stay before ICU admission (days) mean [SD]	6.2 [11.8]	8.0 [12.3]	4.2 [11.4]
Source of admission *n* (%)			
Emergency room	13 (43.3)	7 (22.6)	11 (36.7)
Surgical ward	10 (33.3)	16 (51.6)	5 (16.7)
Medical ward	7 (23.3)	6 (19.4)	14 (46.7)
Other ICU	0 (0.0)	2 (6.5)	0 (0.0)
Surgical status *n* (%)			
Not surgical	17 (56.7)	17 (54.8)	20 (66.7)
Elective surgical	2 (6.7)	3 (9.7)	1 (3.3)
Emergency surgical	11 (36.7)	11 (35.5)	9 (30.0)
Trauma *n* (%)	0 (0.0)	3 (9.7)	2 (6.7)
Comorbidities *n* (%)			
None	4 (13.3)	7 (22.6)	7 (23.3)
Mary Charlson Index median [Q1-Q3]	1 [0-3]	1 [0-2]	1 [0-2]
Reason for admission *n* (%)			
Monitoring/weaning	1 (3.3)	4 (12.9)	2 (6.7)
Respiratory failures	25 (83.3)	21 (67.7)	23 (76.7)
Cardiovascular failures	21 (70.0)	16 (51.6)	21 (70.0)
Neurological failures (GCS≤8)	3 (10.0)	4 (12.9)	2 (6.7)
Renal failure	13 (43.3)	13 (41.9)	7 (23.3)
Multiple organ failures	26 (86.7)	18 (58.1)	21 (70.0)
Top 3 non infectious diseases on admission *n* (%)			
Metabolic disorder	12 (40.0)	8 (25.8)	5 (16.7)
Gastrointestinal perforation	5 (16.7)	3 (10.0)	7 (23.3)
ALI (Acute Lung Injury)	5 (16.7)	5 (16.1)	4 (13.3)
SAPS II on admission, median [Q1-Q3]	61.5 [49-70]	46 [33-62]	51 [44-64]
SOFA at randomization, median [Q1-Q3]	9 [7-12]	9 [8-12]	9 [8-10]
RIFLE at randomization, *n* (%)			
No risk	6 (20.0)	12 (38.7)	11 (36.7)
Risk	8 (26.7)	5 (16.1)	9 (30.0)
Injury	9 (30.0)	8 (25.8)	4 (13.3)
Failure	7 (23.3)	6 (19.4)	6 (20.0)
Septic shock on admission *n* (%)	19 (65.5)	12 (38.7)	12 (40.0)
Missing	1	0	0
Site of infection *n* (%)			
Pneumonia	8 (26.7)	12 (38.7)	10 (33.3)
Peritonitis	7 (23.3)	10 (32.3)	8 (26.7)
Primary bacteraemia	4 (13.3)	1 (3.2)	3 (10.0)
Colecistitis/colangitis	1 (3.3)	1 (3.2)	1 (3.3)
Urinary tract infection	1 (3.3)	1 (3.2)	0 (0.0)
Other	8 (26.7)	5 (16.1)	6 (20.0)
Multisite	1 (3.3)	1 (3.2)	2 (6.7)
Top five microorganisms isolated *n* (%)			
Non-ESBL producing Escherichia coli	6 (20.0)	6 (19.4)	2 (6.7)
Candida albicans	2 (6.7)	2 (6.5)	2 (6.7)
Methicillin-resistant Staphylococcus aureus	0 (0.0)	1 (3.2)	3 (10.0)
Penicillin sensitive Pneumococcus	3 (10.0)	1 (3.2)	0 (0.0)
Ampicillin-resistant vancomycin-sensitive Enterococcus	0 (0.0)	2 (6.5)	1 (3.3)
faecalis	9 (30.0)	9 (29.0)	9 (30.0)
Gram positive bacteria	8 (26.7)	12 (38.7)	7 (23.3)
Gram negative bacteria			

SD: Standard deviation; Q1-Q3: first and third quartiles

Sensitivity analyses

Table S4. Results of the logistic regression model on hospital mortality having limited the evaluation of the volume of plasma treated to the first 3 days

Variable	***OR***	**95% *CI***	***p***
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.47	0.70-3.06	0.064
CPFA, > 0.18 (3° tertile) vs. Controls	0.42	0.16-1.12	
Age (decades)	1.04	1.02-1.07	0.002
Source of admission			
Other ICU vs. Medical ward	0.30	0.05-1.98	0.025
Emergency room vs. Medical ward	0.26	0.10-0.66	
Surgical ward vs. Medical ward	0.37	0.17-0.84	
Renal failure at admission	3.73	1.36-10.22	0.011
Cholecystitis or cholangitis on admission	0.20	0.05-0.83	0.027

Dependent variable: hospital mortality. Number of patients = 184. Prediction: likelihood ratio test: 38.5, degrees of freedom: 8, p<0.0001; % pairs: concordant 76.0%; discordant 23.6%; Somers' *D*: 0.52; receiver operating characteristic (*ROC*) curve area: 0.76. Goodness of fit Hosmer–Lemeshow goodness-of-fit *C* test: 5.7; eight degrees of freedom; *p* value = 0.68. Legend: *OR*, odds ratio; *CI*, confidence interval; ICU, intensive care unit.

Table S5. Results of the logistic regression model on hospital mortality, having excluded, both in the control and the treated groups, patients who died in the first 24 hour from randomization.

Variable	***OR***	**95% *CI***	***p***
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.23	0.51-2.96	0.299
CPFA, > 0.18 (3° tertile) vs. Controls	0.51	0.18-1.43	
Age (decades)	1.05	1.01-1.08	0.006
Source of admission			
Other ICU vs. Medical ward	0.43	0.06-3.14	0.095
Emergency room vs. Medical ward	0.32	0.12-0.90	
Surgical ward vs. Medical ward	0.36	0.15-0.91	
Renal failure at admission	4.60	1.45-14.61	0.010
Cholecystitis or cholangitis on admission	0.20	0.04-1.18	0.075

Dependent variable: hospital mortality. Number of patients = 149. Prediction: likelihood ratio test: 29.1, degrees of freedom: 8, *p*=0.0003; % pairs: concordant 76.8%; discordant 22.9%; Somers' *D*: 0.54; receiver operating characteristic (*ROC*) curve area: 0.77. Goodness of fit Hosmer–Lemeshow goodness-of-fit *C* test: 10.99; eight degrees of freedom; *p* value = 0.20. Legend: *OR*, odds ratio; *CI*, confidence interval; ICU, intensive care unit.

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