BMJ open

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-003536
Article Type:	Research
Date Submitted by the Author:	17-Jul-2013
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Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES, Clinical trials < THERAPEUTICS

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial

GiViTI

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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.

ClinicalTrials.gov, NCT00332371; ISRCTN24534559

Text word count: 3,084

Keywords: septic shock, intensive care medicine, coupled plasma filtration-adsorption

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Summary

Article focus

- Coupled plasmafiltration-adsorption (CPFA) is a blood purification technique specifically proposed for the treatment of severe infections, which provided promising results.
- This is an open label, multicentre, randomized, superiority, clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock.

Key messages

- We found no difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution.
- Patients who had a larger volume of plasma treated with CPFA seemed to have a reduced hospital mortality, but this hypothesis should be confirmed in future trials.

Strengths and Limitations

- The study was prematurely terminated on the grounds of futility.
- A large part of patients randomized to CPFA were undertreated as per protocol stipulation, underlying the difficulty of performing such a technique.
- For this reason, it is difficult to say whether the ineffectiveness was due to the impracticability of the technique or to a lack of effect.
- The preplanned subgroup analysis suggesting efficacy if a high volume of plasma was treated, was aimed at minimizing potential biases, but they cannot be completely excluded.

Abstract

Objectives

Coupled plasma filtration-adsorption (CPFA), removing inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomized, 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, superiority clinical trial

Setting

18 Italian adult, general, intensive care units (ICUs)

Participants

Of the planned 330 adult patients with septic shock, 192 were randomized to either have CPFA added to the standard care, or not. The External Monitoring Committee excluded 8 ineligible patients who were erroneously included.

Interventions

CPFA was to be performed performed daily for 5 days, lasting at least 10 hours per day.

Primary and secondary outcome measures

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

Results

There was no difference in hospital mortality (47.3% controls, 45.1% CPFA; p=0.76), nor in secondary endpoints, namely 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 randomized 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 to controls (OR 0.36, 95%-CI 0.13-0.99).

Conclusions

CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested CPFA could reduce mortality, if a high volume of plasma is treated. Due 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 number, NCT00332371; ISRCTN24534559).

Text word count: 296

Introduction

The immune response against pathogens is modulated through the production of numerous mediators, like cytokines, that promote both pro- and anti-inflammatory responses[1-3]. The overall efficacy is dictated by the balance between these two responses[4 5], attained through a combination of different factors: 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 under- or over-expression of their receptors[1 6-8]. In some circumstances, the release of inflammatory mediators is so over-abundant that the immune response goes out of control, initiating systemic response that leads to organ dysfunction, and septic shock, heavily influencing the prognosis[9-12].

Following observations that plasma cytokine levels are elevated in critically ill septic patients and this may relate to eventual outcome, numerous therapeutic attempts have been made to neutralize specific molecules[8]. The repeated failure of this strategy suggested 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 plasmafiltration-adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators[13].

Early experience with CPFA showed increased survival in a rabbit model of endotoxin-induced septic shock[14]. The first clinical study showed that a single treatment lasting 10 hours significantly improved hemodynamic status [15]. These preliminary observations were confirmed in a study of ten 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 randomized multi-center clinical trial to assess the efficacy of CPFA in the treatment of critically ill patients with septic shock.

Methods

Ethics Statement and data sharing

The protocol was approved by each hospital's ethics committee. Written consent was obtained from the patient when possible, otherwise physicians enrolled patients according to the European Guidelines for Good Clinical Practice[17].

Raw data are available upon justified request.

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 ICU were eligible for study entry, provided that CPFA could be commenced within 6 hours from diagnosis. This was made by the attending physician (present 24/7) using explicit criteria[18]. Reasons for exclusion included: 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 hours, and lack of informed consent.

The Project Margherita electronic case report form (eCRF) was used for this study[12 19]. 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[20] 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 enrolled patients, their clinical condition, including the Sequential Organ Failure Assessment (SOFA) score[21], the RIFLE criteria for acute renal dysfunction, and CPFA parameters were collected at the time of randomization 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 supplement.

Randomization and Interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enroll the patient or to register reasons for not doing so. Enrolled patients were randomly allocated by the eCRF on a 1:1 basis to either have CPFA added to the standard care, or not. The allocation was securely saved in the database and revealed once baseline additional data collection was completed. According to the available clinical evidence, CPFA was intended to be repeated daily for the first 5 days, lasting at least 10 hours each day, with a plasma flow of 30-40 ml/min and a minimum of 10 liters of plasma treated per day (see the online supplement).

Outcomes, Follow-up and Plan of analysis

The primary endpoint was mortality at discharge from the last hospital in which the patients were treated. Thus, for patients transferred to other hospital, mortality was assessed at the discharge from the last hospital in which the patients stayed. To minimize 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, 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 randomization; the proportion of patients who developed ≥1 new organ failures during their ICU stay (defined by an organ SOFA score of 3 or 4 [21]); ICU-free days during the first 30 days from randomization.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely 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 hours of randomization.

The study was sized to have 80% power to detect an improvement in hospital mortality from an expected 63% to 47% with CPFA (25% relative improvement), with a two-tailed 5% type I error. A total of 330 patients were required. A blocked randomization schedule (randomly permuting blocks of four and six) was adopted[22], with stratification according to the center and the presence of septic shock on admission. A Bayesian approach (see online supplement) was adopted for interim analyses[22].

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted 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 analyzed using the χ^2 test. Effect size was expressed in terms of absolute risk difference with its 95% confidence interval (95%-CI)[23]. With regard to secondary endpoints and subgroup

analyses, categorical variables were compared with χ^2 or Fisher exact tests, while a Student's t test was used for continuous variables. Mortality within 90 days of randomization 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.* 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 randomization was lost, comparison with the control group was performed through a logistic regression model that adjusted for possible confounders (see online supplement for details).

Results

Between January 2007 and November 2010 a total of 192 patients had been randomized. Recruitment in each ICU lasted a median of 22 months (interquartile range, 13-26). Central monitoring subsequently identified 14 enrolled patients whose eligibility criteria were doubtful. Further clinical information was retrieved and provided to the EDSMC who determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled (see online supplement). Analysis was performed by intention-to-treat on the 184 remaining patients[24]. Figure 1 denotes the flow of participants.

Table 1 shows the patients' characteristics, further details are provided in the online supplement. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving treatment with 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 centers. To better express and investigate the phenomenon of under-treatment, and following the emerging concept of dose of renal replacement therapy[25], we computed the volume of plasma treated in l/kg/day. 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 under-treatment. Four patients died during CPFA, one before initiating the treatment, two in the very first moment, and one after the first 0.09 l/kg of plasma treated. The mean time to commencement of CPFA after septic shock identification was 5.7 hours (SD 3.8); 38 patients started within 4 hours. In the control group, in violation of the protocol, 3 patients were treated with CPFA, one of whom died at 7 days post-

randomization.

No difference was seen in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95%-CI, -12.2–16.6%). The 90-day survival curves of the two groups substantially overlapped (logrank test, p=0.48) (Figure 2). Secondary endpoints did not differ: the occurrence of new organ failure was 55.9% in control versus 56.0% for CPFA patients (p=0.99); the free-ICU days during the first 30 days post-randomization were 6.8 in the control group versus 7.5 in the CPFA group (p=0.35). There were also no differences in the *a priori* determined subgroups. Hospital mortality in patients with septic shock on ICU admission was comparable (16/39 [41.0%] 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 difference in mortality was observed between controls 44/93 (47.3%), and patients starting CPFA within 4 hours from randomization (17/38 [44.7%]; p=0.88), nor in those who started CPFA after 4 hours (20/46 [43.5%]; p=0.76). In 7 patients the timing of CPFA initiation was missing. Eventually, no effect of the number of patients per ICU was observed.

The per protocol analysis revealed a near significant trend in hospital mortality according to the tertiles of volume of plasma treated per kg per day over the first 5 days (Figure 3). Table 3 compares characteristics of the groups defined by the tertiles. 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 0.13-0.99). On the other hand, there was no evidence that outcome in patients who received lower volume treatment was statistically better or worse than controls, as the 95%-CI did include the null value of 1 (OR=1.52, 95%-CI=0.73-3.17). We performed two sensitivity analyses, namely: limiting the evaluation of the volume of plasma treated to the first 3 days, and by excluding, both in the control and treated groups, patients who died in the first 24 hours post-randomization. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days, the second was intended to minimize 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 supplement) confirmed the same estimates, even though statistical significance was lost for lack of power.

Discussion

The prognosis of critically ill patients with septic shock remains poor, with mortality rates still around 50-60%[12 26]. All attempts to find a "magic bullet" to restore immune derangements during sepsis and improve outcomes have failed, highlighting the complexity of the immune response, including a marked intra-patient 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 27], GiViTI performed this randomized clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock. After randomizing more than half the planned number of patients, we found no difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution. To overturn these results with the sample still to be randomized, 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 1), the eligibility criteria selected a subgroup where mortality was sensibly lower (47.3%), so reducing the power of the study. Thus, the EDSMC considered that continue to spend money in a clinical trial that had little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, nonbinding Bayesian futility criteria for stopping the trial were not fulfilled.

The correct primary endpoint of clinical trials in septic shock is still debated[28]. Most have adopted 28-day mortality due to FDA stipulations. However, the mortality rate attributable to sepsis continues long after the initiation of the acute event[29]; indeed, 16.8% of our study patients were still in the ICU beyond 28 days after randomization. On the other hand, over-extending 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 last hospital into which they were admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialized, interdisciplinary care, which means he or she had survived the septic shock episode. 90-day mortality was anyway recorded and considered as secondary endpoint.

Nearly half the patients randomized to CPFA were undertreated as per protocol stipulation. This poses two crucial questions: the true feasibility of the technique in the ICU, and the possible relationship between

the overall negative result and such under-treatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centers. CPFA involves a complex circuit that includes a hemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions, and anticoagulation. We used heparin for anticoagulation (see online supplement), 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 centers may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be insufficient antithrombin substrate for the heparin to be effective[30].

Of note, patients who had a larger volume of plasma treated seemed to have a 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 found, a number of potential problems threatens the validity of this result. Firstly, subgroup definition for the per-protocol analysis (i.e., tertiles of plasma treated) was based upon characteristics measured after randomization. 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 (Table 3). We adjusted for possible confounders in the multivariate model to minimize this risk, but we were limited to prognostic factors collected in the database. Secondly, 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 standardized 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 supplement), the first hours were characterized by a certain degree of under-treatment 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 1 more death in the highest tertile subgroup would have rendered the difference in hospital mortality non-significant.

In conclusion, CPFA was not able to reduce mortality in patients with septic shock. The subgroup analysis was suggestive of efficacy, if a high volume of plasma was treated. Although we have taken counter-measures to minimize potential biases, these cannot be completely excluded. Unfortunately, we have no data on the immuno-inflammatory status of the patients to account for. Hence, this result can only be viewed as hypothesis generating and should be confirmed in future trials. Regional anticoagulation with citrate represents a valid alternative as its anticoagulatory effect is limited to the extracorporeal circuit, without any systemic effect, and can be safely applied in the ICU[31 32]. In a feasibility study carried out in thirteen patients at high-risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with heparin[33]. 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[34]. Should these preliminary results be established, a confirmatory trial should be considered to avoid the risk of dismissing a potentially effective treatment for such a high mortality condition as septic shock, as a consequence of the present negative results.

Acknowledgments

The study was funded by GiViTI and by 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.

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript. None of the authors has any conflict of interest in relation to this work. The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Registration number: ClinicalTrials.gov NCT00332371; ISRCTN24534559. Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis.

Funding

Bellco SpA

Competing Interests

None

Contributorship

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript.

Data sharing

Further analyses will be provided upon request to the corresponding author.

References

1. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 2003;**71**(10):5803-13
2. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007;**11**(2):R49 doi: cc5783 [pii]
10.1186/cc5783[published Online First: Epub Date]|.
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007;**167**(15):1655-63 doi: 167/15/1655 [pii]
10.1001/archinte.167.15.1655[published Online First: Epub Date]|.
4. Weighardt H, Heidecke CD, Emmanuilidis K, et al. Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. Surgery 2000;**127**(3):309-15 doi: S0039-6060(00)91079-1 [pii]
10.1067/msy.2000.104118[published Online First: Epub Date]|.
5. Tamayo E, Fernandez A, Almansa R, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw;**22**(2):82-7 doi: ecn.2011.0281 [pii]
10.1684/ecn.2011.0281[published Online First: Epub Date]|.
6. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006;**12**(3):151-70 doi: 10.1179/096805106X102246[published Online First: Epub Date]|.
7. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 2000;**275**(27):20260-7 doi: 10.1074/jbc.M909168199
M909168199 [pii][published Online First: Epub Date]|.
8. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991;**21**(9):2177-84 doi: 10.1002/eji.1830210928[published Online First: Epub Date]|.
9. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005;**365**(9453):63-78
10. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. Bmj 2003;**326**(7383):262-6
11. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;**348**(2):138-50
12. Malacarne P, Langer M, Nascimben E, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;**36**(4):1105-13
13. Tetta C, Bellomo R, Inguaggiato P, Wratten ML, Ronco C. Endotoxin and cytokine removal in sepsis. Ther

Apher 2002;**6**(2):109-15 doi: tap413 [pii][published Online First: Epub Date]|.
14. Tetta C, Gianotti L, Cavaillon JM, et al. Coupled plasma filtration-adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000;**28**(5):1526-33
15. Ronco C, Brendolan A, Lonnemann G, et al. A pilot study of coupled plasma filtration with adsorption in septic shock*. Critical Care Medicine 2002;**30**(6):1250-55
16. Formica M, Olivieri C, Livigni S, et al. Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003;**29**(5):703-8
17. Human Medicines Evaluation Unit. Guidelines for good clinical practice. London: European Agency for the Evaluation of Medical Products, 1996:17-20.
18. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;**29**(4):530-8
19. Boffelli S, Rossi C, Anghileri A, et al. Continuous quality improvement in intensive care medicine. The GiViTI Margherita Project - Report 2005. Minerva Anestesiol 2006;**72**(6):419-32
20. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993;**270**(24):2957-63
21. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;**26**(11):1793-800
22. Piantadosi S. *Clinical trial. A methodological perspective*. New York: Jonh Wiley & Sons, 1997.
23. Kleinbaum D, Kupper L, Morgenstern H. *Epidemiologic research*. New York, NY: Van Nostrand Reynhold, 1982.
24. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002;**325**(7365):652-4
25. Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. Critical Care 2011;**15**(2):207
26. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med 2003;**168**(2):165-72
27. Page M, Rimmele T. [Coupled plasma filtration adsorption: rationale and perspectives in septic shock]. Can J Anaesth 2008;**55**(12):847-52 doi: 55/12/847 [pii]
10.1007/BF03034056[published Online First: Epub Date]|.
28. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;**29**(4):880-6
29. Perl TM, Dvorak L, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;**274**(4):338-45
30. Singer M, McNally T, Screaton G, Mackie I, Machin S, Cohen SL. Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 1994;**20**(3):212-5
31. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med;**38**(1):20-8 doi: 10.1007/s00134-011-2438-3[published Online First: Epub Date]|.
32. Mariano F, Bergamo D, Gangemi EN, Hollo Z, Stella M, Triolo G. Citrate Anticoagulation for Continuous Renal Replacement Therapy in Critically Ill Patients: Success and Limits. International Journal of Nephrology 2011;**2011** doi: 10.4061/2011/748320[published Online First: Epub Date]|.
33. Mariano F, Tetta C, Stella M, Biolino P, Miletto A, Triolo G. Regional Citrate Anticoagulation in Critically Ill Patients Treated with Plasma Filtration and Adsorption. Blood Purification 2004;**22**(3):313-19
34. Pozzato M, Ferrari F, Cecere P, et al. Safety and efficacy of citrate anticoagulation in spetic shock patients treated with couplet plasma filtration adsorbtion (CPFA). J Am Soc Nephrol 2011;**22 - Congress Proceeding**:605A

Table 1. Characteristics of the patients before randomization

	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)
Body Mass Index N (%)		
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 (Acute Lung Injury)	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1-Q3]	53 [43-67]	51 [42-65]
SOFA at randomization, median [Q1-Q3]	9 [8-11]	9 [8-11]
RIFLE at randomization, 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 (%)	39 (42.4)	43 (47.8)
Missing	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 (Extended-spectrum β-lactamase) producing E. coli	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)
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)

SD=Standard deviation; Q1-Q3=first and third quartiles; Underweight=for male, BMI<20, for female, BMI<19; Normal weight=for male, BMI 20-25, for female, 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.

Table 2. Reasons for under treatment in the CPFA arm (N = 44)

	N	%
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organizational problems	4	9.1
Patient's death	4	9.1
Lack of specialized personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

Table 3. 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/day) N = 30	**2nd tertile of volume of plasma treated** (0.12-0.18 l/kg/day) N = 31	**3rd tertile of volume of plasma treated** (>0.18 l/kg/day) 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

Table 4. Results of the logistic regression model on hospital mortality

Variable	OR	95%-CI	*p*
Volume of plasma treated (l/kg/day)			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.52	0.73-3.17	0.033
CPFA, > 0.18 (3° tertile) vs. Controls	0.36	0.13-0.99	
Age (decades)	1.57	1.19-2.07	0.001
Source of admission			
Other ICU vs. Medical ward	0.28	0.04-1.89	0.021
Emergency room vs. Medical ward	0.27	0.11-0.67	
Surgical ward vs. Medical ward	0.34	0.15-0.77	
Renal failure at admission	4.08	1.47-11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04-0.75	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. Legend: OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Figure 1. Flow chart of participants.

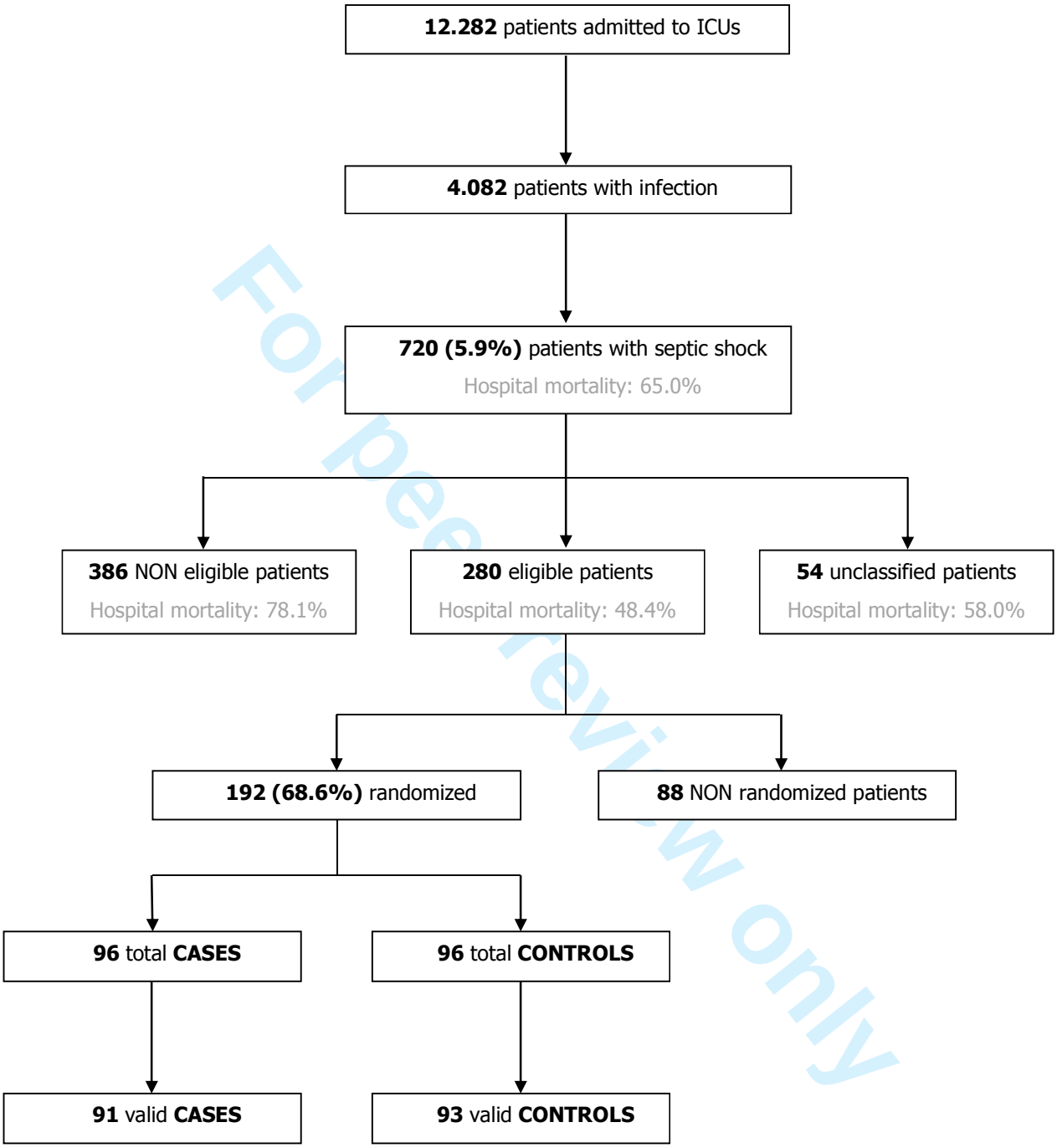
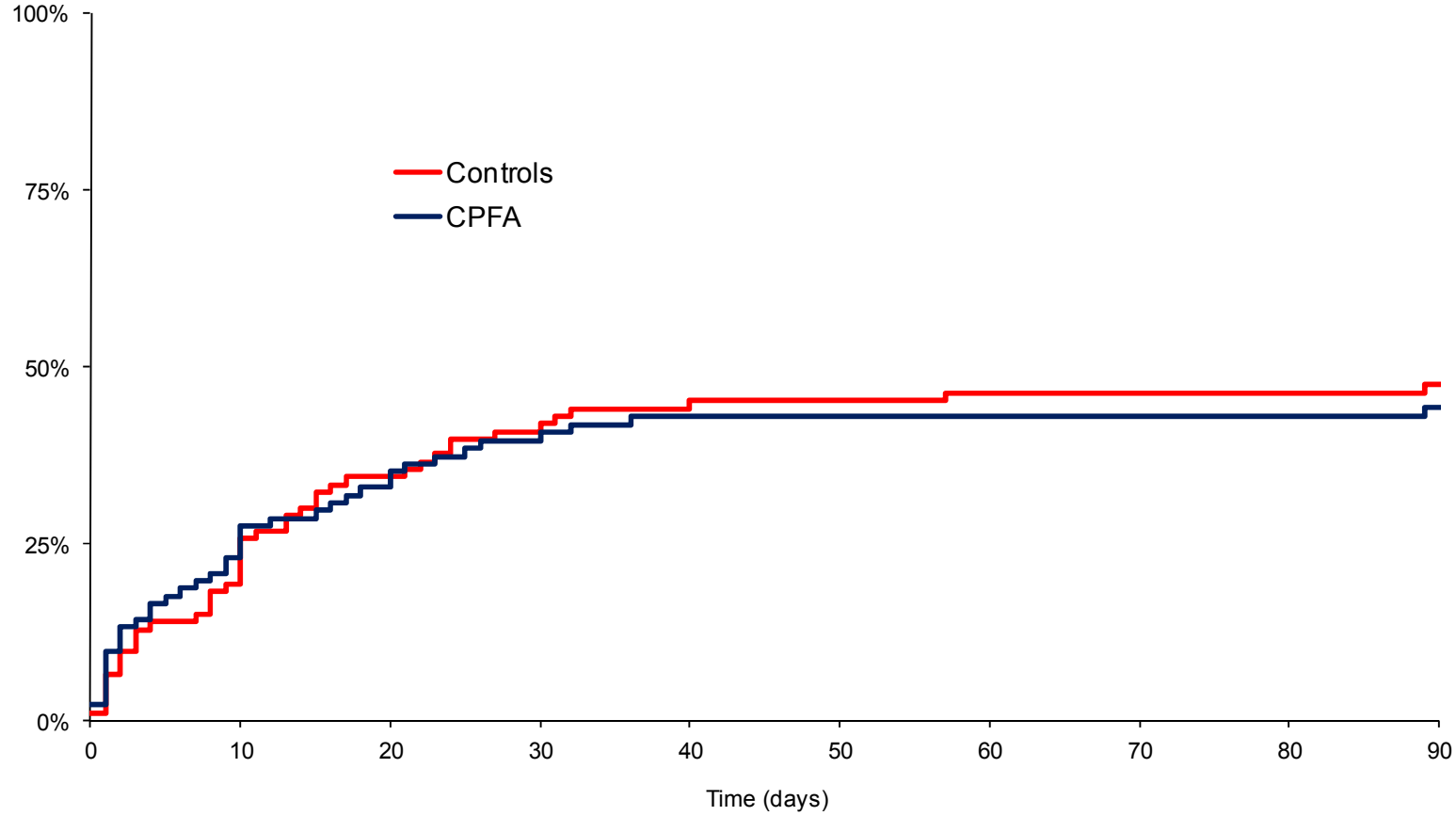
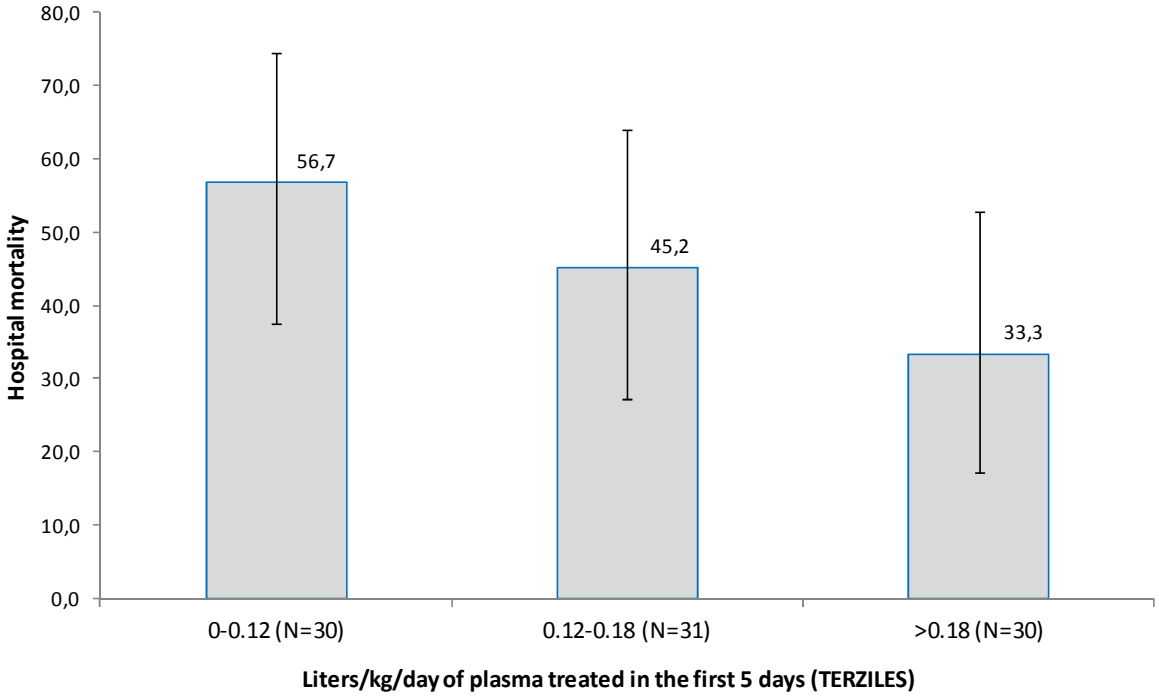


Figure 2. Survival curves.



Patients at risk										
Controls	93	75	61	55	51	50	48	48	47	46
CPFA	91	70	61	54	48	47	46	44	44	43

Figure 3. Hospital mortality according to the quantity of volume of plasma treated (whiskers represent 95% confidence interval).



χ^2 test for general association, 3.26; $p = 0.20$
Cochran-Armitage test for trend, 1.82; $p = 0.069$

Appendix

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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 one author (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; ad hoc site visits to ICUs with specific problems were performed during the study.

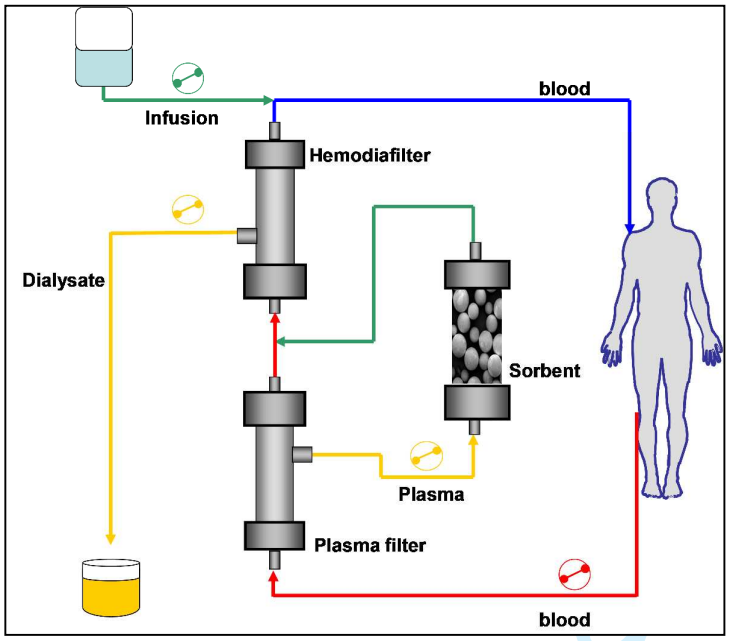
Central monitoring of the study identified 14 randomized patients whose eligibility criteria were in doubt. Further clinical information were retrieved for each patient and provided to the EDSMC, without revealing the randomization arm. According to internationally accepted criteria[1], the EDSMC determined 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, with life expectancy less than two weeks (exclusion criterion). In one case the patient was in coma following an operated spontaneous intra-cerebral hemorrhage (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.

Coupled plasma filtration adsorption (CPFA)

Coupled plasma filtration adsorption (CPFA) was developed to non-specifically remove larger cytokines and mediators during systemic inflammation with an extracorporeal circuit consisting of a plasma filter, a resin cartridge and a high flux dialyzer [2].

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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 hemofilter, in which convective exchanges may be applied in a post-dilution mode (see Figure S1) [3].

Figure S1. CPFA



The post-dilution reinfusion rate could be set up to 4 l/hr. 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 [4]. More specifically, the filtration fraction should be set to 10% in the first hour, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, 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, 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.

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

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.

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/min 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/min).

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

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) [5] and can be adopted.

Treatment schedule

Prefilter:

- heparin 1000 U/h
- Prostacyclin (Flolan) 4 ng/kg/min

Postfilter:

- Protamine sulphate 1 mg/100 IU 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 [6]. 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) [7]. 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 [8]. 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 [8].

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 S1. Characteristics of the patients before randomization

	Controls (N = 93)	**CPFA (N = 91)**	**1st tertile of volume of plasma treated** (<0.12 l/kg/day) N = 30	**2nd tertile of volume of plasma treated** (0.12-0.18 l/kg/day) N = 31	**3rd tertile of volume of plasma treated** (>0.18 l/kg/day) N = 30
Physiological parameters, mean [SD]					
PaO_2/FiO_2	167 [69]	197 [95]	189 [96]	186 [80]	215 [108]
INR	1.6 [0.5]	1.5 [0.4]	1.6 [0.4]	1.4 [0.3]	1.6 [0.4]
PTT	40.9 [12.0]	42.5 [15.4]	45.2 [19.4]	39.3 [14.0]	43.3 [12.0]
Platelet count (x 10^3)	196 [137]	156 [122]	119 [99]	159 [113]	190 [143]
Fibrinogen	575 [241]	534 [249]	502 [275]	633 [223]	463 [227]
Bilirubin	2.2 [2.5]	2.0 [3.7]	1.5 [1.7]	2.8 [5.9]	1.6 [1.2]
Creatinine	2.0 [1.4]	2.3 [1.5]	2.5 [1.7]	2.3 [1.5]	2.2 [1.3]
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/min or epinephrine or norepinephrine > 0.1 μg/kg/min
** = CVVH couldn't overcome the dose of 25 ml/kg/hr
SD=Standard deviation; Q1-Q3=first and third quartiles

Sensitivity analyses

Table S1. 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/day)			
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 S2. 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/day)			
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.

References

1. Fergusson, D., et al., *Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis.* Bmj, 2002. **325**(7365): p. 652-4.
2. Page, M. and T. Rimmele, *[Coupled plasma filtration adsorption: rationale and perspectives in septic shock].* Can J Anaesth, 2008. **55**(12): p. 847-52.
3. Ronco, C., et al., *Coupled plasma filtration adsorption: rationale, technical development and early clinical experience.* Blood Purif, 2003. **21**(6): p. 409-16.
4. Formica, M., et al., *Coupled plasma filtration adsorption.* Contrib Nephrol, 2007. **156**: p. 405-10.
5. Fabbri, L.P., et al., *Regional anticoagulation and antiaggregation for CVVH in critically ill patients: a prospective, randomized, controlled pilot study.* Acta Anaesthesiol Scand, 2010. **54**(1): p. 92-7.
6. Piantadosi, S., *Clinical trial. A methodological perspective*. 1997, New York: Jonh Wiley & Sons.
7. Freedman, L.S., D.J. Spiegelhalter, and M.K. Parmar, *The what, why and how of Bayesian clinical trials monitoring.* Stat Med, 1994. **13**(13-14): p. 1371-83; discussion 1385-9.
8. Hosmer, D. and S. Lemeshow, *Applied logistic regression*. 1989, New York: John Wiley and Sons, Inc.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
		Title and abstract	
	1a	Identification as a randomised trial in the title	page 1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	p. 2
		Introduction	
Background and objectives	2a	Scientific background and explanation of rationale	p. 3, row 2-22
	2b	Specific objectives or hypotheses	p. 3, r. 22-23
		Methods	
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	p. 4, r. 21-22
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	p. 4, r. 3-9
	4b	Settings and locations where the data were collected	p. 4, r.2
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	p. 4, r. 23-26 + Online suppl.
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	p. 4, r. 28-p. 5, r. 8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	p. 5, r. 13-15
	7b	When applicable, explanation of any interim analyses and stopping guidelines	p. 5, r. 17 + Online suppl.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	p. 4, r. 21-22
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	p. 5, r. 15-16
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	p. 4, r. 22-23
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	p. 4, r. 22-23; p. 4, r. 4-5;
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers,	NA

		those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	p. 5, r. 25-29
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	p. 6, r. 1-7 + Online suppl
		Results	
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	p. 6, r. 10-13+ Online suppl
Recruitment	14a	Dates defining the periods of recruitment and follow-up	p. 6, r. 9-10
	14b	Why the trial ended or was stopped	p. 5, r. 19-23; p. 8, r. 8-15
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 1; p. 5 r. 4-6
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	p. 6 r. 28 to p. 7 r. 4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	p. 6 r. 28-29
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	p. 7 r. 4-11
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	p. 6 r. 15-17
		Discussion	
Limitations	20	Trial limitations, sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	p. 8 r. 25 to p. 9 r. 28
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	p. 8 r. 16-24; p. 9 r. 29 to p. 10 r. 3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	p. 10 r. 3-12
Other information			
Registration	23	Registration number and name of trial registry	p. 10 r. 22
Protocol	24	Where the full trial protocol can be accessed, if available	p. 10 r. 21
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	p. 10 r. 14-17

BMJ open

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-003536.R1
Article Type:	Research
Date Submitted by the Author:	07-Nov-2013
Complete List of Authors:	Livigni, Sergio; Ospedale San Giovanni Bosco, Servizio Anestesia e Rianimazione B-DEA Bertolini, Guido; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Rossi, Carlotta; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Ferrari, Fiorenza; Ospedale San Giovanni Bosco, Servizio Anestesia e Rianimazione B-DEA Giardino, Michele; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Pozzato, Marco; Ospedale San Giovanni Bosco, Servizio di Nefrologia e Dialisi Remuzzi, Giuseppe; IRCCS-"Mario Negri" Institute for Pharmacological Research,
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES, Clinical trials < THERAPEUTICS

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva (Italian Group for the Evaluation of Interventions in Intensive Care Medicine) is an independent collaboration network of Italian intensive care units.

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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.

ClinicalTrials.gov, NCT00332371; ISRCTN24534559

Text word count: 3,821

Keywords: septic shock, intensive care medicine, coupled plasma filtration-adsorption

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Abstract

Objectives

Coupled plasma filtration-adsorption (CPFA), removing inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomized, 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, superiority clinical trial

Setting

18 Italian adult, general, intensive care units (ICUs)

Participants

Of the planned 330 adult patients with septic shock, 192 were randomized to either have CPFA added to the standard care, or not. The External Monitoring Committee excluded 8 ineligible patients who were erroneously included.

Interventions

CPFA was to be performed performed daily for 5 days, lasting at least 10 hours per day.

Primary and secondary outcome measures

The primary endpoint was mortality at discharge from the last hospital at which the patient stayed. Secondary endpoints were: 90-day mortality; new organ failures; 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 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 randomized to CPFA were subsequently found to be undertreated. An *a priori* planned subgroup analysis showed those receiving a CPFA dose >0.18 L kg^{-1} day^{-1} had a lower mortality compared to controls (OR 0.36, 95%-CI 0.13-0.99).

Conclusions

CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested CPFA could reduce mortality, if a high volume of plasma is treated. Due 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 number, NCT00332371; ISRCTN24534559).

Text word count: 299

Summary

Article focus

- Coupled plasmafiltration-adsorption (CPFA) is a blood purification technique specifically proposed for the treatment of severe infections, which provided promising results.
- This is an open label, multicentre, randomized, superiority, clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock.

Key messages

- We found no statistical difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution.
- Patients who had a larger volume of plasma treated with CPFA seemed to have a reduced hospital mortality, but this hypothesis should be confirmed in future trials.

Strengths and Limitations

- The study was prematurely terminated on the grounds of futility.
- A large part of patients randomized to CPFA were undertreated as per protocol stipulation, underlying the difficulty of performing such a technique.
- For this reason, it is difficult to say whether the ineffectiveness was due to the impracticability of the technique or to a lack of effect.
- The preplanned subgroup analysis suggesting efficacy if a high volume of plasma was treated, was aimed at minimizing potential biases, but they cannot be completely excluded.

Introduction

The host response against pathogens is a complex one. It is modulated through the production of numerous mediators that, among other mechanisms, promote both pro- 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, the under- or over-expression of their receptors[1 10-12], all these factors have negatively affected the numerous therapeutic attempts to neutralize specific molecules[12]. The repeated failure of this strategy suggested 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 plasmafiltration-adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators[13].

Early experience with CPFA showed increased survival in a rabbit model of endotoxin-induced septic shock[14]. The first clinical study showed that a single treatment lasting 10 hours significantly improved hemodynamic status [15]. These preliminary observations were confirmed in a study of ten 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 randomized multi-center clinical trial to assess the efficacy of CPFA in reducing mortality of critically ill patients with septic shock.

Methods

Ethics Statement and data sharing

The protocol was approved by each hospital's ethics committee. Written consent was obtained from the patient when possible, otherwise physicians enrolled patients according to the article 4.8.15 of the Guidelines for Good Clinical Practice[17]. Raw data are available upon justified request.

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 ICU were eligible for study

entry, provided that CPFA could be commenced within 6 hours from occurrence of hypotension refractory to fluids resuscitation. This was made by the attending physician (present 24/7) using explicit criteria[18]. Reasons for exclusion prior to randomization 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 hours, 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 enrolled patients, their clinical condition, including the Sequential Organ Failure Assessment (SOFA) score[22], the RIFLE criteria for acute renal dysfunction, and CPFA parameters were collected at the time of randomization 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 supplement.

Randomization and Interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enroll the patient or to register reasons for not doing so. Once enrolled, 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 randomization schedule (randomly permuting blocks of four and six)[23] was implemented in the eCRF, with stratification according to the center and the presence of septic shock on admission. The allocation was securely saved in the database and revealed only once baseline additional data collection was completed. All these procedures were implemented to guarantee allocation concealment[24].

Coupled plasma filtration adsorption (CPFA)

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 dialyzer [25].

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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^{-1}), and a final passage of the reconstituted blood through a high-permeability 1.4 m^2 polyethersulfone hemofilter, in which convective exchanges may be applied in a post-dilution mode (Figure 1) [26].

The post-dilution reinfusion rate could be set up to 4 L hr^{-1}. The blood flow was maintained between 150 and 200 ml min^{-1}, 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, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, corresponding to a blood flow of 150 ml min^{-1} and a filtration fraction of 12%.

The reinfusion solution, sterile and pyrogen-free, with bicarbonate buffer, contained the following composition (mmol L^{-1}): Na 140, K 1.5, Ca 2, Mg 0.75, Cl 108, bicarbonate 35, acetate 4, 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 supplement.

According to the available clinical evidence, CPFA was to be repeated daily for the first 5 days, lasting at least 10 hours each time, so that an average of 0.15 L kg^{-1} day^{-1} of plasma should have been treated per day.

Outcomes, Follow-up and Plan of analysis

The primary endpoint was mortality at discharge from the last hospital in which the patients were treated. Thus, for patients transferred to other hospital, mortality was assessed at the discharge from the last hospital in which the patients stayed. To minimize 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, 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 randomization; 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]); ICU-free days during the first 30 days from randomization.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely 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 hours of randomization.

The study was sized to have 80% power to detect an improvement in hospital mortality from an expected 63% 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 supplement) was adopted for interim analyses[23].

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted 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 analyzed using the χ^2 test. Effect size was expressed in terms of absolute risk difference with its 95% confidence interval (95%-*CI*)[28]. With regard to secondary endpoints and subgroup analyses, categorical variables were compared with χ^2 or Fisher exact tests, while a Student's *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 randomization 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 that 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 randomization was lost, comparison with the control group was performed through a logistic regression model that adjusted for possible confounders (see online supplement for details).

Results

Between January 2007 and November 2010 a total of 192 patients had been randomized. Recruitment in each ICU lasted a median of 22 months (interquartile range, 13-26). During this period, 386 patients with septic shock were excluded being non-eligible (see online supplement for details). Central monitoring subsequently identified 14 enrolled patients whose eligibility criteria were doubtful. Further clinical

information was retrieved and provided to the EDSMC who determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled (see online supplement). 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 supplement. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving treatment with 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 centers. To better express and investigate the phenomenon of under-treatment, and following the emerging concept of dose of renal replacement therapy[30], we computed the volume of plasma treated in L kg^{-1} day^{-1}. In the 91 patients randomized in the CPFA arm, a mean of 0.15 L kg^{-1} day^{-1} 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 under-treatment. Four patients died during CPFA, one before initiating the treatment, two in the very first moment, and one after the first 0.09 L kg^{-1} of plasma treated. The mean time to commencement of CPFA after septic shock identification was 5.7 hours (*SD* 3.8); 38 patients started within 4 hours. In the control group, in violation of the protocol, 2 patients were treated with CPFA, one died at 7 days post-randomization, the other was discharged alive from the hospital 37 days after randomization.

No statistical difference was found in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95%-*CI*, -12.2–16.6%). 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 control versus 56.0% for CPFA patients (p=0.99); the free-ICU days during the first 30 days post-randomization 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 [41.0%] 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 hours from randomization (17/38 [44.7%]; p=0.88), nor in those who started CPFA after 4 hours (20/46 [43.5%]; p=0.76). In 7 patients the timing of CPFA initiation was missing. Eventually, no effect of 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). Table 3 compares characteristics of the groups defined by the tertiles. 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^{-1} day^{-1} of plasma treated over the first 5 days) was statistically lower than in the control group (*OR* 0.36, 95%-*CI* 0.13-0.99). We then performed two sensitivity analyses, namely: limiting the evaluation of the volume of plasma treated to the first 3 days, and by excluding, both in the control and treated groups, patients who died in the first 24 hours post-randomization. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days, the second was intended to minimize 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 supplement) confirmed the same estimates, even though statistical significance was lost for lack of power.

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 outcomes have failed, highlighting the complexity of the immune response, including a marked intra-patient 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 randomized clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock.

The main findings

After randomizing 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 reverse these results with the sample still to be randomized, 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%), so reducing the power of the study. Thus, the EDSMC considered that continue to spend money in a clinical trial that had little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, nonbinding 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 have adopted 28-day mortality due to FDA 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 randomization. On the other hand, over-extending 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 last hospital into which they were admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialized, interdisciplinary care, which means he or she had survived the septic shock episode. 90-day mortality was anyway recorded and considered as secondary endpoint.

The problem of under-treatment

Nearly half the patients randomized to CPFA were undertreated as per protocol stipulation. This poses two crucial questions: the true feasibility of the technique in the ICU, and the possible relationship between the overall negative result and such under-treatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centers.

Why did the training not have effect? Many factors could have contributed. First, CPFA involves a complex circuit that includes a hemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions, and anticoagulation. We used heparin for anticoagulation (see online supplement), 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 centers may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be 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 cases the physicians did not start a new course of CPFA in 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. And it was often difficult to involve the nephrologists, that in many centers are those in charge of the procedure. On the other hand, despite excellent feedbacks from 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.

The per-protocol analysis and its limits

Of note, patients who had a larger volume of plasma treated seemed to have a 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 found, a number of potential problems threatens the validity of this result. Firstly, subgroup definition for the per-protocol analysis (i.e., tertiles of plasma treated) was based upon characteristics measured after randomization. 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 (Table 3). We adjusted for possible confounders in the multivariate model to minimize this risk, but we were limited to prognostic factors collected in the database. Particularly, we have no data on the immuno-inflammatory status of the patients to account for. Secondly, 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 standardized 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 supplement), the first hours were characterized by a certain degree of under-treatment 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 1 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 eligible patients were not randomized due to the very narrow window (6 hours) for patient recruitment and initiation of treatment. This would have particularly hampered the generalizability 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 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 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. 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 minimize potential biases, these cannot be completely excluded. Hence, this result can only be viewed as hypothesis generating. Regional anticoagulation with citrate represents a valid alternative 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 thirteen patients at high-risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with 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. Hence, we have designed a confirmatory, adaptive trial whose first step will be to prove citrate regional anticoagulation easily allows high volume of plasma treated with CPFA.

Acknowledgments

The study was funded by GiViTI and by 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.

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript. None of the authors has any conflict of interest in relation to this work. The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Registration number: ClinicalTrials.gov NCT00332371; ISRCTN24534559. Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis.

Contributorship Statement

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Competing Interests

None

Funding

This work was supported by the Bellco SpA

References

1. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 2003;**71**(10):5803-13
2. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007;**11**(2):R49 doi: cc5783 [pii] 10.1186/cc5783[published Online First: Epub Date]|.
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007;**167**(15):1655-63 doi: 167/15/1655 [pii] 10.1001/archinte.167.15.1655[published Online First: Epub Date]|.
4. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;**369**(9):840-51 doi: 10.1056/NEJMra1208623[published Online First: Epub Date]|.
5. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. Bmj 2003;**326**(7383):262-6
6. Weighardt H, Heidecke CD, Emmanuilidis K, et al. Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. Surgery 2000;**127**(3):309-15 doi: S0039-6060(00)91079-1 [pii] 10.1067/msy.2000.104118[published Online First: Epub Date]|.
7. Tamayo E, Fernandez A, Almansa R, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw;**22**(2):82-7 doi: ecn.2011.0281 [pii] 10.1684/ecn.2011.0281[published Online First: Epub Date]|.
8. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005;**365**(9453):63-78
9. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;**348**(2):138-50
10. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006;**12**(3):151-70 doi: 10.1179/096805106X102246[published Online First: Epub Date]|.
11. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 2000;**275**(27):20260-7 doi: 10.1074/jbc.M909168199 M909168199 [pii][published Online First: Epub Date]|.
12. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991;**21**(9):2177-84 doi: 10.1002/eji.1830210928[published Online First: Epub Date]|.
13. Tetta C, Bellomo R, Inguaggiato P, Wratten ML, Ronco C. Endotoxin and cytokine removal in sepsis. Ther Apher 2002;**6**(2):109-15 doi: tap413 [pii][published Online First: Epub Date]|.
14. Tetta C, Gianotti L, Cavaillon JM, et al. Coupled plasma filtration-adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000;**28**(5):1526-33
15. Ronco C, Brendolan A, Lonnemann G, et al. A pilot study of coupled plasma filtration with adsorption in septic shock*. Critical Care Medicine 2002;**30**(6):1250-55
16. Formica M, Olivieri C, Livigni S, et al. Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003;**29**(5):703-8
17. Human Medicines Evaluation Unit. Guidelines for good clinical practice. London: European Agency for the Evaluation of Medical Products, 1996:17-20.
18. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;**29**(4):530-8
19. Boffelli S, Rossi C, Anghileri A, et al. Continuous quality improvement in intensive care medicine. The GiViTI Margherita Project - Report 2005. Minerva Anestesiol 2006;**72**(6):419-32
20. Malacarne P, Langer M, Nascimben E, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;**36**(4):1105-13
21. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993;**270**(24):2957-63
22. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ

dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;**26**(11):1793-800
23. Piantadosi S. *Clinical trial. A methodological perspective*. New York: Jonh Wiley & Sons, 1997.
24. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet 2002;**359**(9306):614-8 doi: S0140-6736(02)07750-4 [pii] 10.1016/S0140-6736(02)07750-4[published Online First: Epub Date]|.
25. Page M, Rimmele T. [Coupled plasma filtration adsorption: rationale and perspectives in septic shock]. Can J Anaesth 2008;**55**(12):847-52 doi: 55/12/847 [pii] 10.1007/BF03034056[published Online First: Epub Date]|.
26. Ronco C, Brendolan A, d'Intini V, Ricci Z, Wratten ML, Bellomo R. Coupled plasma filtration adsorption: rationale, technical development and early clinical experience. Blood Purif 2003;**21**(6):409-16 doi: 10.1159/000073444 73444 [pii][published Online First: Epub Date]|.
27. Formica M, Inguaggiato P, Bainotti S, Wratten ML. Coupled plasma filtration adsorption. Contrib Nephrol 2007;**156**:405-10 doi: 102131 [pii] 10.1159/0000102131[published Online First: Epub Date]|.
28. Kleinbaum D, Kupper L, Morgenstern H. *Epidemiologic research*. New York, NY: Van Nostrand Reynhold, 1982.
29. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002;**325**(7365):652-4
30. Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. Critical Care 2011;**15**(2):207
31. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med 2003;**168**(2):165-72
32. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;**29**(4):880-6
33. Perl TM, Dvorak L, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;**274**(4):338-45
34. Singer M, McNally T, Screaton G, Mackie I, Machin S, Cohen SL. Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 1994;**20**(3):212-5
35. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med;**38**(1):20-8 doi: 10.1007/s00134-011-2438-3[published Online First: Epub Date]|.
36. Mariano F, Bergamo D, Gangemi EN, Hollo Z, Stella M, Triolo G. Citrate Anticoagulation for Continuous Renal Replacement Therapy in Critically Ill Patients: Success and Limits. International Journal of Nephrology 2011;**2011** doi: 10.4061/2011/748320[published Online First: Epub Date]|.
37. Mariano F, Tetta C, Stella M, Biolino P, Miletto A, Triolo G. Regional Citrate Anticoagulation in Critically Ill Patients Treated with Plasma Filtration and Adsorption. Blood Purification 2004;**22**(3):313-19
38. Pozzato M, Ferrari F, Cecere P, et al. Safety and efficacy of citrate anticoagulation in spetic shock patients treated with couplet plasma filtration adsorbtion (CPFA). J Am Soc Nephrol 2011;**22 - Congress Proceeding**:605A

Table 1. Characteristics of the patients before randomization

	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)
Body Mass Index *n* (%)		
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 (Acute Lung Injury)	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1-Q3]	53 [43-67]	51 [42-65]
SOFA at randomization, median [Q1-Q3]	9 [8-11]	9 [8-11]
RIFLE at randomization, *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* (%)	39 (42.4)	43 (47.8)
Missing	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 (Extended-spectrum □-lactamase) producing E. coli	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)
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)

SD=Standard deviation; Q1-Q3=first and third quartiles; Underweight=for male, BMI<20, for female, BMI<19; Normal weight=for male, BMI 20-25, for female, 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.

Table 2. Reasons for under treatment in the CPFA arm (n = 44)

	n	**%**
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organizational problems	4	9.1
Patient's death	4	9.1
Lack of specialized personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

Table 3. 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

Table 4. Results of the logistic regression model on hospital mortality

Variable	OR	95%-CI	*p*
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.52	0.73-3.17	0.033
CPFA, > 0.18 (3° tertile) vs. Controls	0.36	0.13-0.99	
Age (decades)	1.57	1.19-2.07	0.001
Source of admission			
Other ICU vs. Medical ward	0.28	0.04-1.89	0.021
Emergency room vs. Medical ward	0.27	0.11-0.67	
Surgical ward vs. Medical ward	0.34	0.15-0.77	
Renal failure at admission	4.08	1.47-11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04-0.75	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. Legend: OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Figure 1. CPFA schema

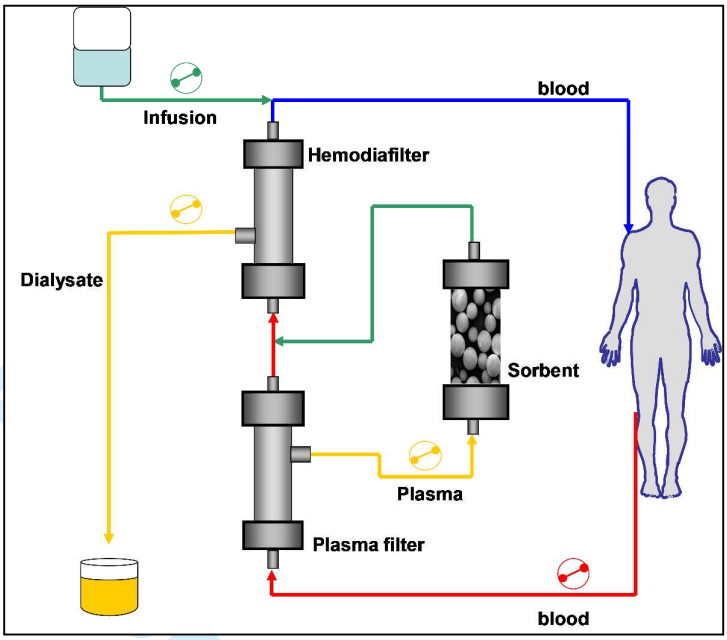


Figure 2. Flow chart of participants.

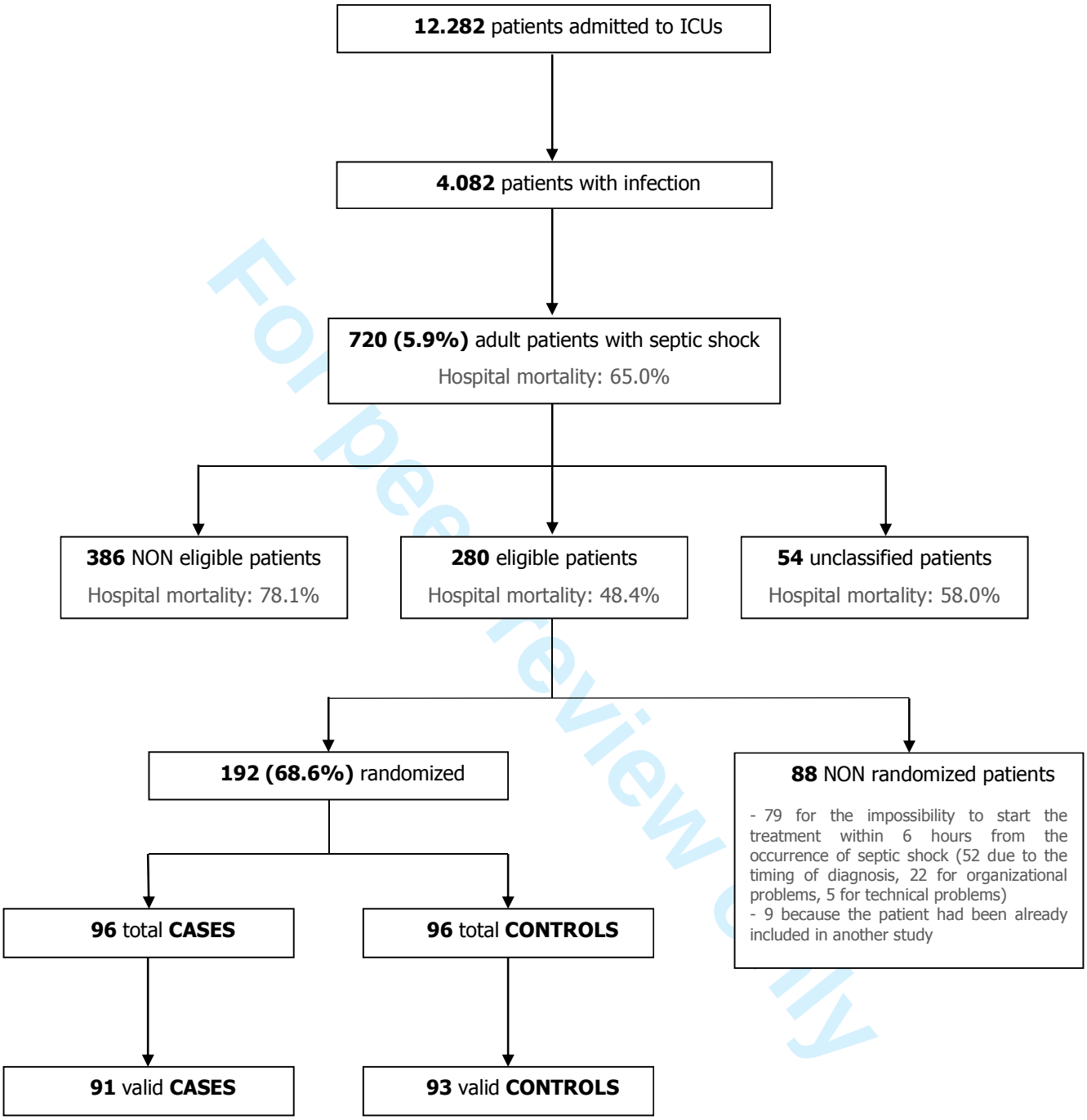
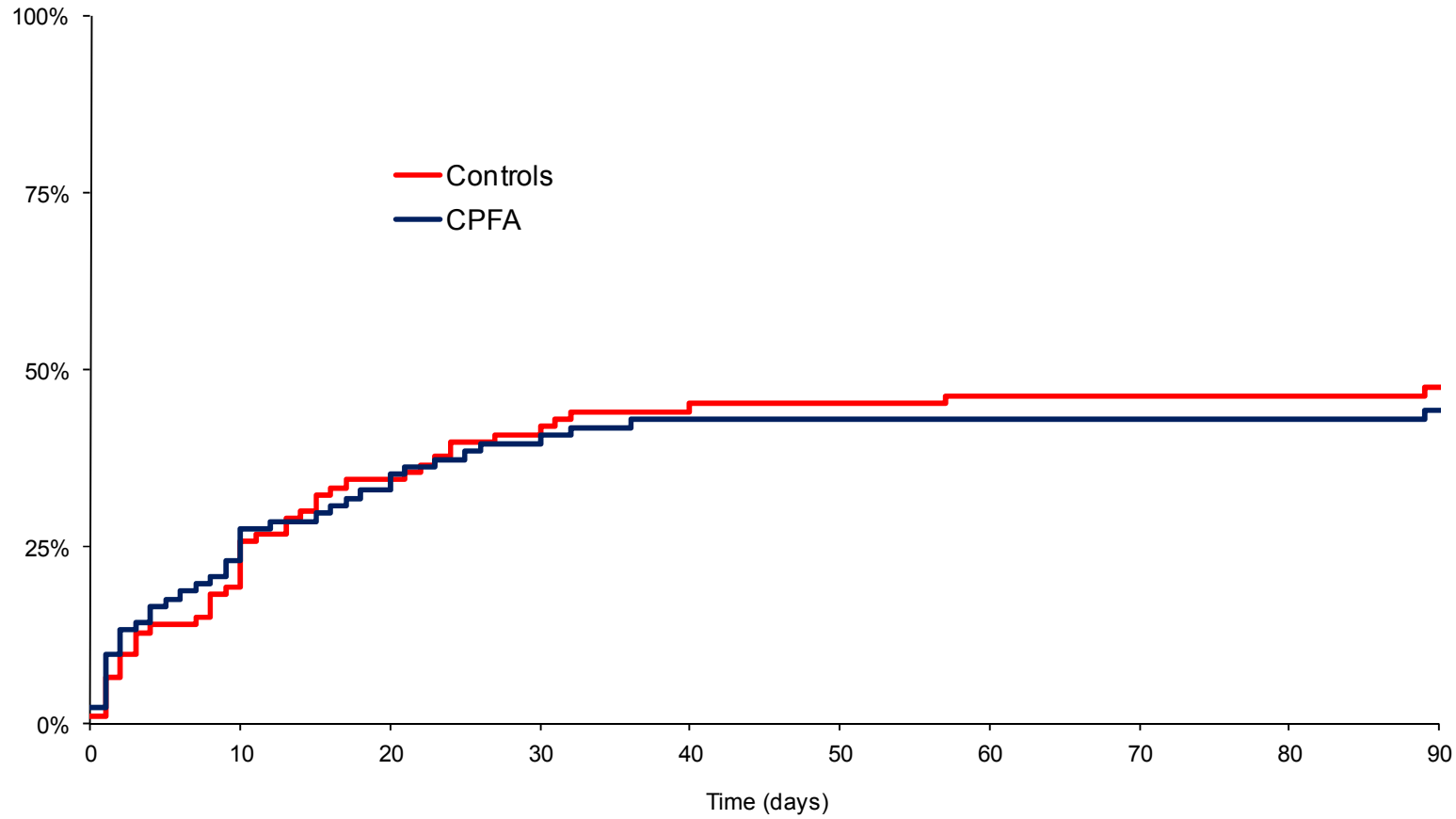
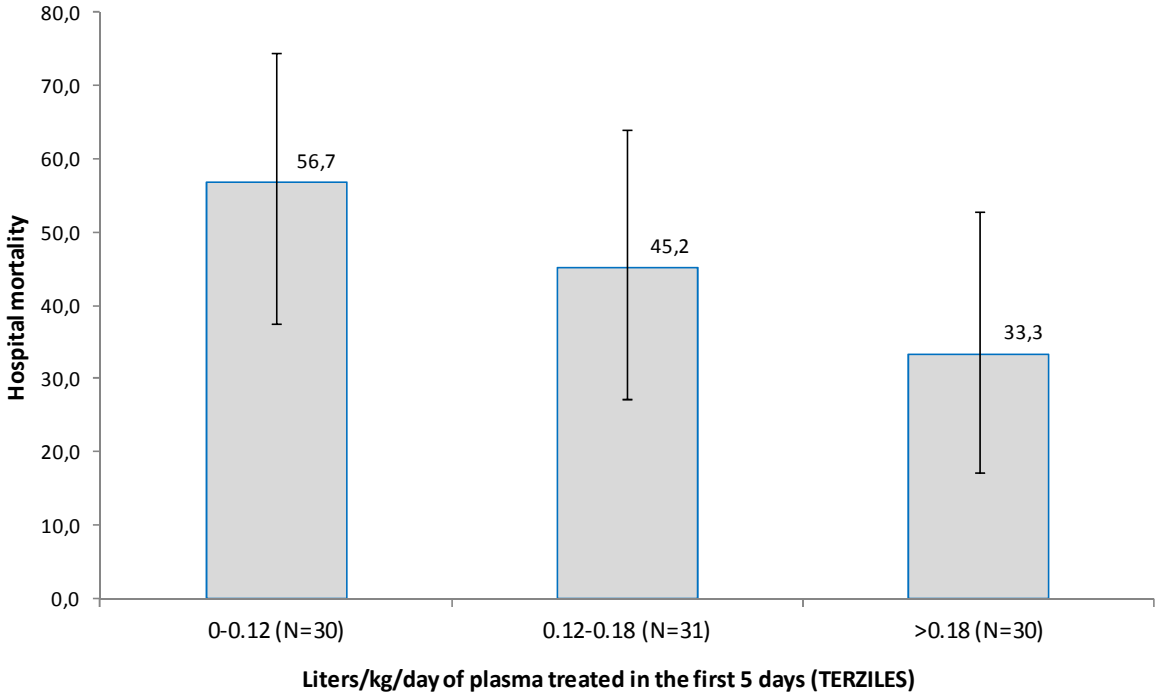


Figure 3. Survival curves.



Patients at risk										
Controls	93	75	61	55	51	50	48	48	47	46
CPFA	91	70	61	54	48	47	46	44	44	43

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



χ^2 test for general association, 3.26; $p = 0.20$
Cochran-Armitage test for trend, 1.82; $p = 0.069$

Appendix

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Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva (Italian Group for the Evaluation of Interventions in Intensive Care Medicine) is an independent collaboration network of Italian intensive care units.

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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.

ClinicalTrials.gov, NCT00332371; ISRCTN24534559

Text word count: 3,~~084~~821

Keywords: septic shock, intensive care medicine, coupled plasma filtration-adsorption

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Summary

Article focus

- Coupled plasmafiltration-adsorption (CPFA) is a blood purification technique specifically proposed for the treatment of severe infections, which provided promising results.
- This is an open label, multicentre, randomized, superiority, clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock.

Key messages

- We found no statistical difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution.
- Patients who had a larger volume of plasma treated with CPFA seemed to have a reduced hospital mortality, but this hypothesis should be confirmed in future trials.

Strengths and Limitations

- The study was prematurely terminated on the grounds of futility.
- A large part of patients randomized to CPFA were undertreated as per protocol stipulation, underlying the difficulty of performing such a technique.
- For this reason, it is difficult to say whether the ineffectiveness was due to the impracticability of the technique or to a lack of effect.
- The preplanned subgroup analysis suggesting efficacy if a high volume of plasma was treated, was aimed at minimizing potential biases, but they cannot be completely excluded.

Abstract

Objectives

Coupled plasma filtration-adsorption (CPFA), removing inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomized, 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, superiority clinical trial

Setting

18 Italian adult, general, intensive care units (ICUs)

Participants

Of the planned 330 adult patients with septic shock, 192 were randomized to either have CPFA added to the standard care, or not. The External Monitoring Committee excluded 8 ineligible patients who were erroneously included.

Interventions

CPFA was to be performed performed daily for 5 days, lasting at least 10 hours per day.

Primary and secondary outcome measures

The primary endpoint was mortality at discharge from the last hospital at which the patient stayed. Secondary endpoints were: 90-day mortality; new organ failures; 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 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 randomized to CPFA were subsequently found to be undertreated. An *a priori* planned subgroup analysis showed those receiving a CPFA dose >0.18 ~~l~~L ~~/~~kg^{-1}~~/~~ day^{-1} had a lower mortality compared to controls (OR 0.36, 95%-CI 0.13-0.99).

Conclusions

CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested CPFA could reduce mortality, if a high volume of plasma is treated. Due 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 number, NCT00332371; ISRCTN24534559).

Text word count: ~~296~~299

Introduction

The ~~immune~~ host response against pathogens is a complex one. It is modulated through the production of numerous mediators~~, like cytokines,~~ that, among other mechanisms, promote both pro- and anti-inflammatory responses[1-4]. The ~~overall efficacy is dictated by the~~ balance between these two ~~responses~~ pathways heavily influences the outcome[4-9]. ~~, attained through a combination of different factors: t~~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 under- or over-expression of their receptors[1 10-12], all these factors have negatively affected the~~. In some circumstances, the release of inflammatory mediators is so over-abundant that the immune response goes out of control, initiating systemic response that leads to organ dysfunction, and septic shock, heavily influencing the prognosis.~~

~~Following observations that plasma cytokine levels are elevated in critically ill septic patients and this may relate to eventual outcome,~~ numerous therapeutic attempts ~~have been made~~ to neutralize specific molecules[12]. The repeated failure of this strategy suggested 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 plasmafiltration-adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators[13].

Early experience with CPFA showed increased survival in a rabbit model of endotoxin-induced septic shock[14]. The first clinical study showed that a single treatment lasting 10 hours significantly improved hemodynamic status [15]. These preliminary observations were confirmed in a study of ten 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 randomized multi-center clinical trial to assess the efficacy of CPFA in ~~the treatment~~reducing mortality of critically ill patients with septic shock.

Methods

Ethics Statement and data sharing

The protocol was approved by each hospital's ethics committee. Written consent was obtained from the patient when possible, otherwise physicians enrolled patients according to the article 4.8.15 of the ~~European~~

Guidelines for Good Clinical Practice[17]. Raw data are available upon justified request.

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 ICU were eligible for study entry, provided that CPFA could be commenced within 6 hours from ~~diagnosis~~occurrence of hypotension refractory to fluids resuscitation. This was made by the attending physician (present 24/7) using explicit criteria[18]. Reasons for exclusion prior to randomization were~~included~~: 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 hours, 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 enrolled patients, their clinical condition, including the Sequential Organ Failure Assessment (SOFA) score[22], the RIFLE criteria for acute renal dysfunction, and CPFA parameters were collected at the time of randomization 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 supplement.

Randomization and Interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enroll the patient or to register reasons for not doing so. ~~Enrolled~~ Once enrolled, 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 randomization schedule (randomly permuting blocks of four and six)[23] was implemented in the eCRF, with stratification according to the center and the presence of septic shock on admission. The allocation was securely saved in the database and revealed only once baseline additional data collection was completed. All these procedures were implemented to guarantee allocation concealment[24]. ~~According to the available clinical evidence, CPFA was intended to be repeated daily for the first 5 days, lasting at least 10 hours each day, with a plasma flow of 30-40 ml/min and a minimum of 10 liters of plasma treated per day (see the online~~

~~supplement).~~

Coupled plasma filtration adsorption (CPFA)

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 dialyzer [25]~~[24]~~.

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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^{-1}), and a final passage of the reconstituted blood through a high-permeability 1.4 m^2 polyethersulfone hemofilter, in which convective exchanges may be applied in a post-dilution mode (Figure 1) [26]~~[25]~~.

The post-dilution reinfusion rate could be set up to 4 L hr^{-1}. The blood flow was maintained between 150 and 200 ml min^{-1}, while the plasma flow was controlled by a filtration fraction ranging from 10 to 18% of blood flow [27]~~[26]~~. More specifically, the filtration fraction should be set to 10% in the first hour, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, corresponding to a blood flow of 150 ml min^{-1} and a filtration fraction of 12%.

The reinfusion solution, sterile and pyrogen-free, with bicarbonate buffer, contained the following composition (mmol L^{-1}): Na 140, K 1.5, Ca 2, Mg 0.75, Cl 108, bicarbonate 35, acetate 4, 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 supplement.

According to the available clinical evidence, CPFA was to be repeated daily for the first 5 days, lasting at least 10 hours each time, so that an average of 0.15 L kg^{-1} day^{-1} of plasma should have been treated per day.

Outcomes, Follow-up and Plan of analysis

The primary endpoint was mortality at discharge from the last hospital in which the patients were treated. Thus, for patients transferred to other hospital, mortality was assessed at the discharge from the last hospital in which the patients stayed. To minimize 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, 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 randomization; 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]); ICU-free days during the first 30 days from randomization.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely 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 hours of randomization.

The study was sized to have 80% power to detect an improvement in hospital mortality from an expected 63% to 47% with CPFA (25% relative improvement), with a two-tailed 5% type I error. A total of 330 patients were required. ~~A blocked randomization schedule (randomly permuting blocks of four and six) was adopted[26], with stratification according to the center and the presence of septic shock on admission.~~ A Bayesian approach (see online supplement) was adopted for interim analyses[23].

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted 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 analyzed using the χ^2 test. Effect size was expressed in terms of absolute risk difference with its 95% confidence interval (95%-*CI*)[28]~~[27]~~. With regard to secondary endpoints and subgroup analyses, categorical variables were compared with χ^2 or Fisher exact tests, while a Student's *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 randomization 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 that 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 randomization was lost, comparison with the control group was performed through a logistic regression model that adjusted for possible confounders (see online

supplement for details).

Results

Between January 2007 and November 2010 a total of 192 patients had been randomized. Recruitment in each ICU lasted a median of 22 months (interquartile range, 13-26). During this period, 386 patients with septic shock were excluded being non-eligible (see online supplement for details). Central monitoring subsequently identified 14 enrolled patients whose eligibility criteria were doubtful. Further clinical information was retrieved and provided to the EDSMC who determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled (see online supplement). Analysis was performed by intention-to-treat on the 184 remaining patients[29][28]. Figure 12 denotes the flow of participants.

Table 1 shows the patients' characteristics, further details are provided in the online supplement. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving treatment with 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 centers. To better express and investigate the phenomenon of under-treatment, and following the emerging concept of dose of renal replacement therapy[30][29], we computed the volume of plasma treated in L/kg^{-1}/day^{-1}. In the 91 patients randomized in the CPFA arm, a mean of 0.15 L kg^{-1}/day^{-1} 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 under-treatment. Four patients died during CPFA, one before initiating the treatment, two in the very first moment, and one after the first 0.09 L kg^{-1} of plasma treated. The mean time to commencement of CPFA after septic shock identification was 5.7 hours (*SD* 3.8); 38 patients started within 4 hours. In the control group, in violation of the protocol, 3 2 patients were treated with CPFA, one of whom died at 7 days post-randomization, the other was discharged alive from the hospital 37 days after randomization.

No statistical difference was foundseen in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95%-*CI*, -12.2–16.6%). The 90-day survival curves of the two groups substantially overlapped (logrank test, p=0.48) (Figure 23). Secondary endpoints did not statistically differ: the occurrence of new organ failure was 55.9% in control versus 56.0% for CPFA patients (p=0.99); the free-ICU days during the first 30 days post-randomization 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 [41.0%] 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 hours from randomization (17/38 [44.7%]; p=0.88), nor in those who started CPFA after 4 hours (20/46 [43.5%]; p=0.76). In 7 patients the timing of CPFA initiation was missing. Eventually, no effect of the number of patients per ICU was observed.

The per-protocol analysis revealed a ~~near~~ 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 ~~3~~4). Table 3 compares characteristics of the groups defined by the tertiles. The logistic regression model, aimed at adjusting for possible confounders, verified that hospital mortality in patients falling within the third tertile (≥0.18 ~~l~~L kg^{-1} ~~/~~day^{-1} of plasma treated over the first 5 days) was statistically lower than in the control group (*OR* 0.36, 95%-*CI* 0.13-0.99). ~~On the other hand, there was no evidence that outcome in patients who received lower volume treatment was statistically better or worse than controls, as the 95%-CI did include the null value of 1 (OR=1.52, 95% CI=0.73-3.17).~~ We then performed two sensitivity analyses, namely: limiting the evaluation of the volume of plasma treated to the first 3 days, and by excluding, both in the control and treated groups, patients who died in the first 24 hours post-randomization. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days, the second was intended to minimize 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 supplement) confirmed the same estimates, even though statistical significance was lost for lack of power.

Discussion

The prognosis of critically ill patients with septic shock remains poor, with mortality rates still around 50-60%[20 31]~~[20-30]~~. All attempts to find a "magic bullet" to restore immune derangements during sepsis and improve outcomes have failed, highlighting the complexity of the immune response, including a marked intra-patient 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][~~15 16 24~~], GiViTI performed this randomized clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock.

The main findings

After randomizing 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 ~~overturn~~ reverse these results with the sample still to be randomized, 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 ~~1~~2), the eligibility criteria selected a subgroup where mortality was sensibly lower (47.3%), so reducing the power of the study. Thus, the EDSMC considered that continue to spend money in a clinical trial that had little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, nonbinding 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][~~31~~]. Most have adopted 28-day mortality due to FDA stipulations. However, the mortality rate attributable to sepsis continues long after the initiation of the acute event[33][~~32~~]; indeed, 16.8% of our study patients were still in the ICU beyond 28 days after randomization. On the other hand, over-extending 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 last hospital into which they were admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialized, interdisciplinary care, which means he or she had survived the septic shock episode. 90-day mortality was anyway recorded and considered as secondary endpoint.

The problem of under-treatment

Nearly half the patients randomized to CPFA were undertreated as per protocol stipulation. This poses two crucial questions: the true feasibility of the technique in the ICU, and the possible relationship between the overall negative result and such under-treatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centers.

Why did the training not have effect? Many factors could have contributed. First, CPFA involves a complex circuit that includes a hemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions, and anticoagulation. We used heparin for anticoagulation (see online supplement), 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 centers may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be insufficient antithrombin substrate for the heparin to be effective[34][~~33~~]. Second, because of the high cost of the procedure (about 1.200 € per treatment), in most cases the physicians did not start a new course of CPFA in 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. And it was often difficult to involve the nephrologists, that in many centers are those in charge of the procedure. On the other hand, despite excellent feedbacks from 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.

The per-protocol analysis and its limits

Of note, patients who had a larger volume of plasma treated seemed to have a 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 found, a number of potential problems threatens the validity of this result. Firstly, subgroup definition for the per-protocol analysis (i.e., tertiles of plasma treated) was based upon characteristics measured after randomization. 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 (Table 3). We adjusted for possible confounders in the multivariate model to minimize this risk, but we were limited to prognostic factors collected in the database. Particularly, we have no data on the immuno-inflammatory status of the patients to account for. Secondly, 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 standardized 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 supplement), the first hours were characterized by a certain degree of under-treatment 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 1 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 eligible patients were not randomized due to the very narrow window (6 hours) for patient recruitment and initiation of treatment. This would have particularly hampered the generalizability 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 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 more robust with a larger sample.

Conclusion

~~In 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. 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 minimize potential biases, these cannot be completely excluded. ~~Unfortunately, we have no data on the immuno inflammatory~~

~~status of the patients to account for.~~ Hence, this result can only be viewed as hypothesis generating ~~and should be confirmed in future trials~~. Regional anticoagulation with citrate represents a valid alternative as its anticoagulatory effect is limited to the extracorporeal circuit, without any systemic effect, and can be safely applied in the ICU[35 36]~~[34 35]~~. In a feasibility study carried out in thirteen patients at high-risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with heparin[37]~~[36]~~. 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]~~[37]~~. Should these preliminary results be ~~established~~confirmed, the question whether the reason of our negative result was a problem of feasibility or efficacy would become essential, ~~a confirmatory trial should be considered~~ to avoid the risk of dismissing a potentially effective treatment for such a high mortality condition as septic shock~~, as a consequence of the present negative results~~. Hence, we have designed a confirmatory, adaptive trial whose first step will be to prove citrate regional anticoagulation easily allows high volume of plasma treated with CPFA.

Acknowledgments

The study was funded by GiViTI and by 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.

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript. None of the authors has any conflict of interest in relation to this work. The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Registration number: ClinicalTrials.gov NCT00332371; ISRCTN24534559. Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis.

References

1. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 2003;**71**(10):5803-13
2. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007;**11**(2):R49 doi: cc5783 [pii]
10.1186/cc5783[published Online First: Epub Date]|.
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and

sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007;**167**(15):1655-63 doi: 167/15/1655 [pii]
10.1001/archinte.167.15.1655[published Online First: Epub Date]|.
4. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;**369**(9):840-51 doi: 10.1056/NEJMra1208623[published Online First: Epub Date]|.
5. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. Bmj 2003;**326**(7383):262-6
6. Weighardt H, Heidecke CD, Emmanuilidis K, et al. Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. Surgery 2000;**127**(3):309-15 doi: S0039-6060(00)91079-1 [pii]
10.1067/msy.2000.104118[published Online First: Epub Date]|.
7. Tamayo E, Fernandez A, Almansa R, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw;**22**(2):82-7 doi: ecn.2011.0281 [pii]
10.1684/ecn.2011.0281[published Online First: Epub Date]|.
8. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005;**365**(9453):63-78
9. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;**348**(2):138-50
10. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006;**12**(3):151-70 doi: 10.1179/096805106X102246[published Online First: Epub Date]|.
11. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 2000;**275**(27):20260-7 doi: 10.1074/jbc.M909168199
M909168199 [pii][published Online First: Epub Date]|.
12. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991;**21**(9):2177-84 doi: 10.1002/eji.1830210928[published Online First: Epub Date]|.
13. Tetta C, Bellomo R, Inguaggiato P, Wratten ML, Ronco C. Endotoxin and cytokine removal in sepsis. Ther Apher 2002;**6**(2):109-15 doi: tap413 [pii][published Online First: Epub Date]|.
14. Tetta C, Gianotti L, Cavaillon JM, et al. Coupled plasma filtration-adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000;**28**(5):1526-33
15. Ronco C, Brendolan A, Lonnemann G, et al. A pilot study of coupled plasma filtration with adsorption in septic shock*. Critical Care Medicine 2002;**30**(6):1250-55
16. Formica M, Olivieri C, Livigni S, et al. Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003;**29**(5):703-8
17. Human Medicines Evaluation Unit. Guidelines for good clinical practice. London: European Agency for the Evaluation of Medical Products, 1996:17-20.
18. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;**29**(4):530-8
19. Boffelli S, Rossi C, Anghileri A, et al. Continuous quality improvement in intensive care medicine. The GiViTI Margherita Project - Report 2005. Minerva Anestesiol 2006;**72**(6):419-32
20. Malacarne P, Langer M, Nascimben E, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;**36**(4):1105-13
21. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993;**270**(24):2957-63
22. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;**26**(11):1793-800
23. Piantadosi S. *Clinical trial. A methodological perspective*. New York: Jonh Wiley & Sons, 1997.
24. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet 2002;**359**(9306):614-8 doi: S0140-6736(02)07750-4 [pii]
10.1016/S0140-6736(02)07750-4[published Online First: Epub Date]|.
25. Page M, Rimmele T. [Coupled plasma filtration adsorption: rationale and perspectives in septic shock]. Can J Anaesth 2008;**55**(12):847-52 doi: 55/12/847 [pii]
10.1007/BF03034056[published Online First: Epub Date]|.
26. Ronco C, Brendolan A, d'Intini V, Ricci Z, Wratten ML, Bellomo R. Coupled plasma filtration adsorption: rationale, technical development and early clinical experience. Blood Purif 2003;**21**(6):409-16 doi: 10.1159/000073444

73444 [pii][published Online First: Epub Date]|.
27. Formica M, Inguaggiato P, Bainotti S, Wratten ML. Coupled plasma filtration adsorption. Contrib Nephrol 2007;**156**:405-10 doi: 102131 [pii]
10.1159/0000102131[published Online First: Epub Date]|.
28. Kleinbaum D, Kupper L, Morgenstern H. *Epidemiologic research*. New York, NY: Van Nostrand Reynhold, 1982.
29. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002;**325**(7365):652-4
30. Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. Critical Care 2011;**15**(2):207
31. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med 2003;**168**(2):165-72
32. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;**29**(4):880-6
33. Perl TM, Dvorak L, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;**274**(4):338-45
34. Singer M, McNally T, Screaton G, Mackie I, Machin S, Cohen SL. Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 1994;**20**(3):212-5
35. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med;**38**(1):20-8 doi: 10.1007/s00134-011-2438-3[published Online First: Epub Date]|.
36. Mariano F, Bergamo D, Gangemi EN, Hollo Z, Stella M, Triolo G. Citrate Anticoagulation for Continuous Renal Replacement Therapy in Critically Ill Patients: Success and Limits. International Journal of Nephrology 2011;**2011** doi: 10.4061/2011/748320[published Online First: Epub Date]|.
37. Mariano F, Tetta C, Stella M, Biolino P, Miletto A, Triolo G. Regional Citrate Anticoagulation in Critically Ill Patients Treated with Plasma Filtration and Adsorption. Blood Purification 2004;**22**(3):313-19
38. Pozzato M, Ferrari F, Cecere P, et al. Safety and efficacy of citrate anticoagulation in spetic shock patients treated with couplet plasma filtration adsorbtion (CPFA). J Am Soc Nephrol 2011;**22 - Congress Proceeding**:605A

Table 1. Characteristics of the patients before randomization

	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)
Body Mass Index *n* (%)		
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 (Acute Lung Injury)	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1-Q3]	53 [43-67]	51 [42-65]
SOFA at randomization, median [Q1-Q3]	9 [8-11]	9 [8-11]
RIFLE at randomization, *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* (%)	39 (42.4)	43 (47.8)
Missing	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 (Extended-spectrum β-lactamase) producing E. coli	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)
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)

SD=Standard deviation; Q1-Q3=first and third quartiles; Underweight=for male, BMI<20, for female, BMI<19; Normal weight=for male, BMI 20-25, for female, 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.

Table 2. Reasons for under treatment in the CPFA arm (n = 44)

	n	**%**
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organizational problems	4	9.1
Patient's death	4	9.1
Lack of specialized personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

Table 3. 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

Table 4. Results of the logistic regression model on hospital mortality

Variable	OR	95%-CI	*p*
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.52	0.73-3.17	0.033
CPFA, > 0.18 (3° tertile) vs. Controls	0.36	0.13-0.99	
Age (decades)	1.57	1.19-2.07	0.001
Source of admission			
Other ICU vs. Medical ward	0.28	0.04-1.89	0.021
Emergency room vs. Medical ward	0.27	0.11-0.67	
Surgical ward vs. Medical ward	0.34	0.15-0.77	
Renal failure at admission	4.08	1.47-11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04-0.75	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. Legend: OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Figure 1. CPFA schema

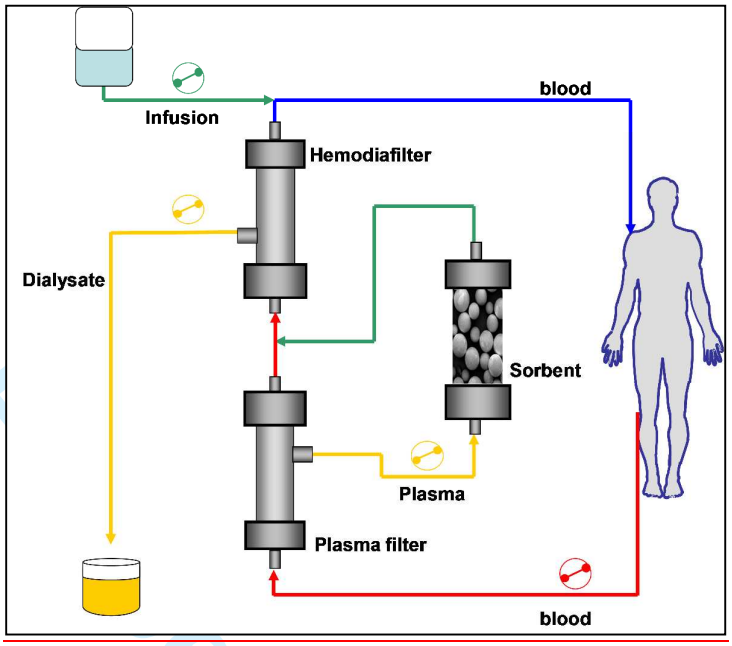


Figure 2. Flow chart of participants.

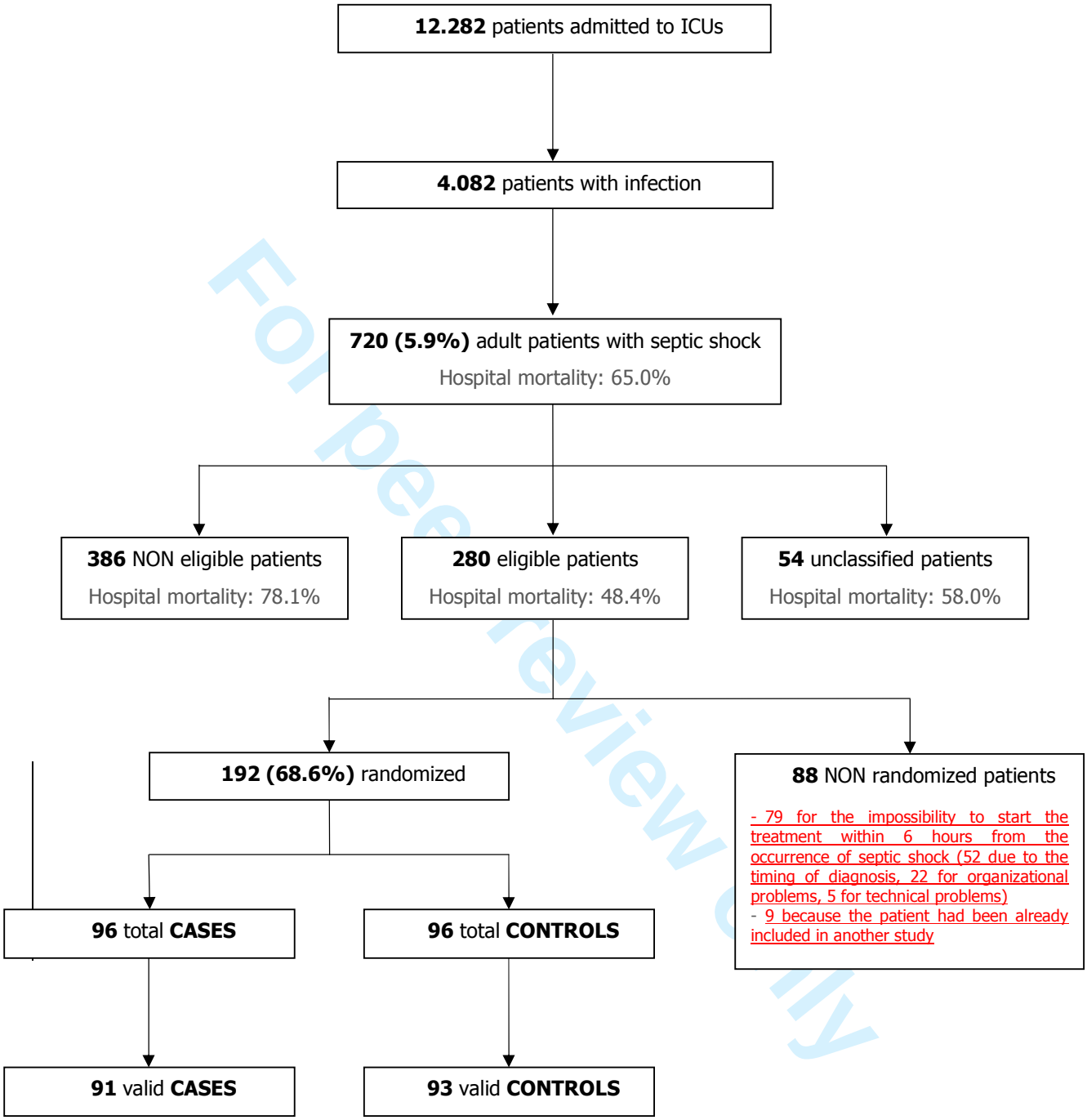
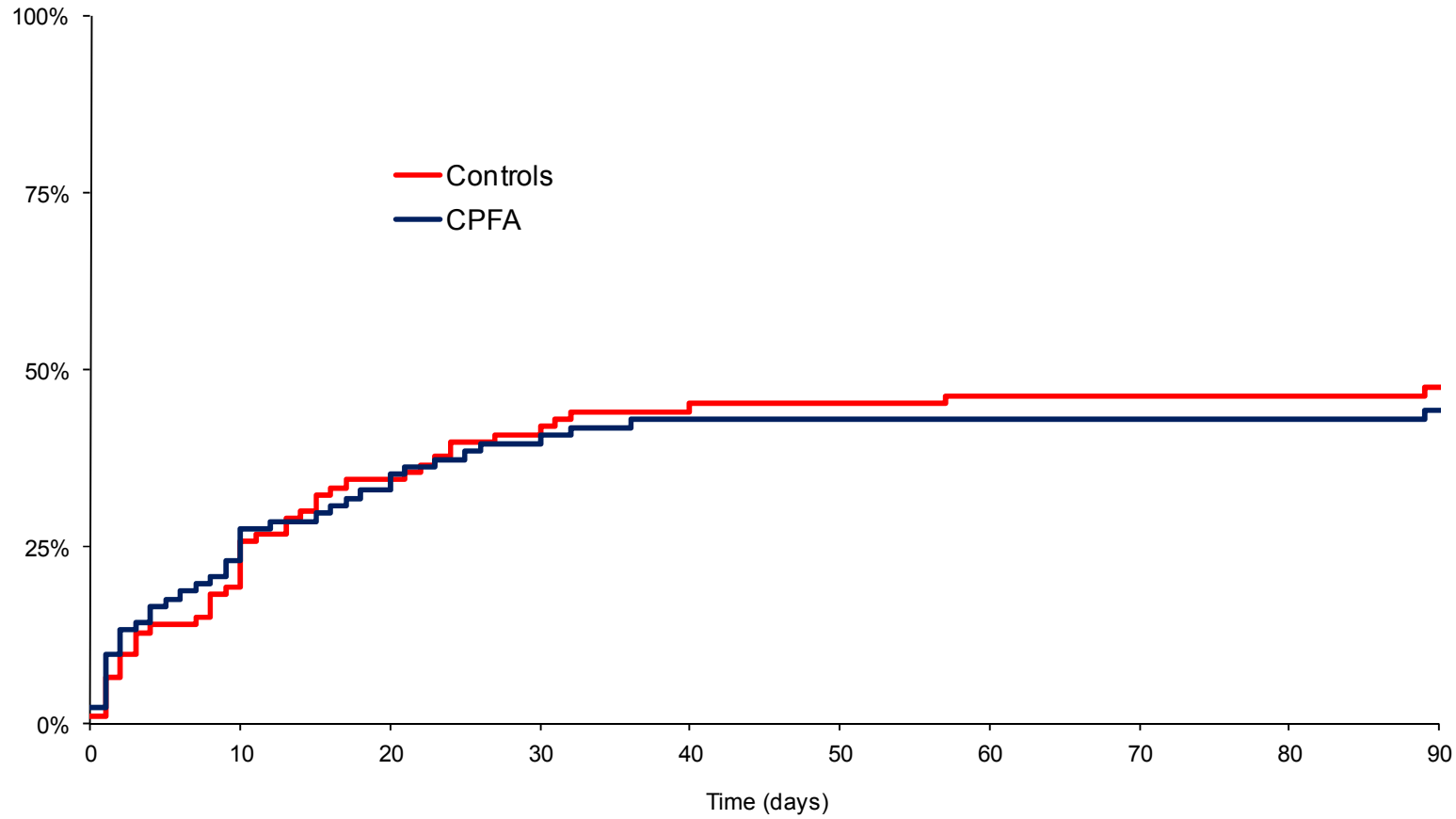
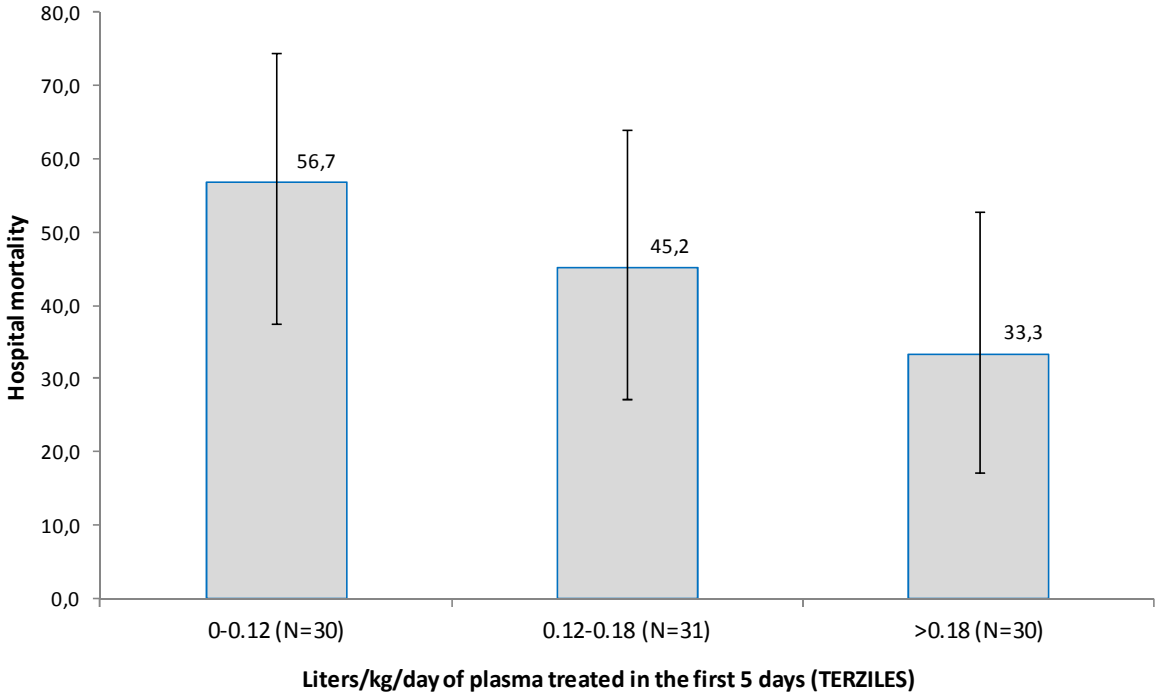


Figure 3. Survival curves.



Patients at risk										
Controls	93	75	61	55	51	50	48	48	47	46
CPFA	91	70	61	54	48	47	46	44	44	43

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



χ^2 test for general association, 3.26; $p = 0.20$
Cochran-Armitage test for trend, 1.82; $p = 0.069$

Appendix

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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)
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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

Sensitivity analyses

Table S3. 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 S4. 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.

References

1. Fergusson, D., et al., *Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis.* Bmj, 2002. **325**(7365): p. 652-4.
2. Fabbri, L.P., et al., *Regional anticoagulation and antiaggregation for CVVH in critically ill patients: a prospective, randomized, controlled pilot study.* Acta Anaesthesiol Scand, 2010. **54**(1): p. 92-7.
3. Piantadosi, S., *Clinical trial. A methodological perspective*. 1997, New York: Jonh Wiley & Sons.
4. Freedman, L.S., D.J. Spiegelhalter, and M.K. Parmar, *The what, why and how of Bayesian clinical trials monitoring.* Stat Med, 1994. **13**(13-14): p. 1371-83; discussion 1385-9.
5. Hosmer, D. and S. Lemeshow, *Applied logistic regression*. 1989, New York: John Wiley and Sons, Inc.

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

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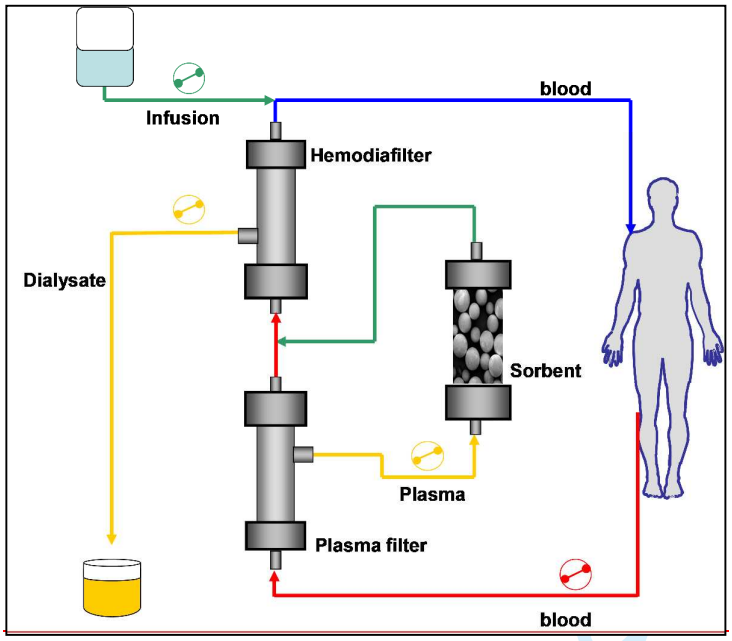
Cardiopulmonary resuscitation	4 (1.0)
Metastatic cancer	3 (0.8)
Not reported	21 (5.4)

Coupled plasma filtration adsorption (CPFA)

Coupled plasma filtration adsorption (CPFA) was developed to non-specifically remove larger cytokines and mediators during systemic inflammation with an extracorporeal circuit consisting of a plasma filter, a resin cartridge and a high flux dialyzer [2].

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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 hemofilter, in which convective exchanges may be applied in a post-dilution mode (see Figure S1) [3].

Figure S1. CPFA



The post dilution reinfusion rate could be set up to 4 l/hr. 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 [4]. More specifically, the filtration fraction should be set to 10% in the first hour, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, 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, 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.

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

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.

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

Regional anticoagulation

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Treatment schedule

Prefilter:

- heparin 1000 U/h^{-1}
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Postfilter:

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

Important advices:

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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).

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Table ~~S1~~S2. Characteristics of the patients before randomization

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Platelet count (x 10^3)	575 [241]	534 [249]	502 [275]	633 [223]	463 [227]
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Bilirubin	2.0 [1.4]	2.3 [1.5]	2.5 [1.7]	2.3 [1.5]	2.2 [1.3]
Creatinine					
Treatments, ~~N~~ *n* (%)					
Steroids	21 (23.9)	29 (34.1)	7 (29.2)	12 (38.7)	10 (33.3)
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* = 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}
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Sensitivity analyses

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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 ~~S2~~S4. 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.

References

1. Fergusson, D., et al., *Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis.* Bmj, 2002. **325**(7365): p. 652-4.
2. Page, M. and T. Rimmele, *[Coupled plasma filtration adsorption: rationale and perspectives in septic shock].* Can J Anaesth, 2008. **55**(12): p. 847-52.
3. Ronco, C., et al., *Coupled plasma filtration adsorption: rationale, technical development and early clinical experience.* Blood Purif, 2003. **21**(6): p. 409-16.
4. Formica, M., et al., *Coupled plasma filtration adsorption.* Contrib Nephrol, 2007. **156**: p. 405-10.
5. Fabbri, L.P., et al., *Regional anticoagulation and antiaggregation for CVVH in critically ill patients: a prospective, randomized, controlled pilot study.* Acta Anaesthesiol Scand, 2010. **54**(1): p. 92-7.
6. Piantadosi, S., *Clinical trial. A methodological perspective*. 1997, New York: Jonh Wiley & Sons.
7. Freedman, L.S., D.J. Spiegelhalter, and M.K. Parmar, *The what, why and how of Bayesian clinical trials monitoring.* Stat Med, 1994. **13**(13-14): p. 1371-83; discussion 1385-9.
8. Hosmer, D. and S. Lemeshow, *Applied logistic regression*. 1989, New York: John Wiley and Sons, Inc.

BMJ open

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-003536.R2
Article Type:	Research
Date Submitted by the Author:	09-Dec-2013
Complete List of Authors:	Livigni, Sergio; Ospedale San Giovanni Bosco, Servizio Anestesia e Rianimazione B-DEA Bertolini, Guido; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Rossi, Carlotta; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Ferrari, Fiorenza; Ospedale San Giovanni Bosco, Servizio Anestesia e Rianimazione B-DEA Giardino, Michele; IRCCS-"Mario Negri" Institute for Pharmacological Research, Public Health, Laboratory of Clinical Epidemiology Pozzato, Marco; Ospedale San Giovanni Bosco, Servizio di Nefrologia e Dialisi Remuzzi, Giuseppe; IRCCS-"Mario Negri" Institute for Pharmacological Research,
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES, Clinical trials < THERAPEUTICS

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva (Italian Group for the Evaluation of Interventions in Intensive Care Medicine) is an independent collaboration network of Italian intensive care units.

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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.

ClinicalTrials.gov, NCT00332371; ISRCTN24534559

Text word count: 3,843

Keywords: septic shock, intensive care medicine, coupled plasma filtration-adsorption

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Abstract

Objectives

Coupled plasmafiltration-adsorption (CPFA, Bellco, Italy), to remove inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomized, 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, superiority clinical trial

Setting

18 Italian adult, general, intensive care units (ICUs)

Participants

Of the planned 330 adult patients with septic shock, 192 were randomized to either have CPFA added to the standard care, or not. The External Monitoring Committee excluded 8 ineligible patients who were erroneously included.

Interventions

CPFA was to be performed daily for 5 days, lasting at least 10 hours per day.

Primary and secondary outcome measures

The primary endpoint was mortality at discharge from the last hospital at which the patient stayed. Secondary endpoints were: 90-day mortality; new organ failures; 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 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 randomized to CPFA were subsequently found to be undertreated. An *a priori* planned subgroup analysis showed those receiving a CPFA dose >0.18 L kg^{-1} day^{-1} had a lower mortality compared to controls (OR 0.36, 95%-CI 0.13-0.99).

Conclusions

CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested CPFA could reduce mortality, when a high volume of plasma is treated. Due 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 number, NCT00332371; ISRCTN24534559).

Text word count: 300

Summary

Article focus

- Coupled plasmafiltration-adsorption (CPFA) is a blood purification technique specifically proposed for the treatment of severe infections, which provided promising results.
- This is an open label, multicentre, randomized, superiority, clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock.

Key messages

- We found no statistical difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution.
- Patients who had a larger volume of plasma treated with CPFA seemed to have a reduced hospital mortality, but this hypothesis should be confirmed in future trials.

Strengths and Limitations

- The study was prematurely terminated on the grounds of futility.
- A large part of patients randomized to CPFA were undertreated as per protocol stipulation, underlying the difficulty of performing such a technique.
- For this reason, it is difficult to say whether the ineffectiveness was due to the impracticability of the technique or to a lack of effect.
- The preplanned subgroup analysis suggesting efficacy if a high volume of plasma was treated, was aimed at minimizing potential biases, but they cannot be completely excluded.

Introduction

The host response against pathogens is a complex one. It is modulated through the production of numerous mediators that, among other mechanisms, promote both pro- 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, the under- or over-expression of their receptors[1 10-12], all these factors have negatively affected the numerous therapeutic attempts to neutralize specific molecules[12]. The repeated failure of this strategy suggested 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 plasmafiltration-adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators[13].

Early experience with CPFA showed increased survival in a rabbit model of endotoxin-induced septic shock[14]. The first clinical study showed that a single treatment lasting 10 hours significantly improved hemodynamic status [15]. These preliminary observations were confirmed in a study of ten 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 randomized multi-center clinical trial to assess the efficacy of CPFA in reducing mortality of critically ill patients with septic shock.

Methods

Ethics Statement and data sharing

The protocol was approved by each hospital's ethics committee. Written consent was obtained from the patient when possible, otherwise physicians enrolled patients according to the article 4.8.15 of the Guidelines for Good Clinical Practice[17]. Raw data are available upon justified request.

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 ICU were eligible for study entry, provided that CPFA could be commenced within 6 hours from occurrence of hypotension refractory to

fluids resuscitation. This was made by the attending physician (present 24/7) using explicit criteria[18]. Reasons for exclusion prior to randomization 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 hours, 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 enrolled patients, their clinical condition, including the Sequential Organ Failure Assessment (SOFA) score[22], the RIFLE criteria for acute renal dysfunction, and CPFA parameters were collected at the time of randomization 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 supplement.

Randomization and Interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enroll the patient or to register reasons for not doing so. Once enrolled, 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 randomization schedule (randomly permuting blocks of four and six)[23] was implemented in the eCRF, with stratification according to the center and the presence of septic shock on admission. The allocation was securely saved in the database and revealed only once baseline additional data collection was completed. All these procedures were implemented to guarantee allocation concealment[24].

Coupled plasma filtration adsorption (CPFA)

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 dialyzer [25].

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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^{-1}), and a final passage of the reconstituted blood through a high-permeability 1.4 m^2 polyethersulfone hemofilter, in which convective

exchanges may be applied in a post-dilution mode (Figure 1) [26].

The post-dilution reinfusion rate could be set up to 4 L hr^{-1}. The blood flow was maintained between 150 and 200 ml min^{-1}, 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, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, corresponding to a blood flow of 150 ml min^{-1} and a filtration fraction of 12%.

The reinfusion solution, sterile and pyrogen-free, with bicarbonate buffer, contained the following composition (mmol L^{-1}): Na 140, K 1.5, Ca 2, Mg 0.75, Cl 108, bicarbonate 35, acetate 4, 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 supplement.

According to the available clinical evidence, CPFA was to be repeated daily for the first 5 days, lasting at least 10 hours each time, so that an average of 0.15 L kg^{-1} day^{-1} of plasma should have been treated per day.

Outcomes, Follow-up and Plan of analysis

The primary endpoint was mortality at discharge from the last hospital in which the patients were treated. Thus, for patients transferred to other hospital, mortality was assessed at the discharge from the last hospital in which the patients stayed. To minimize 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, 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 randomization; 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]); ICU-free days during the first 30 days from randomization.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely 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 hours of randomization.

The study was sized to have 80% power to detect an improvement in hospital mortality from an

expected 63% 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 supplement) was adopted for interim analyses[23].

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted 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 analyzed using the χ^2 test. Effect size was expressed in terms of absolute risk difference with its 95% confidence interval (95%-*CI*)[28]. With regard to secondary endpoints and subgroup analyses, categorical variables were compared with χ^2 or Fisher exact tests, while a Student's *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 randomization 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 that 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 randomization was lost, comparison with the control group was performed through a logistic regression model that adjusted for possible confounders (see online supplement for details).

Results

Between January 2007 and November 2010 a total of 192 patients had been randomized. Recruitment in each ICU lasted a median of 22 months (interquartile range, 13-26). During this period, 386 patients with septic shock were excluded being non-eligible (see online supplement for details). Central monitoring subsequently identified 14 enrolled patients whose eligibility criteria were doubtful. Further clinical information was retrieved and provided to the EDSMC who determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled (see online supplement). 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 supplement. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving treatment with 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 centers. To better express and investigate the phenomenon of under-treatment, and following the emerging concept of dose of renal replacement therapy[30], we computed the volume of plasma treated in L kg^{-1} day^{-1}. In the 91 patients randomized in the CPFA arm, a mean of 0.15 L kg^{-1} day^{-1} 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 under-treatment. Four patients died during CPFA, one before initiating the treatment, two in the very first moment, and one after the first 0.09 L kg^{-1} of plasma treated. The mean time to commencement of CPFA after septic shock identification was 5.7 hours (*SD* 3.8); 38 patients started within 4 hours. In the control group, in violation of the protocol, 2 patients were treated with CPFA, one died at 7 days post-randomization, the other was discharged alive from the hospital 37 days after randomization.

No statistical difference was found in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95%-*CI*, -12.2–16.6%). 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 control versus 56.0% for CPFA patients (p=0.99); the free-ICU days during the first 30 days post-randomization 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 [41.0%] 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 hours from randomization (17/38 [44.7%]; p=0.88), nor in those who started CPFA after 4 hours (20/46 [43.5%]; p=0.76). In 7 patients the timing of CPFA initiation was missing. Eventually, no effect of 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). Characteristics of the groups defined by the tertiles are showed in the online supplement. 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^{-1} day^{-1} of plasma treated over the first 5 days) was statistically lower than in the control group (*OR* 0.36, 95%-*CI* 0.13-0.99; 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 by excluding, both in the control and treated groups, patients who died in the first 24 hours post-randomization. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days, the second was intended to minimize 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 supplement) confirmed the same estimates, even though statistical significance was lost for lack of power.

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 outcomes have failed, highlighting the complexity of the immune response, including a marked intra-patient 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 randomized clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock.

The main findings

After randomizing 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 reverse these results with the sample still to be randomized, 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%), so reducing the power of the study. Thus, the EDSMC considered that continue to spend money in a clinical trial that had little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, nonbinding 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 have adopted 28-day mortality due to FDA 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 randomization. On the other hand, over-extending 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 last hospital into which they were admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialized, interdisciplinary care, which means he or she had survived the septic shock episode. 90-day mortality was anyway recorded and considered as secondary endpoint.

The problem of under-treatment

Nearly half the patients randomized to CPFA were undertreated as per protocol stipulation. This poses two crucial questions: the true feasibility of the technique in the ICU, and the possible relationship between the overall negative result and such under-treatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centers.

Why did the training not have effect? Many factors could have contributed. First, CPFA involves a complex circuit that includes a hemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions, and anticoagulation. We used heparin for anticoagulation (see online supplement), 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 centers may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be 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 cases the physicians did not start a new course of CPFA in 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. And it was often difficult to involve the nephrologists, that in many centers are those in charge of the procedure. On the other hand, despite excellent feedbacks from 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 thirteen patients at high-risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with 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 a 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 found, a number of potential problems threatens the validity of this result. Firstly, subgroup definition for the per-protocol analysis (i.e., tertiles of plasma treated) was based upon characteristics measured after randomization. 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

supplement). We adjusted for possible confounders in the multivariate model to minimize this risk, but we were limited to prognostic factors collected in the database. Particularly, we have no data on the immuno-inflammatory status of the patients to account for. Secondly, 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 standardized 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 supplement), the first hours were characterized by a certain degree of under-treatment 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 1 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 eligible patients were not randomized due to the very narrow window (6 hours) for patient recruitment and initiation of treatment. This would have particularly hampered the generalizability 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 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 minimize 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.

Acknowledgments

The study was funded by GiViTI and by 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.

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript. None of the authors has any conflict of interest in relation to this work. The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Registration number: ClinicalTrials.gov NCT00332371; ISRCTN24534559. Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis.

Collaborators

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Funding

This work was supported by the Bellco SpA

Data Sharing Statement

Further analyses will be provided upon request to the corresponding author.

Competing interests

None

Contributorship

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript.

References

1. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 2003;**71**(10):5803-13
2. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007;**11**(2):R49 doi: cc5783 [pii]
10.1186/cc5783[published Online First: Epub Date]|.
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007;**167**(15):1655-63 doi: 167/15/1655 [pii]
10.1001/archinte.167.15.1655[published Online First: Epub Date]|.
4. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;**369**(9):840-51 doi: 10.1056/NEJMra1208623[published Online First: Epub Date]|.
5. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. Bmj 2003;**326**(7383):262-6
6. Weighardt H, Heidecke CD, Emmanuilidis K, et al. Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. Surgery 2000;**127**(3):309-15 doi: S0039-6060(00)91079-1 [pii]
10.1067/msy.2000.104118[published Online First: Epub Date]|.
7. Tamayo E, Fernandez A, Almansa R, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw;**22**(2):82-7 doi: ecn.2011.0281 [pii]
10.1684/ecn.2011.0281[published Online First: Epub Date]|.
8. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005;**365**(9453):63-78
9. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;**348**(2):138-50
10. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006;**12**(3):151-70 doi: 10.1179/096805106X102246[published Online First: Epub Date]|.
11. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 2000;**275**(27):20260-7 doi: 10.1074/jbc.M909168199
M909168199 [pii][published Online First: Epub Date]|.
12. Munoz C, Misset B, Fitting C, et al. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991;**21**(9):2177-84 doi: 10.1002/eji.1830210928[published Online First: Epub Date]|.
13. Tetta C, Bellomo R, Inguaggiato P, Wratten ML, Ronco C. Endotoxin and cytokine removal in sepsis. Ther

Apher 2002;**6**(2):109-15 doi: tap413 [pii][published Online First: Epub Date]|.
14. Tetta C, Gianotti L, Cavaillon JM, et al. Coupled plasma filtration-adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000;**28**(5):1526-33
15. Ronco C, Brendolan A, Lonnemann G, et al. A pilot study of coupled plasma filtration with adsorption in septic shock*. Critical Care Medicine 2002;**30**(6):1250-55
16. Formica M, Olivieri C, Livigni S, et al. Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003;**29**(5):703-8
17. Human Medicines Evaluation Unit. Guidelines for good clinical practice. London: European Agency for the Evaluation of Medical Products, 1996:17-20.
18. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;**29**(4):530-8
19. Boffelli S, Rossi C, Anghileri A, et al. Continuous quality improvement in intensive care medicine. The GiViTI Margherita Project - Report 2005. Minerva Anestesiol 2006;**72**(6):419-32
20. Malacarne P, Langer M, Nascimben E, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;**36**(4):1105-13
21. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993;**270**(24):2957-63
22. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;**26**(11):1793-800
23. Piantadosi S. *Clinical trial. A methodological perspective*. New York: Jonh Wiley & Sons, 1997.
24. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet 2002;**359**(9306):614-8 doi: S0140-6736(02)07750-4 [pii]
10.1016/S0140-6736(02)07750-4[published Online First: Epub Date]|.
25. Page M, Rimmele T. [Coupled plasma filtration adsorption: rationale and perspectives in septic shock]. Can J Anaesth 2008;**55**(12):847-52 doi: 55/12/847 [pii]
10.1007/BF03034056[published Online First: Epub Date]|.
26. Ronco C, Brendolan A, d'Intini V, Ricci Z, Wratten ML, Bellomo R. Coupled plasma filtration adsorption: rationale, technical development and early clinical experience. Blood Purif 2003;**21**(6):409-16 doi: 10.1159/000073444
73444 [pii][published Online First: Epub Date]|.
27. Formica M, Inguaggiato P, Bainotti S, Wratten ML. Coupled plasma filtration adsorption. Contrib Nephrol 2007;**156**:405-10 doi: 102131 [pii]
10.1159/0000102131[published Online First: Epub Date]|.
28. Kleinbaum D, Kupper L, Morgenstern H. *Epidemiologic research*. New York, NY: Van Nostrand Reynhold, 1982.
29. Fergusson D, Aaron SD, Guyatt G, et al. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002;**325**(7365):652-4
30. Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. Critical Care 2011;**15**(2):207
31. Annane D, Aegerter P, Jars-Guincestre MC, et al. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med 2003;**168**(2):165-72
32. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;**29**(4):880-6
33. Perl TM, Dvorak L, Hwang T, et al. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;**274**(4):338-45
34. Singer M, McNally T, Screaton G, et al. Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 1994;**20**(3):212-5
35. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med;**38**(1):20-8 doi: 10.1007/s00134-011-2438-3[published Online First: Epub Date]|.
36. Mariano F, Bergamo D, Gangemi EN,et al. Citrate Anticoagulation for Continuous Renal Replacement Therapy in Critically Ill Patients: Success and Limits. International Journal of Nephrology 2011;**2011** doi: 10.4061/2011/748320[published Online First: Epub Date]|.
37. Mariano F, Tetta C, Stella M, et al. Regional Citrate Anticoagulation in Critically Ill Patients Treated with Plasma Filtration and Adsorption. Blood Purification 2004;**22**(3):313-19
38. Pozzato M, Ferrari F, Cecere P, et al. Safety and efficacy of citrate anticoagulation in spetic shock patients treated with couplet plasma filtration adsorbtion (CPFA). J Am Soc Nephrol 2011;**22 -**

Congress Proceeding:605A

Table 1. Characteristics of the patients before randomization

		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)
Body Mass Index *n* (%)			
	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 (Acute Lung Injury)	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1-Q3]		53 [43-67]	51 [42-65]
SOFA at randomization, median [Q1-Q3]		9 [8-11]	9 [8-11]
RIFLE at randomization, *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* (%)		
	39 (42.4)	43 (47.8)
Missing	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 (Extended-spectrum β-lactamase) producing E. coli	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)
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)

SD=Standard deviation; Q1-Q3=first and third quartiles; Underweight=for male, BMI<20, for female, BMI<19; Normal weight=for male, BMI 20-25, for female, 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.

Table 2. Reasons for under treatment in the CPFA arm (n = 44)

	n	**%**
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organizational problems	4	9.1
Patient's death	4	9.1
Lack of specialized personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

Table 3. Results of the logistic regression model on hospital mortality

Variable	OR	95%-CI	*p*
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.52	0.73-3.17	0.033
CPFA, > 0.18 (3° tertile) vs. Controls	0.36	0.13-0.99	
Age (decades)	1.57	1.19-2.07	0.001
Source of admission			
Other ICU vs. Medical ward	0.28	0.04-1.89	0.021
Emergency room vs. Medical ward	0.27	0.11-0.67	
Surgical ward vs. Medical ward	0.34	0.15-0.77	
Renal failure at admission	4.08	1.47-11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04-0.75	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. Legend: OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Figure legends

Figure 1. CPFA schema

Figure 2. Flow chart of participants.

Figure 3. Survival curves.

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

Efficacy of Coupled Plasma Filtration Adsorption (CPFA) in patients with septic shock. A multicenter randomized controlled clinical trial

GiViTI

Gruppo Italiano per la Valutazione degli Interventi in Terapia Intensiva (Italian Group for the Evaluation of Interventions in Intensive Care Medicine) is an independent collaboration network of Italian intensive care units.

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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.

ClinicalTrials.gov, NCT00332371; ISRCTN24534559

Text word count: 3,~~821~~843

Keywords: septic shock, intensive care medicine, coupled plasma filtration-adsorption

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Summary

Article focus

- Coupled plasmafiltration-adsorption (CPFA) is a blood purification technique specifically proposed for the treatment of severe infections, which provided promising results.
- This is an open label, multicentre, randomized, superiority, clinical trial to assess the efficacy of CPFA in critically ill patients with septic shock.

Key messages

- We found no statistical difference with the use of CPFA in hospital mortality, the occurrence of new organ failures, or the overall clinical evolution.
- Patients who had a larger volume of plasma treated with CPFA seemed to have a reduced hospital mortality, but this hypothesis should be confirmed in future trials.

Strengths and Limitations

- The study was prematurely terminated on the grounds of futility.
- A large part of patients randomized to CPFA were undertreated as per protocol stipulation, underlying the difficulty of performing such a technique.
- For this reason, it is difficult to say whether the ineffectiveness was due to the impracticability of the technique or to a lack of effect.
- The preplanned subgroup analysis suggesting efficacy if a high volume of plasma was treated, was aimed at minimizing potential biases, but they cannot be completely excluded.

Abstract

Objectives

Coupled plasmafiltration-adsorption (CPFA, Bellco, Italy), to remove~~ing~~ inflammatory mediators from blood, has been proposed as a novel treatment for septic shock. This multicenter, randomized, 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, superiority clinical trial

Setting

18 Italian adult, general, intensive care units (ICUs)

Participants

Of the planned 330 adult patients with septic shock, 192 were randomized to either have CPFA added to the standard care, or not. The External Monitoring Committee excluded 8 ineligible patients who were erroneously included.

Interventions

CPFA was to be performed daily for 5 days, lasting at least 10 hours per day.

Primary and secondary outcome measures

The primary endpoint was mortality at discharge from the last hospital at which the patient stayed. Secondary endpoints were: 90-day mortality; new organ failures; 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 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 randomized to CPFA were subsequently found to be undertreated. An *a priori* planned subgroup analysis showed those receiving a CPFA dose >0.18 L kg^{-1} day^{-1} had a lower mortality compared to controls (OR 0.36, 95%-CI 0.13-0.99).

Conclusions

CPFA did not reduce mortality in patients with septic shock, nor did it positively affect other important clinical outcomes. A subgroup analysis suggested CPFA could reduce mortality, ~~if~~ when a high volume of plasma is treated. Due 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 number, NCT00332371; ISRCTN24534559).

Text word count: ~~299~~300

Introduction

The host response against pathogens is a complex one. It is modulated through the production of numerous mediators that, among other mechanisms, promote both pro- 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, the under- or over-expression of their receptors[1 10-12], all these factors have negatively affected the numerous therapeutic attempts to neutralize specific molecules[12]. The repeated failure of this strategy suggested 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 plasmafiltration-adsorption (CPFA) can non-selectively remove the majority of soluble inflammatory mediators[13].

Early experience with CPFA showed increased survival in a rabbit model of endotoxin-induced septic shock[14]. The first clinical study showed that a single treatment lasting 10 hours significantly improved hemodynamic status [15]. These preliminary observations were confirmed in a study of ten 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 randomized multi-center clinical trial to assess the efficacy of CPFA in reducing mortality of critically ill patients with septic shock.

Methods

Ethics Statement and data sharing

The protocol was approved by each hospital's ethics committee. Written consent was obtained from the patient when possible, otherwise physicians enrolled patients according to the article 4.8.15 of the Guidelines for Good Clinical Practice[17]. Raw data are available upon justified request.

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 ICU were eligible for study

entry, provided that CPFA could be commenced within 6 hours from occurrence of hypotension refractory to fluids resuscitation. This was made by the attending physician (present 24/7) using explicit criteria[18]. Reasons for exclusion prior to randomization 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 hours, 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 enrolled patients, their clinical condition, including the Sequential Organ Failure Assessment (SOFA) score[22], the RIFLE criteria for acute renal dysfunction, and CPFA parameters were collected at the time of randomization 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 supplement.

Randomization and Interventions

Eligibility criteria were flagged up in real time by the eCRF, which prompted the clinician to enroll the patient or to register reasons for not doing so. Once enrolled, 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 randomization schedule (randomly permuting blocks of four and six)[23] was implemented in the eCRF, with stratification according to the center and the presence of septic shock on admission. The allocation was securely saved in the database and revealed only once baseline additional data collection was completed. All these procedures were implemented to guarantee allocation concealment[24].

Coupled plasma filtration adsorption (CPFA)

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 dialyzer [25].

CPFA was performed with the use of a four-pump modular treatment (Lynda®, Bellco, Mirandola, Italy) consisting of a plasmafilter (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^{-1}), and a final passage of the

reconstituted blood through a high-permeability 1.4 m^2 polyethersulfone hemofilter, in which convective exchanges may be applied in a post-dilution mode (Figure 1) [26].

The post-dilution reinfusion rate could be set up to 4 L hr^{-1}. The blood flow was maintained between 150 and 200 ml min^{-1}, 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, then it should be gradually increased to the target value of 18%. The minimum volume of plasma treated per day should be 10 liters, corresponding to a blood flow of 150 ml min^{-1} and a filtration fraction of 12%.

The reinfusion solution, sterile and pyrogen-free, with bicarbonate buffer, contained the following composition (mmol L^{-1}): Na 140, K 1.5, Ca 2, Mg 0.75, Cl 108, bicarbonate 35, acetate 4, 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 supplement.

According to the available clinical evidence, CPFA was to be repeated daily for the first 5 days, lasting at least 10 hours each time, so that an average of 0.15 L kg^{-1} day^{-1} of plasma should have been treated per day.

Outcomes, Follow-up and Plan of analysis

The primary endpoint was mortality at discharge from the last hospital in which the patients were treated. Thus, for patients transferred to other hospital, mortality was assessed at the discharge from the last hospital in which the patients stayed. To minimize 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, 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 randomization; 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]); ICU-free days during the first 30 days from randomization.

Timing of intervention is considered extremely important in septic shock. Thus, two subgroup analyses of the primary endpoint were pre-planned, namely 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 hours of randomization.

The study was sized to have 80% power to detect an improvement in hospital mortality from an expected 63% 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 supplement) was adopted for interim analyses[23].

Premature termination of the trial

In November 2010, the External Data and Safety Monitoring Committee (EDSMC) prompted 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 analyzed using the χ^2 test. Effect size was expressed in terms of absolute risk difference with its 95% confidence interval (95%-*CI*)[28]. With regard to secondary endpoints and subgroup analyses, categorical variables were compared with χ^2 or Fisher exact tests, while a Student's *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 randomization 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 that 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 randomization was lost, comparison with the control group was performed through a logistic regression model that adjusted for possible confounders (see online supplement for details).

Results

Between January 2007 and November 2010 a total of 192 patients had been randomized. Recruitment in each ICU lasted a median of 22 months (interquartile range, 13-26). During this period, 386 patients with septic shock were excluded being non-eligible (see online supplement for details). Central monitoring subsequently identified 14 enrolled patients whose eligibility criteria were doubtful. Further clinical information was retrieved and provided to the EDSMC who determined that 8 of these patients (5 CPFA, 3 control) were erroneously enrolled (see online supplement). 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 supplement. One episode of surgical wound bleeding was registered as possibly related to CPFA in a patient receiving treatment with 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 centers. To better express and investigate the phenomenon of under-treatment, and following the emerging concept of dose of renal replacement therapy[30], we computed the volume of plasma treated in L kg^{-1} day^{-1}. In the 91 patients randomized in the CPFA arm, a mean of 0.15 L kg^{-1} day^{-1} 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 under-treatment. Four patients died during CPFA, one before initiating the treatment, two in the very first moment, and one after the first 0.09 L kg^{-1} of plasma treated. The mean time to commencement of CPFA after septic shock identification was 5.7 hours (*SD* 3.8); 38 patients started within 4 hours. In the control group, in violation of the protocol, 2 patients were treated with CPFA, one died at 7 days post-randomization, the other was discharged alive from the hospital 37 days after randomization.

No statistical difference was found in hospital mortality with 47.3% dying in the control group (44/93) versus 45.1% in the CPFA group (41/91, p=0.76), with an absolute risk difference of 2.2% (95%-*CI*, -12.2–16.6%). 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 control versus 56.0% for CPFA patients (p=0.99); the free-ICU days during the first 30 days post-randomization 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 [41.0%] 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 hours from randomization (17/38 [44.7%]; p=0.88), nor in those who started CPFA after 4 hours (20/46 [43.5%]; p=0.76). In 7 patients the timing of CPFA initiation was missing. Eventually, no effect of 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). ~~Table 3 compares c~~Characteristics of the groups defined by the tertiles are showed in the online supplement. 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^{-1} day^{-1} of plasma treated over the first 5 days) was statistically lower than in the control group (*OR* 0.36, 95%-*CI* 0.13-0.99; see table ~~3~~4). We then performed two sensitivity analyses, namely: limiting the evaluation of the volume of plasma treated to the first 3 days, and by excluding, both in the control and treated groups, patients who died in the first 24 hours post-randomization. The first analysis was aimed at assessing whether any possible benefit of CPFA was obtained before 5 days, the second was intended to minimize 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 supplement) confirmed the same estimates, even though statistical significance was lost for lack of power.

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 outcomes have failed, highlighting the complexity of the immune response, including a marked intra-patient 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 randomized clinical trial to assess the efficacy of CPFA in reducing hospital mortality of patients affected by septic shock.

The main findings

After randomizing 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 reverse these results with the sample still to be randomized, 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%), so reducing the power of the study. Thus, the EDSMC considered that continue to spend money in a clinical trial that had little chance of demonstrating efficacy was undesirable and asked for a premature termination on the grounds of futility, although the anticipated, nonbinding 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 have adopted 28-day mortality due to FDA 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 randomization. On the other hand, over-extending 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 last hospital into which they were admitted following their septic shock episode. At that point, the patient no longer requires aggressive, specialized, interdisciplinary care, which means he or she had survived the septic shock episode. 90-day mortality was anyway recorded and considered as secondary endpoint.

The problem of under-treatment

Nearly half the patients randomized to CPFA were undertreated as per protocol stipulation. This poses two crucial questions: the true feasibility of the technique in the ICU, and the possible relationship between the overall negative result and such under-treatment. The main reason for not reaching the prescribed volume of plasma treated was clotting of the circuit (48%). This problem was encountered by all centers.

Why did the training not have effect? Many factors could have contributed. First, CPFA involves a complex circuit that includes a hemofilter, a plasma filter and an adsorbing cartridge, and requires an adequate balance of flows, dilutions, and anticoagulation. We used heparin for anticoagulation (see online supplement), 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 centers may have been too conservative either with the heparin dosage and/or the blood flow rate through the circuit, or there may be 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 cases the physicians did not start a new course of CPFA in 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. And it was often difficult to involve the nephrologists, that in many centers are those in charge of the procedure. On the other hand, despite excellent feedbacks from 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 thirteen patients at high-risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with 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 a 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 found, a number of potential problems threatens the validity of this result. Firstly, subgroup definition for the per-protocol analysis (i.e., tertiles of plasma treated) was based upon characteristics measured after randomization. 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

supplement~~Table 3~~). We adjusted for possible confounders in the multivariate model to minimize this risk, but we were limited to prognostic factors collected in the database. Particularly, we have no data on the immuno-inflammatory status of the patients to account for. Secondly, 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 standardized 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 supplement), the first hours were characterized by a certain degree of under-treatment 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 1 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 eligible patients were not randomized due to the very narrow window (6 hours) for patient recruitment and initiation of treatment. This would have particularly hampered the generalizability 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 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 minimize potential biases, these cannot be completely excluded. Hence, this result can only be viewed as hypothesis generating. ~~Regional anticoagulation with citrate represents a valid alternative 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 thirteen patients at high risk of bleeding, citrate regional anticoagulation was associated with a significantly lower number of clotted CPFA cartridges than with 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. Hence~~Given the new availability of citrate regional anticoagulation, we have designed a confirmatory, adaptive trial whose first step will be to prove ~~citrate regional anticoagulation~~this new technique easily allows high volume of plasma treated with CPFA.

Acknowledgments

The study was funded by GiViTI and by 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.

The authors substantially contributed to the conception and design (all authors), analysis (GB and CR) and interpretation (all authors) of data, drafting the article (GB) or critically revising it (all authors). All authors approved the final version of the manuscript. None of the authors has any conflict of interest in relation to this work. The full protocol is accessible at: http://www.giviti.marionegri.it/COMPACT.asp Registration number: ClinicalTrials.gov NCT00332371; ISRCTN24534559. Guido Bertolini and Carlotta Rossi had full access to all study data and take responsibility for its integrity and the accuracy of data analysis.

References

1. Feezor RJ, Oberholzer C, Baker HV, et al. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 2003;**71**(10):5803-13
2. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007;**11**(2):R49 doi: cc5783 [pii]
10.1186/cc5783[published Online First: Epub Date]|.
3. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007;**167**(15):1655-63 doi: 167/15/1655 [pii]
10.1001/archinte.167.15.1655[published Online First: Epub Date]|.
4. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;**369**(9):840-51 doi: 10.1056/NEJMra1208623[published Online First: Epub Date]|.
5. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. Bmj 2003;**326**(7383):262-6
6. Weighardt H, Heidecke CD, Emmanuilidis K, et al. Sepsis after major visceral surgery is associated with sustained and interferon-gamma-resistant defects of monocyte cytokine production. Surgery 2000;**127**(3):309-15 doi: S0039-6060(00)91079-1 [pii]
10.1067/msy.2000.104118[published Online First: Epub Date]|.
7. Tamayo E, Fernandez A, Almansa R, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw;**22**(2):82-7 doi: ecn.2011.0281 [pii]
10.1684/ecn.2011.0281[published Online First: Epub Date]|.
8. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005;**365**(9453):63-78
9. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;**348**(2):138-50
10. Cavaillon JM, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006;**12**(3):151-70 doi: 10.1179/096805106X102246[published Online First: Epub Date]|.
11. Wang ZM, Liu C, Dziarski R. Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 2000;**275**(27):20260-7 doi: 10.1074/jbc.M909168199
M909168199 [pii][published Online First: Epub Date]|.
12. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. Eur J Immunol 1991;**21**(9):2177-84 doi: 10.1002/eji.1830210928[published Online First: Epub Date]|.
13. Tetta C, Bellomo R, Inguaggiato P, Wratten ML, Ronco C. Endotoxin and cytokine removal in sepsis. Ther Apher 2002;**6**(2):109-15 doi: tap413 [pii][published Online First: Epub Date]|.
14. Tetta C, Gianotti L, Cavaillon JM, et al. Coupled plasma filtration-adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000;**28**(5):1526-33
15. Ronco C, Brendolan A, Lonnemann G, et al. A pilot study of coupled plasma filtration with adsorption in septic shock*. Critical Care Medicine 2002;**30**(6):1250-55
16. Formica M, Olivieri C, Livigni S, et al. Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003;**29**(5):703-8
17. Human Medicines Evaluation Unit. Guidelines for good clinical practice. London: European Agency for the Evaluation of Medical Products, 1996:17-20.
18. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;**29**(4):530-8
19. Boffelli S, Rossi C, Anghileri A, et al. Continuous quality improvement in intensive care medicine. The GiViTI Margherita Project - Report 2005. Minerva Anestesiol 2006;**72**(6):419-32
20. Malacarne P, Langer M, Nascimben E, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;**36**(4):1105-13
21. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993;**270**(24):2957-63
22. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998;**26**(11):1793-800
23. Piantadosi S. *Clinical trial. A methodological perspective*. New York: Jonh Wiley & Sons, 1997.
24. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering.

Lancet 2002;**359**(9306):614-8 doi: S0140-6736(02)07750-4 [pii]
10.1016/S0140-6736(02)07750-4[published Online First: Epub Date]|.
25. Page M, Rimmele T. [Coupled plasma filtration adsorption: rationale and perspectives in septic shock]. Can J Anaesth 2008;**55**(12):847-52 doi: 55/12/847 [pii]
10.1007/BF03034056[published Online First: Epub Date]|.
26. Ronco C, Brendolan A, d'Intini V, Ricci Z, Wratten ML, Bellomo R. Coupled plasma filtration adsorption: rationale, technical development and early clinical experience. Blood Purif 2003;**21**(6):409-16 doi: 10.1159/000073444
73444 [pii][published Online First: Epub Date]|.
27. Formica M, Inguaggiato P, Bainotti S, Wratten ML. Coupled plasma filtration adsorption. Contrib Nephrol 2007;**156**:405-10 doi: 102131 [pii]
10.1159/0000102131[published Online First: Epub Date]|.
28. Kleinbaum D, Kupper L, Morgenstern H. *Epidemiologic research*. New York, NY: Van Nostrand Reynhold, 1982.
29. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ 2002;**325**(7365):652-4
30. Prowle JR, Schneider A, Bellomo R. Clinical review: Optimal dose of continuous renal replacement therapy in acute kidney injury. Critical Care 2011;**15**(2):207
31. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. Am J Respir Crit Care Med 2003;**168**(2):165-72
32. Cohen J, Guyatt G, Bernard GR, et al. New strategies for clinical trials in patients with sepsis and septic shock. Crit Care Med 2001;**29**(4):880-6
33. Perl TM, Dvorak L, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;**274**(4):338-45
34. Singer M, McNally T, Screaton G, Mackie I, Machin S, Cohen SL. Heparin clearance during continuous veno-venous haemofiltration. Intensive Care Med 1994;**20**(3):212-5
35. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med;**38**(1):20-8 doi: 10.1007/s00134-011-2438-3[published Online First: Epub Date]|.
36. Mariano F, Bergamo D, Gangemi EN, Hollo Z, Stella M, Triolo G. Citrate Anticoagulation for Continuous Renal Replacement Therapy in Critically Ill Patients: Success and Limits. International Journal of Nephrology 2011;**2011** doi: 10.4061/2011/748320[published Online First: Epub Date]|.
37. Mariano F, Tetta C, Stella M, Biolino P, Miletto A, Triolo G. Regional Citrate Anticoagulation in Critically Ill Patients Treated with Plasma Filtration and Adsorption. Blood Purification 2004;**22**(3):313-19
38. Pozzato M, Ferrari F, Cecere P, et al. Safety and efficacy of citrate anticoagulation in spetic shock patients treated with couplet plasma filtration adsorbtion (CPFA). J Am Soc Nephrol 2011;**22 - Congress Proceeding**:605A

Table 1. Characteristics of the patients before randomization

	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)
Body Mass Index *n* (%)		
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 (Acute Lung Injury)	16 (17.2)	14 (15.4)
SAPS II on admission, median [Q1-Q3]	53 [43-67]	51 [42-65]
SOFA at randomization, median [Q1-Q3]	9 [8-11]	9 [8-11]
RIFLE at randomization, *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* (%)	39 (42.4)	43 (47.8)
Missing	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 (Extended-spectrum β-lactamase) producing E. coli	13 (13.7)	14 (15.9)
Candida albicans	4 (4.2)	6 (6.8)
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)

SD=Standard deviation; Q1-Q3=first and third quartiles; Underweight=for male, BMI<20, for female, BMI<19; Normal weight=for male, BMI 20-25, for female, 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.

Table 2. Reasons for under treatment in the CPFA arm (n = 44)

	n	**%**
Clotting of the circuit	21	47.7
Technical problems	5	11.4
Organizational problems	4	9.1
Patient's death	4	9.1
Lack of specialized personnel	3	6.8
Family request to stop CPFA	1	2.3
Other	6	13.6

Table 3. 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 faecalis	0 (0.0)	2 (6.5)	1 (3.3)
Gram positive bacteria	9 (30.0)	9 (29.0)	9 (30.0)
Gram negative bacteria	8 (26.7)	12 (38.7)	7 (23.3)

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

Table 43. Results of the logistic regression model on hospital mortality

Variable	OR	95%-CI	*p*
Volume of plasma treated (L kg^{-1} day^{-1})			
CPFA, ≤ 0.18 (1° and 2° tertiles) vs. Controls	1.52	0.73-3.17	0.033
CPFA, > 0.18 (3° tertile) vs. Controls	0.36	0.13-0.99	
Age (decades)	1.57	1.19-2.07	0.001
Source of admission			
Other ICU vs. Medical ward	0.28	0.04-1.89	0.021
Emergency room vs. Medical ward	0.27	0.11-0.67	
Surgical ward vs. Medical ward	0.34	0.15-0.77	
Renal failure at admission	4.08	1.47-11.32	0.007
Cholecystitis or cholangitis on admission	0.18	0.04-0.75	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. Legend: OR, odds ratio; CI, confidence interval; ICU, intensive care unit.

Figure 1. CPFA schema

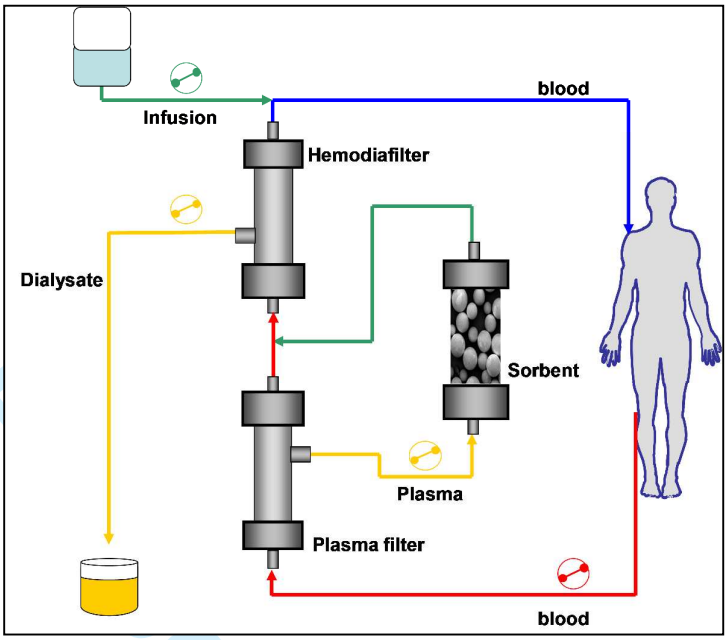


Figure 2. Flow chart of participants.

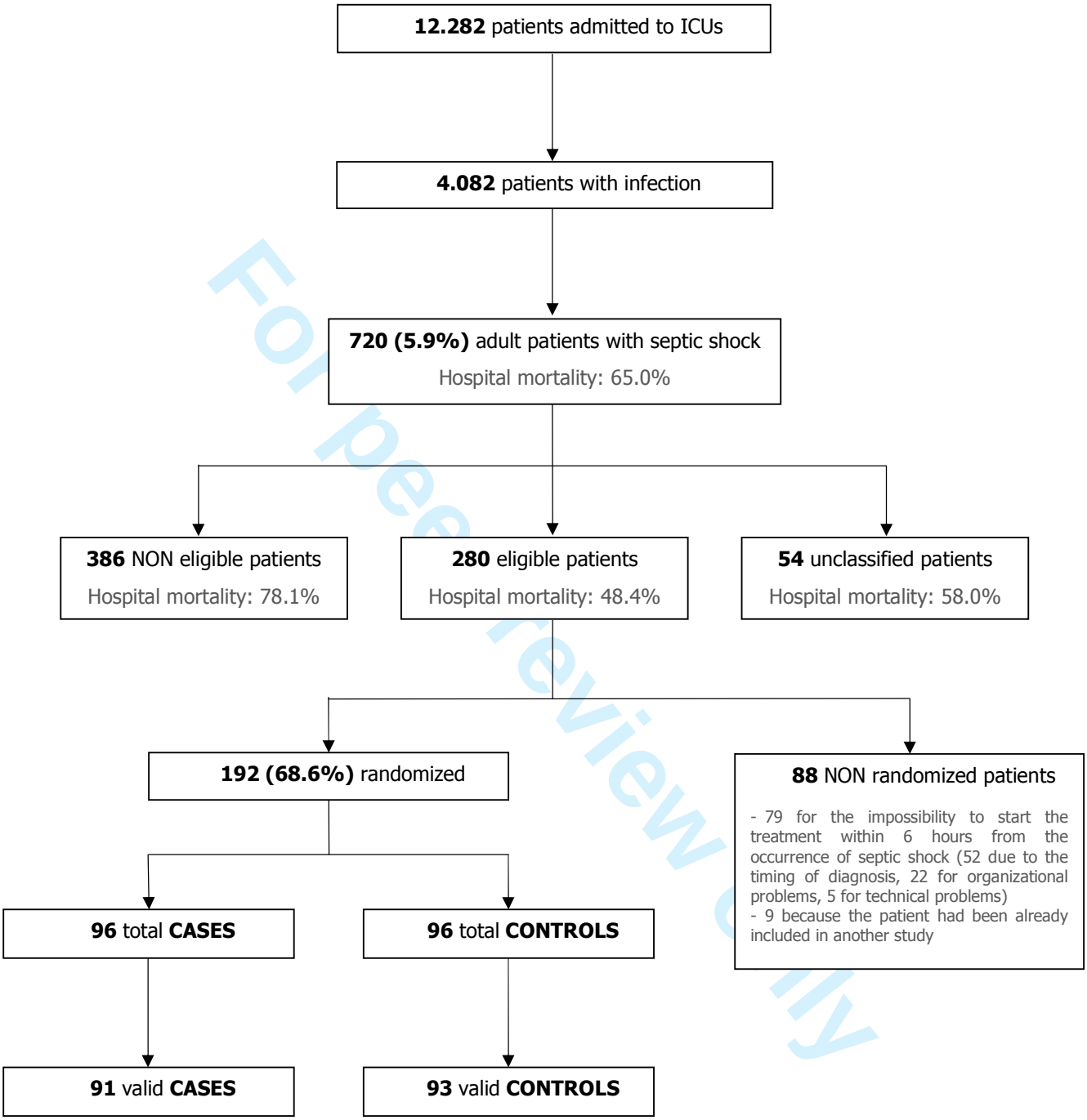
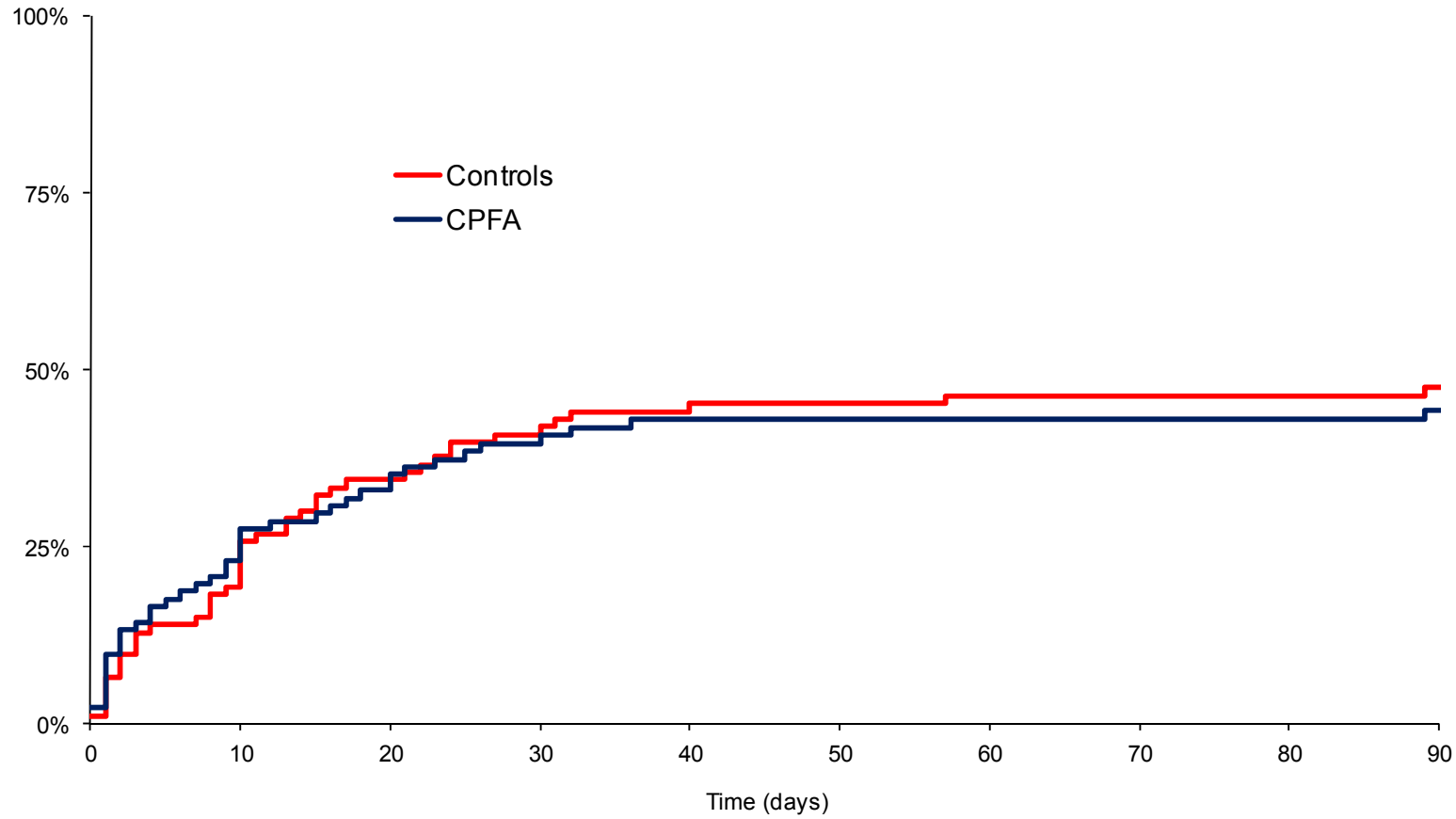
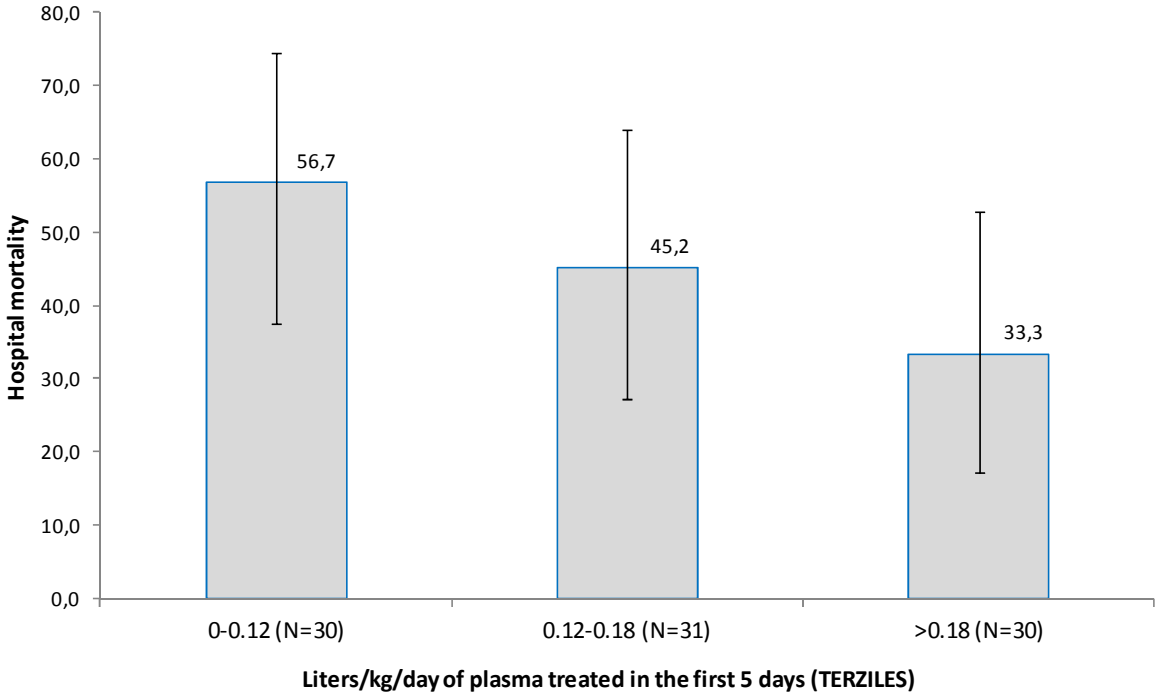


Figure 3. Survival curves.



Patients at risk										
Controls	93	75	61	55	51	50	48	48	47	46
CPFA	91	70	61	54	48	47	46	44	44	43

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



χ^2 test for general association, 3.26; $p = 0.20$
Cochran-Armitage test for trend, 1.82; $p = 0.069$

Appendix

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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 faecalis	0 (0.0)	2 (6.5)	1 (3.3)
Gram positive bacteria	9 (30.0)	9 (29.0)	9 (30.0)
Gram negative bacteria	8 (26.7)	12 (38.7)	7 (23.3)

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.

References

1. Fergusson, D., et al., *Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis.* Bmj, 2002. **325**(7365): p. 652-4.
2. Fabbri, L.P., et al., *Regional anticoagulation and antiaggregation for CVVH in critically ill patients: a prospective, randomized, controlled pilot study.* Acta Anaesthesiol Scand, 2010. **54**(1): p. 92-7.
3. Piantadosi, S., *Clinical trial. A methodological perspective*. 1997, New York: Jonh Wiley & Sons.
4. Freedman, L.S., D.J. Spiegelhalter, and M.K. Parmar, *The what, why and how of Bayesian clinical trials monitoring.* Stat Med, 1994. **13**(13-14): p. 1371-83; discussion 1385-9.
5. Hosmer, D. and S. Lemeshow, *Applied logistic regression*. 1989, New York: John Wiley and Sons, Inc.

Appendix

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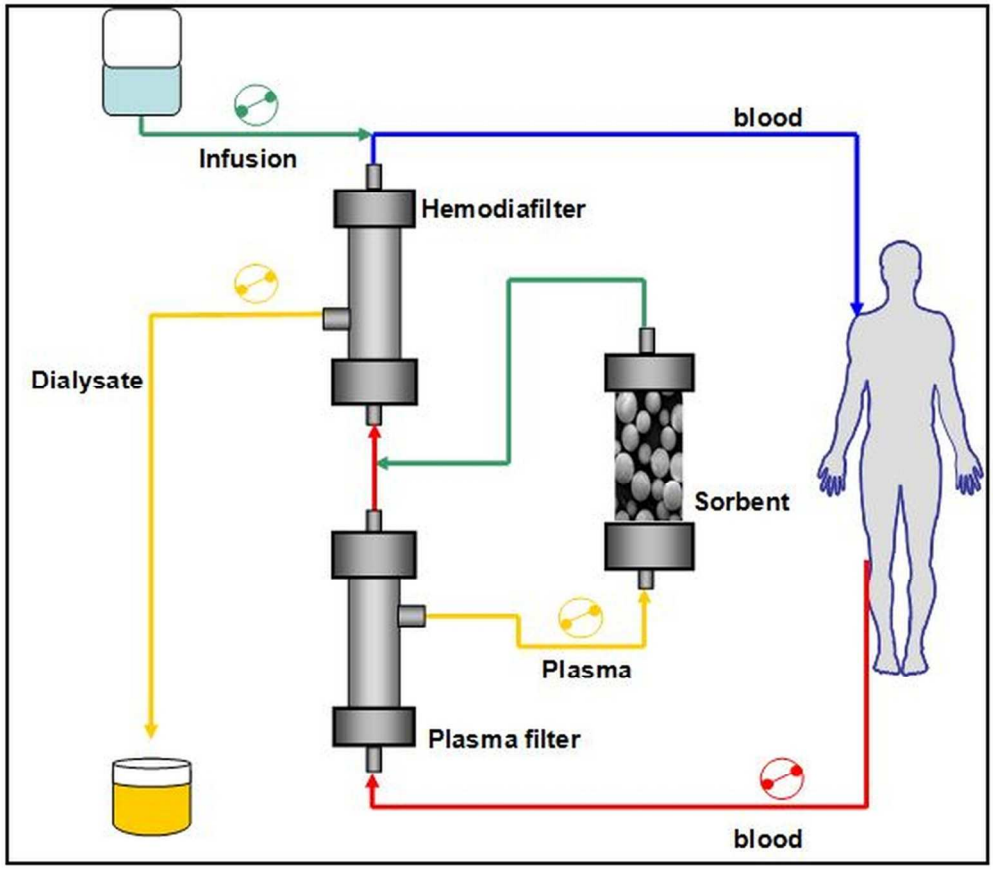

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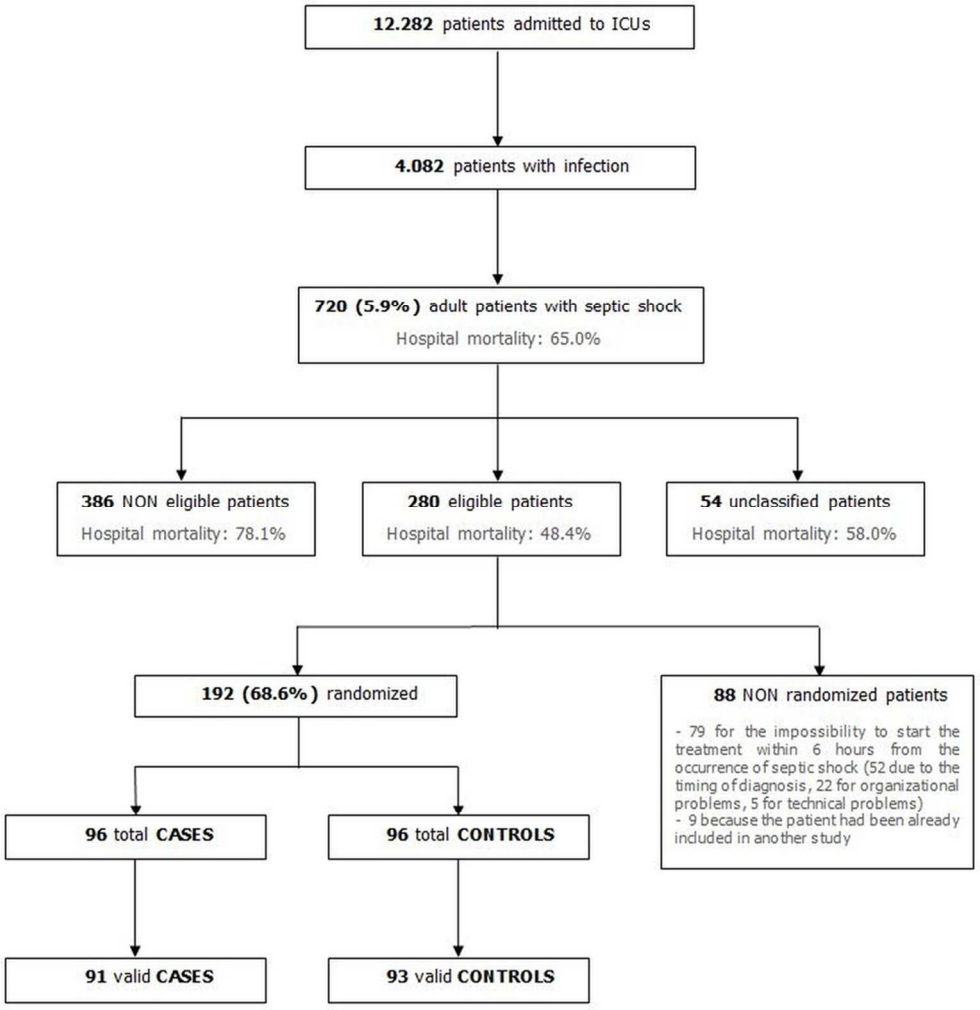

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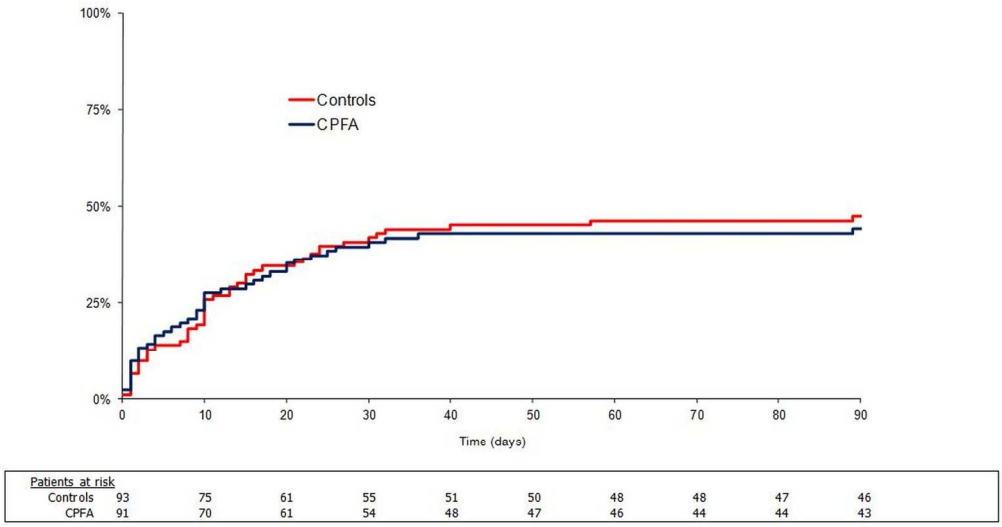

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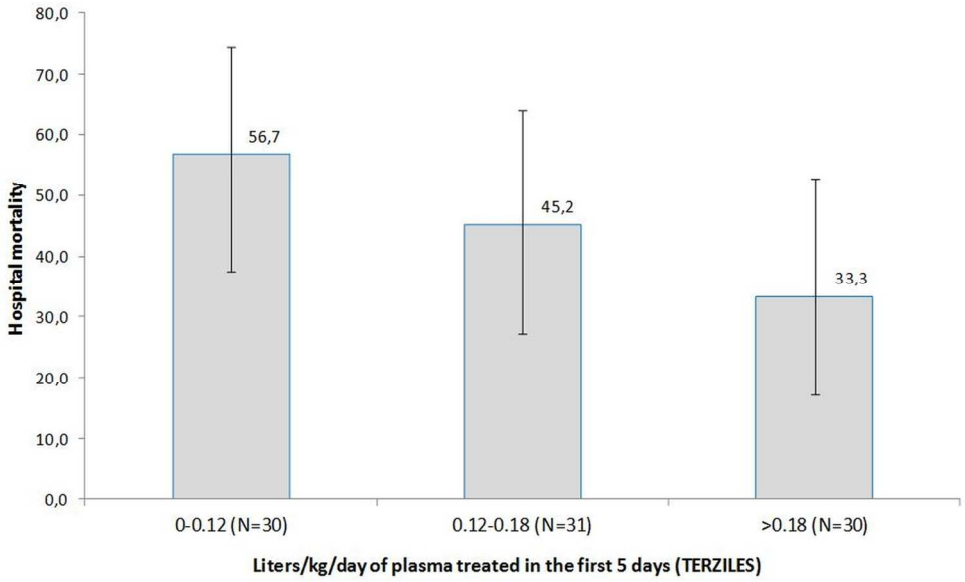

χ^2 test for general association, 3.26; $p = 0.20$
Cochran-Armitage test for trend, 1.82; $p = 0.069$

130x90mm (300 x 300 DPI)